PATH LINKS

BLOOD SCIENCES

USER GUIDE

United by Compassion: Driving for Excellence.

Working in partnership

Hull University Teaching Hospitals NHS Trust

Northern Lincolnshire and Goole NHS Foundation Trust

LOCATIONS OF THE HOSPITAL SITES OF PATH LINKS NHS PATHOLOGY DEPARTMENTS

BOSTON SITE
Pilgrim Hospital
Sibsey Road
BOSTON
Lincolnshire
PE21 9QS
Telephone 01205 364801

GRANTHAM SITE
Grantham & Kesteven District Hospital
101 Manthorpe Road
GRANTHAM
Lincolnshire
NG31 8DG
Telephone 01476 565232

GOOLE SITE
Goole & District Hospital
Woodland Avenue
GOOLE
North Humberside
DN15 6RX
Telephone 01405 720720

GRIMSBY SITE
Diana, Princess of Wales Hospital
Scartho Road
GRIMSBY
North East Lincolnshire
DN33 2BA
Telephone 01472 874111

LINCOLN SITE
Lincoln County Hospital
Greetwell Road
LINCOLN
LN2 5QY
Telephone 01522 512512

LOUTH SITE
County Hospital Louth
High Holme Road
LOUTH
Lincolnshire
LN11 0EU
Telephone 01507 600100

SCUNTHORPE SITE
Scunthorpe General Hospital
Cliff Gardens
SCUNTHORPE
North Lincolnshire
DN15 7BH
Telephone 01724 282282

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SECTION 1	GENERAL PURPOSE OF BLOOD SCIENCE SERVICES

DIRECTORATE PROFILE

The Path Links Blood Sciences Directorate is a clinically led service for the provision of Chemical Pathology, Blood Transfusion, Haematology, Immunology and Andrology services.

Our principal aim is to deliver a high-quality diagnostic, monitoring and health screening service to all service users. Services are provided primarily for the Northern Lincolnshire and Goole NHS Foundation Trust, United Lincolnshire Hospitals NHS Trust, and local Primary Care Trusts. The Laboratory Blood Sciences Directorate is part of the Path Links Pathology Service, a managed Pathology network operating across Lincolnshire since 2001. Laboratory services are provided at each of the Path Links sites at Scunthorpe, Grimsby, Lincoln, Grantham, and Boston offering Chemical Pathology, Blood Transfusion, Haematology, Immunology and Andrology services. Each laboratory is identically equipped, performing standardised procedures to provide a uniform service across Lincolnshire except for Immunology based on the Scunthorpe site and Andrology based at Boston and Grimsby.

GENERAL INFORMATION

Our experienced and motivated team of Consultants, Biomedical Scientists and ancillary staff operate from their own site but meet regularly to provide operational management of the Directorate. The laboratories maintain a comprehensive Management System through Path Links and the Directorate. This is in line with ISO15189:2022 UKAS standards.

The attention given to your request and the scope of analyses performed is partially related to the amount of legible clinical information and test requests written on the form. If a specific abnormality is suspected, please indicate CLEARLY when making the requests.
Laboratory test units and reference ranges have not been included in this user guide as a deliberate policy. The reports issued will have the precise reference range for the age and sex of the patient where appropriate. Abnormal results are highlighted on the report and are available on the enquiry mode via the Web V Trust results reporting system. Advances in technology and methodology can affect some ranges, and therefore to publish in this guide could be misleading.

Please refer to the ranges printed on the report. If you have any subsequent enquiry, please contact the laboratory.

Should any result be unexpected you should contact a senior member of staff and discuss the query with them. Whenever possible we will be happy to re-analyse the sample or suggest further investigations to clarify uncertainties.

Complaints, Concerns, Comments and Compliments
Please refer to the Trust website for information relating to complaints, concerns, comments and compliments. For Northern Lincolnshire and Goole please refer to Patient Advice and Liaison Service (PALS) - Northern Lincolnshire and Goole NHS Foundation Trust

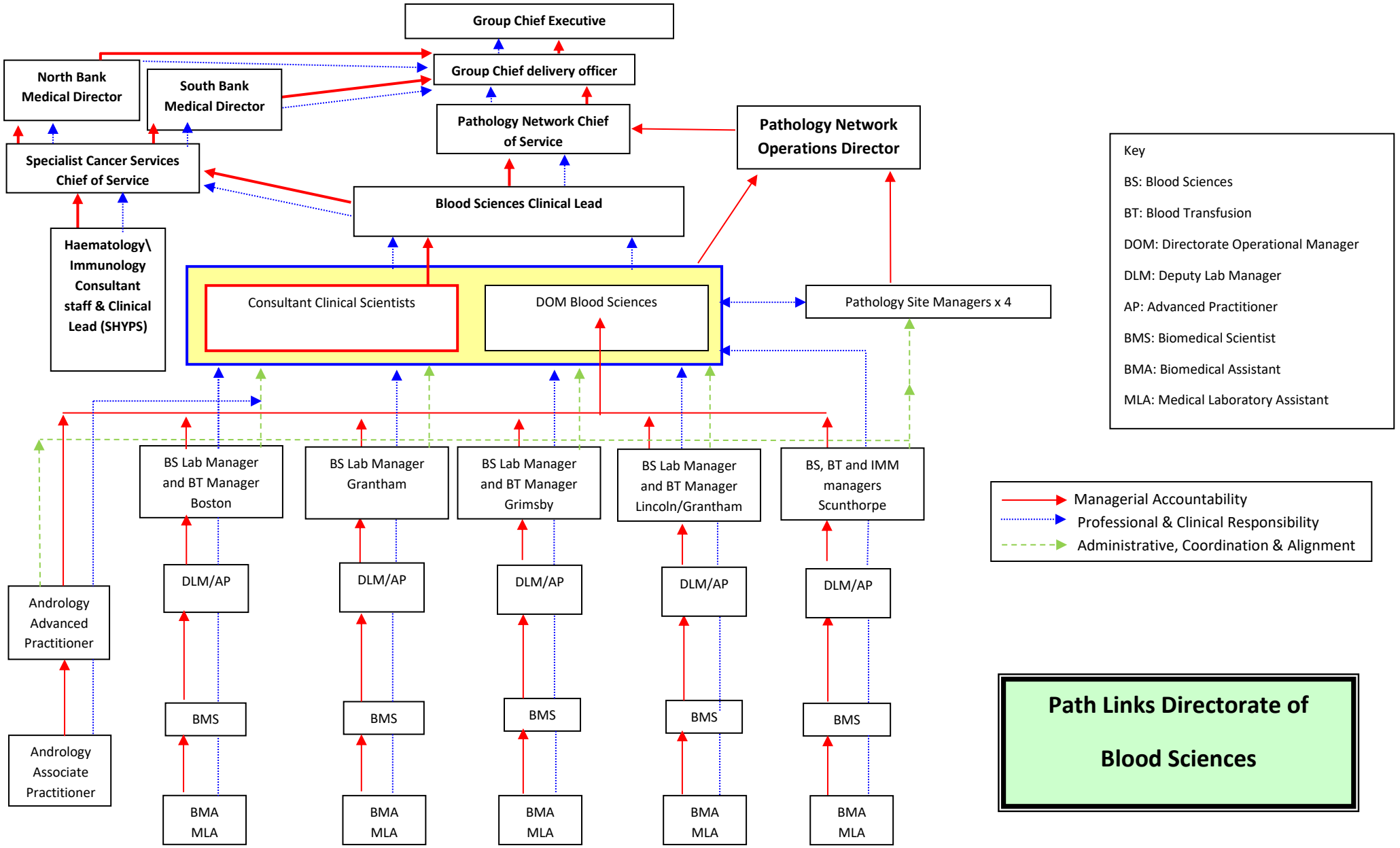
For United Lincolnshire teaching Hospitals NHS trust please refer to Patient advice and liaison service (PALS) - United Lincolnshire Hospitals

Patient Advice and Liaison Service (PALS)
The Patient Advice and Liaison Service offer confidential advice, support, and information on any health-related matters. If you have a comment, concern, complaint or compliment about the care or service you have received from the Trust you can contact the PALS team as follows:

Telephone: NLAG sites 03033 306518 ULH sites – see below
Email: nlg-tr.PALS@nhs.net or ulth.pals@nhs.net

There are offices at:

- **Diana Princess of Wales Hospital** (near the main entrance)
- **Scunthorpe General Hospital** (on the C Floor, near the outpatient department)
- **Grantham Hospital** the PALS office is located outside Ward 6 Tel 01476 464861 or 01476 464862
- **Pilgrim Hospital** the PALS office is in main reception Tel: 01205 446243 or 01205 446244
- **Lincoln County Hospital** the PALS office is located opposite ITU close to main reception Tel: 01522 707071 / 707072


Group Chief Executive
North Bank Medical Director
South Bank Medical Director
Group Chief delivery officer
Pathology Network Chief of Service
Pathology Network Operations Director
Specialist Cancer Services Chief of Service
Blood Sciences Clinical Lead
Haematology\ Immunology Consultant staff & Clinical Lead (SHYPS)
Consultant Clinical Scientists
DOM Blood Sciences
Pathology Site Managers x 4
BS Lab Manager and BT Manager Boston
BS Lab Manager Grantham
BS Lab Manager and BT Manager Grimsby
BS Lab Manager and BT Manager Lincoln/Grantham
BS, BT and IMM managers Scunthorpe
DLM/AP
DLM/AP
DLM/AP
DLM/AP
DLM/AP
Andrology Advanced Practitioner
Andrology Associate Practitioner
BMS
BMS
BMS
BMS
BMS
BMA MLA
BMA MLA
BMA MLA
BMA MLA
BMA MLA
Key
BS: Blood Sciences
BT: Blood Transfusion
DOM: Directorate Operational Manager
DLM: Deputy Lab Manager
AP: Advanced Practitioner
BMS: Biomedical Scientist
BMA: Biomedical Assistant
MLA: Medical Laboratory Assistant
Managerial Accountability
Professional & Clinical Responsibility
Administrative, Coordination & Alignment
Path Links Directorate of Blood Sciences

PATH LINKS CHEMISTRY CONSULTANTS	
Northern Lincolnshire & Goole Hospitals NHS Foundation Trust and United Lincolnshire Hospitals NHS Trust	
Dr Rachel Henderson Consultant Clinical Scientist Lincoln County Hospital and Grantham District Hospital Qualifications: PhD, FRCPath	
Dr Lesley Buswell Clinical Director Consultant Clinical Scientist Pilgrim Hospital, Boston, and Diana princess of Wales Hospital Grimsby Qualifications: BSc., PhD., FRCPath	**Special Interests** Endocrinology, Point of Care Testing (POCT)
Miss Amy Kiley Consultant Clinical Scientist Scunthorpe General Hospital Qualifications: BSc, MSc, FRCPath	

CONSULTANT HAEMATOLOGISTS	
Northern Lincolnshire & Goole Hospitals NHS Foundation Trust – provided by Hull Teaching Hospitals	
Dr James Bailey Laboratory Haematology - Medical Director	**Dr David Allsup** Honorary Consultant Senior Lecturer
Dr Senthilkumar Durairaj Consultant Haematologist - NLaG Transfusion Lead	**Dr Simone Green** Consultant Haematologist
Prof A Fielding Consultant Haematologist	**Prof Russell Patmore** Consultant Haematologist
Dr Zarni Soe Consultant Haematologist	**Dr R Emyeneokpon** Speciality Registrar in Haematology
United Lincolnshire Hospitals NHS Trust	
Dr Gamal Sidra Consultant Haematologist	**Prof Ciro Rinaldi** Consultant Haematologist
Dr P Chudakou Consultant Haematologist	**Dr Susan Levison-Keating** Consultant Haematologist
Dr Charlotte Kallmeyer Consultant Haematologist	**Dr A Boden** Consultant Haematologist
Dr M Gamage Consultant Haematologist	**Dr R Emyeneokpon** Speciality Registrar in Haematology
Dr O Ali Consultant Haematologist	**Dr A Essem** Consultant Haematologist

Path Links Laboratory Technical & Managerial Staff	
Steve Sharp, Scunthorpe IT technical lead Qualifications: BSc, CSci.	**Andrew Cawthra** Laboratory Manager Lincoln and Grantham Qualifications: BSc, MSc (Clin Biochem) Successful Management & Team Leadership programme (at NLaG; endorsed by the Institute of Leadership & Management)
Catherine Dimbleby Blood Sciences Laboratory Manager Blood Sciences Directorate Operational Manager Pathology Site Manager Grimsby Qualifications: BSc MSc Biomedical Science	**James Cragg,** Laboratory Manager Pathology Site Manager Boston Qualifications: BSc MSc MIBMS, PGCert.
Penny Feather Immunology Laboratory Manager Scunthorpe Qualifications: BSc Biomedical Science, HSD	**Andrew Cawthra** Laboratory Manager Lincoln Qualifications: BSc, MSc (Clin Biochem) Successful Management & Team Leadership programme (at NLaG; endorsed by the Institute of Leadership & Management)
Stuart MacDonald Blood Transfusion Manager Lincoln Qualifications: BSc (Hons) Biomedical Science, MSc Biomedical Science, PGCE.	**Ellen Holmes** Blood Transfusion Manager Boston Qualifications: BSc (Hons) Biomedical Science, MSc Biomedical Science, FIBMS, CSci
Abraham Aweda Blood Transfusion Manager Diana Princess of Wales Qualifications: BSc Biomedical science, MSc Haematology, BBTS – SCTSP or Specialist certificate in Transfusion Science Practice	**Kimberley Garnett** Blood Transfusion Manager Scunthorpe Qualifications: BSc Biomedical Sciences, MSc Pathological Sciences, QTS.
Daniel Horwich Blood Sciences Manager (Acting) Scunthorpe Qualifications: HND Biomedical Sciences, BSc Applied Biological Sciences, MSc Pathological Sciences	

See Section 4 for contact details.

Confidentiality and How We Use Data

Personal information on NHS patients is collected and recorded within paper and electronic formats primarily to support high quality care that is safe and effective. To do this, information is also used to support quality improvement activities, investigate any concerns you may raise as well as to support and understand NHS performance. All NHS staff have a legal duty to keep information about you confidential. Information will only ever be shared with people who have a genuine need for it. Other circumstances where information may be shared include administrative teams to plan future care needed, commissioners of Trust services, other NHS or social care providers and in some cases voluntary sector providers.

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SECTION 4	ACCESS TO SERVICES AND SERVICE AVAILABILITY

4.1 Laboratory Blood Science Directorate Contacts

GRIMSBY SITE
Diana, Princess of Wales Hospital
Scartho Road
GRIMSBY
NE Lincs
DN33 2BA

Telephone 01472 874111
Direct dial 03033 followed by extension number.

Contact	E-mail address	
Consultant Haematologists Dr. J Bailey Dr Dr S Durairaj Clinical support is via DPOW/Hull on call	james.bailey6@nhs.net s.durairaj1@nhs.net	Page via switchboard
Clinical Director- Consultant Clinical Scientist/ Dr Lesley Buswell – Boston Pilgrim Hospital /Grimsby – Diana Princess of Wales Hospital	lesleybuswell@nhs.net	03033 304482 or page via switchboard
Clinical Lead Andrology Dr Lesley Buswell – Boston Pilgrim Hospital /Grimsby – Diana Princess of Wales Hospital	lesleybuswell@nhs.net	03033 304482 or page via switchboard
Laboratory staff		
Chief of Service- Pathology care Group James Maclean	james.maclean@nhs.net	
Operations Director - Pathology Care Group **Elaine Seale**	elaine.graham1@nhs.net	
Path Links Blood Science Directorate Operational Manager/ Site manager James Spicer	jamesspicer@nhs.net	304444
Blood Science Manager Catherine Dimbleby	catherine.dimbleby@nhs.net	302672
Blood Transfusion Manager Abraham Aweda	abraham.aweda@nhs.net	304384
BLOOD SCIENCES LABORATORY		304385
BLOOD TRANSFUSION LABORATORY		304387
ANDROLOGY Appointments	Alix Marrows Helen Whaley	304494

SCUNTHORPE SITE
Scunthorpe General Hospital
Cliff Gardens
SCUNTHORPE
N Lincs
DN15 7BH

Telephone 01724 282282
Direct dial 03033 followed by extension number

Contact	E-mail address	extension
Consultant Haematologists Clinical support is via DPOW/Hull on call		Page via switchboard
Consultant Clinical Scientist Miss Amy Kiley Scunthorpe General Hospital	amykiley@nhs.net	03033 302199 or page via switchboard
Consultant Immunologist **Dr Pavels Gordins**	Pavels.Gordins@hey.nhs.uk	01482 461397
Consultant Immunologist **Dr Sujoy Khan**	Sujoy.Khan@hey.nhs.uk	01482 461444
Visiting Immunology **Consultant clinical scientist** Anna McHugh	anna.mchugh@nhs.net	01482 607710
Haematology Consultant Secretary **Chemistry Secretary** Susan Hampshire	 susanhampshire@nhs.net	305192 302365
Laboratory staff **Blood Sciences Manager** Daniel Horwich **Blood Transfusion Manager** Kimberley Garnett **Immunology Laboratory Manager** Penny Feather **Blood Sciences Laboratory** **Blood Transfusion Laboratory** **Andrology** Enquiries Appointments	 Daniel.Horwich@nhs.net kimberley.garnett@nhs.net p.feather1@nhs.net	 302639 302120 302050 306610 303788 03033 306610 03033 304494

LINCOLN SITE
County Hospital
Greetwell Road
LINCOLN
LN2 5QY

Telephone: 01522 512512

Direct dial: 01522 + extension no.
Fax: 01522 573757

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Contact	E-mail address	extension
Consultant Haematologists		
Dr C Kallmeyer	charlotte.kallmeyer@nhs.net	573077
Dr G Sidra	g.sidra@nhs.net	573750
Dr A Boden	alison.boden5@nhs.net	308722
Dr O Ali	osama.ali@nhs.net	597698
Dr H Fernandez	Harberth.Fernandez@nhs.net	308722
Dr P Chudakou	Pavel.Chudakou@nhs.net	307859
Consultant Clinical Scientist		
Rachel Henderson	rachel.henderson12@nhs.net	01522 573267 or page via switchboard
Consultant Secretaries		
Carol Edwards	Caroledwards3@nhs.net	597790
Debbie Nattrass	Debbie.nattrass@nhs.net	573724
Sue Naylor	Sue.naylor1@nhs.net	307296
Secretarial Assistants		
Sharon Allen	Sharon.allen32@nhs.net	573724
Wendy Cooper	Wendy.Cooper26@nhs.net	307297
Juliet Preston	Juliet.Preston@nhs.net	573724
Debbie Issott	Margaret.nunn2@nhs.net	597791
Laboratory staff		
Path Links Quality Assurance Manager Alison Gresty	Alison.Gresty1@nhs.net	
Pathology site Manager Rob Hughes	Robert.Hughes25@nhs.net	582532
Blood Science Laboratory Manager Andrew Cawthra	A.cawthra@nhs.net	597885
Blood Transfusion Manager Stuart MacDonald	Stuart.MacDonald11@nhs.net	597885
BLOOD SCIENCES LABORATORY		573269
BLOOD TRANSFUSION		573747
ANDROLOGY Appointments		01205 446314

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BOSTON
Pilgrim hospital
Sibsey Road
BOSTON
Lincs
PE21 9QS

Telephone 01205 364801
Direct dial 01205 + extension no.

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Contact	E-mail address	extension
Consultant Haematologists Dr C Rinaldi Dr S Levison-Keating	Crinaldi@nhs.net susan.levison-keating@nhs.net	446311 or page via switchboard page via switchboard
Consultant Clinical Scientist Dr Lesley Buswell	lesleybuswell@nhs.net	01205 446339 or page via switchboard
Clinical Lead Andrology Dr Lesley Buswell	lesleybuswell@nhs.net	01205 446339 or page via switchboard
Consultant Secretaries Alethea Baxter **Secretarial Assistants** Maggie Smith	Alethea.baxter1@nhs.net Maggie.Smith16@nhs.net	 446307 446319
Laboratory staff **Pathology site Manager/** **Blood Science Laboratory Manager** James Cragg **Blood Transfusion Manager** Ellen Holmes **HAEMATOLOGY LABORATORY** **BLOOD TRANSFUSION LABORATORY** **COAGULATION LABORATORY** **ANDROLOGY LABORATORY** **Andrology Appointments**	 James.cragg3@nhs.net EllenHolmes@nhs.net susan.parker57@nhs.net	 445837 446310 446328 446332 446329 446314 446314

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GRANTHAM SITE

Grantham & District General Hospital
101 Manthorpe Road
GRANTHAM
Lincs
NG31 8DG
Telephone 01476 565232
Direct dial 01476 + extension no.
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Contact	E-mail address	extension
Consultant Haematologist Dr G Sidra Dr H Fernandez	g.sidra@nhs.net Harberth.Fernandez@nhs.net	Page via switchboard
Consultant Clinical Scientist Rachel Henderson	rachel.henderson12@nhs.net	01522 573627 or page via switchboard
Consultant Secretaries Maria Joynt		464460
Laboratory staff		
Path Links Director James Maclean		464704
Pathology Site Manager Robert Hughes	Robert.Hughes25@nhs.net	01522 572249
Path Links Quality Assurance Manager Alison Gresty	Alison.Gresty1@nhs.net	464459
Blood Sciences Manager Andrew Cawthra	a.cawthra@nhs.net	464459
Blood Transfusion Manager Stuart MacDonald	Stuart.MacDonald11@nhs.net	464464/ 01522 597885
Blood Sciences Laboratory		464463
Blood Transfusion Laboratory		464464

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Hours of service (all sites)

Hospital Site	Service Area	Hours of Service	Daily Availability
Boston	Reception	09:00 – 17:30	Monday to Friday
	Laboratory	24-hour service	All Days
	Andrology	09:00 – 17:00	Monday to Friday
	Phlebotomy	08:45 – 16:45	Monday to Friday
Lincoln	Reception	09:00 - 17:00	Monday to Friday
	Laboratory	24-hour service	All Days
	Phlebotomy	08:00 – 17:00	Monday to Friday
Louth	Reception	08:30 – 16:30	Monday to Friday
	Phlebotomy	08:30 – 16:30	Monday to Friday
Goole	Reception	08:30 – 17:00	Monday to Friday
	Phlebotomy	08:30 – 17:00	Monday to Thursday
	Phlebotomy	08:30 – 16:30	Friday
Grantham	Reception	0830 – 1700	Monday to Friday
	Laboratory	24-hour service	All Days
	Phlebotomy	0830 – 1645	Monday to Friday
Grimsby	Reception	0800 – 1700	Monday to Friday
	Laboratory	24-hour service	All Days
	Phlebotomy	0800 – 1700	Monday to Friday
	Phlebotomy	Ward collection	Sat/Sun am
Scunthorpe	Reception	08:00 -17:00	Monday to Friday
	Laboratory	24-hour service	All days
	Immunology	09:00 – 17:30	Monday to Friday
	Phlebotomy	08:00 – 17:00	Monday to Friday
	Phlebotomy	Ward collection	Saturday am. Sunday am

SECTION 5	SCOPE OF SERVICES

5.1 Laboratory Equipment, Analysis, Results and Reporting

General Haematology, coagulation, blood transfusion, Chemistry and Immunoassay equipment is the same across all sites. This is beneficial for the users as the majority of tests can be undertaken at any site and the results will be comparable. It also provides service resilience during, for example, periods of bad weather, when transport access to the northern laboratories can be limited and work can be transferred to those laboratories less affected.

The laboratory computer allows the staff to access results from any Path Links site. This enables Consultant cross site cover, and authorisation of reports.

The in-house software, Web View, allows all wards and clinics in all hospitals to access patient results from both primary and secondary care. This results archive files all patient results temporally regardless of where the sample(s) has been analysed. It has also been developed to allow electronic requesting in both primary and secondary care. Paperless reporting is in place for the majority of general practice surgeries and is being developed in secondary care too.

5.2 Clinical services by site

i. **Haematology**

All Path Links Consultant Haematologists provide a proportion of their time to laboratory haematology activities. Their principal role is to provide a clinical haematology service which is managed separately through the respective Division of Medicine of their Trust.

Details of clinical haematology services including outpatient sessions and referral guidelines can be obtained from the appropriate Trust:

United Lincolnshire Hospitals NHS Trust	Northern Lincolnshire & Goole Hospitals NHS Foundation Trust
• Lincoln County Hospital	• Diana Princes of Wales Hospital Grimsby
• County Hospital Louth	• Scunthorpe General Hospital
• Pilgrim Hospital Boston	• Goole District Hospital
• Grantham & District Hospital	
nww.ulh.nhs.uk	nww.nlg.nhs.uk

All consultants contribute to locality multi-disciplinary team meetings and attend other appropriate local, regional or national meetings or conferences.

A number of specialist tests may be referred to other laboratories across the UK. Please contact your local laboratory for further details. Within Path Links certain specialist services have been centralised onto the following laboratory sites:

Lincoln: Specialist Coagulation, Hemophilia & Thrombophilia Testing

Scunthorpe: Sickle, Haemoglobinopathy and Thalassaemia Testing

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ii. **Clinical chemistry**

Clinical chemistry meetings by site

Boston	Weekly Grand Round and weekly endocrine meetings
Grantham	Monthly Grand Round meeting
Grimsby	Weekly Grand Round, monthly endocrine meetings
Lincoln	Weekly Physicians Meetings, quarterly Grand Rounds, weekly diabetes/endocrine review
Scunthorpe	Weekly Grand Round monthly endocrine meetings

All consultants attend other appropriate local, regional, or national meetings or conferences.

All sites - Diabetic clinics; weekly for adults and monthly for adolescents.

Lipid Clinics - Patients needing investigation or monitoring for dyslipidaemias are followed up at all hospital sites by the physicians running the general endocrine clinics.

Other off-site provision

Ward-based blood gas analysers on all sites, managed jointly between the 2 Trusts and the 2 POCT committees.

Ward-based blood glucose meters on all sites, managed jointly between the 2 Trusts and the 2 POCT committees.

Ward-based Hemocue haemoglobin analysers on some sites, managed jointly between the 2 Trusts and the 2 POCT committees.

Ward- based urine pregnancy testing kits on all sites, managed jointly between the 2 Trusts and the 2 POCT committees.

Ward- based INR testing on some sites, managed jointly between the 2 Trusts and the 2 POCT committees.

iii. **Immunology**

Clinics

General

The Immunology/Allergy Clinical Service is run by Hull Teaching Hospitals, providing a general immunology and allergy service for the diagnosis and management of immunologically mediated diseases.

Examples of conditions managed in the clinic include:

Antibody deficiency syndromes, urticaria, angioedema, anaphylaxis, and investigation of suspected immunodeficiency in patients with recurrent or unusual infections.

The clinics do not look after patients with the following conditions:

Children <16 years old (Scunthorpe: Dr S Kapoor). Children <16 are seen as tertiary referrals from Consultant Paediatricians only.
Chronic fatigue syndrome (please refer to specialist CFS services)
HIV/AIDS (please refer to Sexual Health Clinics)

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iv. **Andrology**

The Andrology Service is a clinically led service for the provision of fertility and post vasectomy semen analyses. Sample analysis takes place within Path Links Pathology laboratories based at Pilgrim Hospital, Boston and Diana, Princess of Wales Hospital, Grimsby.

Site	Department	Service Offered	Staff Contact	Telephone
Pilgrim Hospital, Boston Sibsey Road Boston Lincolnshire PE21 9QS	Pathology	Fertility & post vasectomy	Susan Parker Lindsay Pacey Appointments	01205 446314 01205 446314
Diana, Princess of Wales Hospital, Grimsby Scartho Road Grimsby North East Lincolnshire DN33 2BA	Pathology	Fertility & post vasectomy	Alix Marrows Helen Whaley Appointments	03033 304494 03033 304494
Lincoln County Hospital Greetwell Road Lincoln Lincolnshire LN2 5QY	Pathology	Receipt of routine post vasectomy samples only	Appointments	01205 446314
Scunthorpe General Hospital Cliff Gardens Scunthorpe North Lincolnshire DN15 7BH	Pathology	Receipt of routine post vasectomy samples only	Enquiries Appointments	03033 306610 03033 304494

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SECTION 6	QUALITY STANDARDS

ACCREDITATION

For a list of accredited tests provided by Path Links laboratories please refer to United Kingdom Accreditation Service (UKAS) Schedule of Accreditation; https://www.ukas.com/wp-content/uploads/schedule_uploads/00007/8833%20Medical%20Multiple%20.pdf

Laboratories have developed local standards for turnaround times that are monitored in accordance with the user's needs. Importance is placed on the service to A&E and EAU. The laboratory IT system can be interrogated for statistical analysis of turnaround times by test or source - as required by a user.

Path Links laboratories participate in regional and national audit activities as required e.g., Audit Commission.

National & local standards (UKAS/NEQAS)

QUALITY ASSURANCE AND AUDIT

At all sites there is full participation in External Quality Assurance schemes (EQA) where such a scheme exists for the test repertoire. Daily Internal Quality Control (IQC) sera are also analysed at specified intervals for all analytes on all analysers.

Evaluation of performance is monitored and reviewed through IQC and EQA and an audit timetable is set up on QPulse (an electronic quality management system) to store EQA reports and CAPA reports. When an EQA exception is noted, this is investigated and reported in a standardised manner in QPulse and tabled at the monthly Directorate meeting. All Path Links laboratories maintain close working relationships within county, within Trent region, nationally, and with UKNEQAS personnel to ensure the quality of the service is continually reviewed and improved as opportunity arises. The majority of equipment is supplied through a Managed Service Contract and ensures a very close working relationship with all of our major suppliers.

Adverse findings are reported through the appropriate channels, e.g., Medicines and Healthcare products Regulatory Agency (MHRA)

The Chemical Pathology Directorate has in place a planned programme of clinical audits and laboratory audits based on local, regional, and national initiatives.

Lesley Buswell is currently the co-ordinator for the clinical audit programme for the Directorate of Chemical Pathology. Audits are tabled at the monthly Directorate meeting and discussed in depth at either the Consultant away days or the technical working group meetings.

Additionally, there is an internal programme of vertical, horizontal and examination audits and assessments based on the CPA UK Ltd/UKAS assessment tools. Findings and corrective actions are recorded and actioned.

TURNAROUND TIMES

The time taken to produce a test report from collection of a sample is dependent on many variables, not least of which is the time taken for it to reach the laboratory. Turnaround times are therefore calculated from the time a sample is received in the laboratory to the time a result is available to clinical staff.

Most routine tests requests in Chemical Pathology are tested on the day of receipt; some may wait until the following morning. Reports will be generated as soon as these have been technically validated and where appropriate clinically authorised. In many cases this will not be a printed hard copy report, but via access to our electronic results service WebV. Some tests e.g., non-urgent therapeutic drugs and tumour markers, have been rationalised to one or two sites only, and may be analysed as a 'batch' because of the low volume of work.

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These will normally generate a report within 4-5 days. As a rule, target times are as follows:
Urgent biochemistry from Accident & Emergency Dept, Medical admissions: within 1 hour. Routine non-urgent inpatient biochemistry: within 4 hours
Routine non-urgent GP and Outpatient biochemistry: same day
Andrology reports: five working days
Samples referred to specialist laboratories: usually 1-2 weeks, but some may take several months.

Some tests cannot be analysed in Path Links laboratories. These include specialist tests that can only be analysed by a reference centre, tests that are only requested infrequently, or those where a regional centre has been established. Please refer to the appendix at the back of this book for an indication of how long it will take from sending a sample to receiving a report. These are only general guidelines. The actual time depends on the circumstances of the laboratories they are sent to, and delays can occur for several reasons.

For details of all turnaround test times, please refer to INT-INS-141, accessible via the NLaG intranet.

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SECTION 7	BLOOD SCIENCE SAMPLE COLLECTION

1. Introduction

It is a legal requirement that all health care workers ensure that ALL specimens are submitted to the laboratory in a safe manner. All samples and request forms should be correctly labelled. Please read the Trust sample labelling policy BEFORE collecting samples. If collecting **high risk samples,** please read section 5 of this guide. If only one sample has been collected for more than one departmental analysis, please ensure that forms are filled in for every department and are sent to the laboratory with the sample. It is impossible to guarantee tests will be performed if separate forms are not received. *Please ensure that any consumables or reagents supplied by Path links are returned for safe disposal where required, e.g., for exceeded expiration.*

When making a request:

- Every specimen container and every request form must describe the nature of the specimen and the identity and location of the patient, so that staff can identify the source quickly.
- The person who sends the specimen must ensure that the container used is the appropriate one for the purpose, is properly closed and not externally contaminated.
- The specimen should be placed in the sealable bag provided with the request form.
- If the hospital transport is to be used, the sample should be placed in the transport boxes provided. In the event of a sample breakage, the material leaked must be safely contained and collected. Decontamination kits with instructions are available separately from the laboratory for the vans and hospital sample collection trolleys.
- If a patient collects or transports a specimen to the laboratory themselves, it is the requesting Health Care Worker's responsibility to provide the appropriate containers, instructions, and collection facilities.
- If a specimen is to be posted, it must be in an individual sealed plastic bag, with the form kept separate, packed as per UN guidelines.
- Leaking or incorrectly labelled specimens will be discarded.

2. Test requests

For advice on the requesting of tests, please refer to the pdf version of the department handbook, contact the lab, or visit www.labtestsonline.org.uk. Lab tests on-line is a website that has been developed to provide patients and carers with information about medical laboratory tests.

Some tests are not performed in Path Links laboratories; these are referred to reference laboratories. For full information on these tests and laboratories please refer to the Path Links Send Away List which has a separate link.

The Turnaround Times (TATs) of all tests analysed in Path Links laboratories, and those sent to reference laboratories are audited and monitored. These are presented in a separate document. As a minimum target we aim to achieve these times for 95% of requests.

Laboratory test repertoire and reference ranges

The laboratory reference ranges are given on all printed and electronic reports and are tailored for age and sex as and where appropriate. They are taken either from manufacturer test data appropriate for the method and analyser used; from internally assayed patient material where appropriate or where reagent changes necessitate; from UK or international guidelines. When new ranges are introduced, an advisory note (including date of application) is included with the electronic record (Ward V and

pathology computer) and on printed reports. Immunology test reference ranges, where applicable, are
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included in the immunology user guide. Chemical Pathology reference ranges, due to the complexity of age and sex related ranges, are presented in a separate document.

Interpretative comments, including cut-off targets, are appended by consultant staff during clinical authorisation. Reference ranges and interpretative comments on test results from referral laboratories are similarly reproduced on all printed reports and within the electronic record. These are amended when necessary. Access to all this information is available to any authorised clinical user having password access to the electronic patient results, as well as on receipt of printed reports.

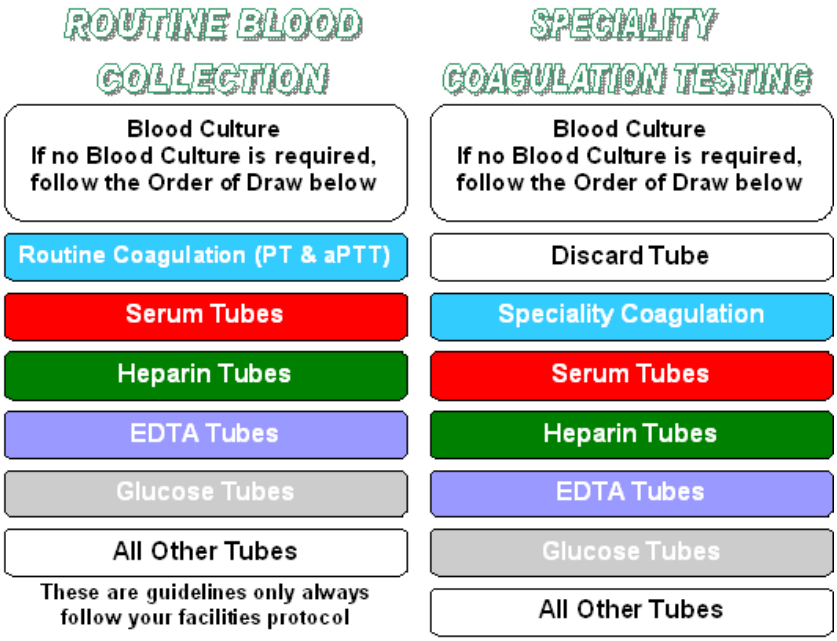
3. Blood Collection

The current blood collection system used throughout the whole of the Path Links area is the Greiner Bio-One Vacuette system. If you have difficulty collecting blood, please seek advice BEFORE sending the sample (or the patient) to the laboratory. Samples from IV infusions in the arm will give false results on all tests. Please read the Trust labelling policy BEFORE sending samples.

Please refer to separate links for:
Path Links Adult tube collection guide
Path Links Paediatric tube collection guide

Please ensure that tubes are collected in the following order: “blood culture, coagulation, serum, heparin, EDTA, glucose, all other tubes.

For speciality coagulation testing, if no blood culture is required, a discard tube prior to the order of draw is recommended."



Phlebotomy Service for Tests with Special Requirements

A routine phlebotomy service is provided for Outpatients. In cases where the blood sample must be separated and frozen quickly, e.g., PTH and Gut Hormones it is better for the patient to make an

appointment to attend so that the suitable arrangements can be made. It is essential to ensure the correct samples are collected and processed in the appropriate way to avoid any degradation of the analyte(s).

4. Centrifuge and Sample Handling Instructions for GP Surgeries

ALL samples that require centrifugation must be left for 30 minutes before spinning as this will allow the clot to form. They should then be centrifuged at 2000g for 10 minutes at 21oC.

Samples Requiring Special Handling—Not for Processing at Surgery
Contact the laboratory if you require further information

- ACTH
- Aldosterone
- Aluminium
- Amino Acids plasma. Urine Amino / organic acids / MPS
- Ammonia
- Alpha-1-antitrysin faecal
- Anti Mullerian Hormone
- Biotinidase
- Calcitonin
- Carotene
- Chromogranin A & or B
- Copper 24 hour urine
- Chromium / Cobalt
- C-Peptide
- Cysteine (leucocyte)
- Gastrin
- Glucagon
- Gut Hormone Profile (inc. chromogranin A&B)
- Guanidinoacetate / creatinine
- Homocysteine
- IGF2, IGF BP3, Proinsulin
- Lactate
- Metanephrines (Plasma)
- Neurotensin
- NSE
- Pancreatic Polypeptide
- Parathyroid related peptide
- Plasma 5HIAA
- Porphyrins
- Procollagen III
- Pyruvate
- Renin
- Serotonin
- Somatostatin
- TSH receptor antibody
- VIP
- Vitamin A
- Vitamin B1 (or B complex)
- Vitamin C
- Vitamin E

Trace elements sample requirements vary according to metal.

VACUETTE® tube serum 456089

- Copper
- Selenium
- Zinc

VACUETTE® tube 6ml Trace Elements— Sodium Heparin 456080

- Cobalt
- Chromium

Samples for Glucose analysis should be taken in the specific tube
(VACUETTE® tube 2nl FX Sodium Fluoride / Ptassium Oxalate 13x75 grey cap-white ring, non-ridged)
DO NOT CENTRIDGE

Please also be aware of the stability of the following tests:
DO NOT CENTRIFUGE

FBC	18hrs
Coagulation	10hrs
Specialist Coagulation	4hrs
D-Dimer	8hrs
Erythrocyte Sedimentation rate (ESR)	12hrs

5. 24hr urine collection

When collecting 24-hour urine samples, please ensure that correct and appropriately labelled bottles are used. The bottles can be obtained from Pathology reception.
General Instructions:

1. At a set time, e.g., 8am, empty the bladder completely and discard the urine. The start time may be altered for convenience, but the collection must finish 24hours after the start.
2. Collect ALL the urine passed after this time into the bottle(s) provided.
3. At 8am (or the start time chosen) again empty the bladder and ADD this to the bottle.
4. Return bottles to laboratory.

NB: IMPORTANT SAFETY NOTE - Some bottles will contain preservatives, including acid. Please ensure this is not discarded. Follow the instructions provided on the bottle and avoid all physical contact with the preservative.

6. High Risk Samples

If a patient is suspected of having rabies or viral haemorrhagic fever, no specimens should be collected without prior discussion with the Consultant Microbiologist.

Specimens from patients who have, or are suspected of having the following diseases constitute a risk of infection to persons handling the specimens (nursing, portering, Laboratory Reception and Technical staff):

- HIV (Aids related complexes)
- Amoebic dysentery
- Brucellosis
- Creutzfeldt-Jakob disease
- Psittacosis
- Q fever
- Tuberculosis (mycobacterium species)
- Typhoid/paratyphoid fever
- Viral hepatitis (including type B and C antigen carriers)

To minimise this risk, we ask you to ensure that such specimens are packaged as follows:

- Attach a Danger of Infection label to the specimen container.
- Place the specimen in a mini-grip bag (one sample per bag) and close the seal.
- Attach a Danger of Infection label to the request form.
- Specify on the request form the nature of the risk.
- Place the request form in the external pocket of the bag.

For out of hours specimens - these should not be transported via an air tube.

- Please make the On Call BMS staff aware and discuss with them the arrangements for the handover of the sample.
- Danger of Infection labels are available from the Laboratory.

If there is any doubt as to whether a specimen should be labelled "Danger of Infection ", the Consultant Microbiologist should be consulted.

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7. **Requests for additional tests on a sample that has already been sent to the laboratory:**

Requests for additional tests on the original sample can sometimes be made. These will depend on the sample stability and the tests required. Most Haematology tests must be performed on the day of collection. Coagulation samples should be tested fresh and additional tests are not normally accepted. Chemical Pathology and Immunology samples that have been collected into yellow cap or white cap (gel) tubes will be suitable for additional tests to be added up to 7 days after receipt but there are exceptions to this.

Wherever possible an additional test should be added electronically using the WebV or DART OCM requesting software. This automatically requests the test in the lab computer system and ensures a full audit trail. Rules set up in the database will restrict tests that are not suitable for testing due to stability issues.

If it is not possible to add a request electronically then please contact the laboratory for further advice. Where it is suitable to add the required test, the laboratory will book this on the system. The laboratory will make a note of the person requesting the additional test(s) and ask for a paper request form to be submitted with full patient details and the test(s) required. This should be sent to the laboratory as soon as possible. Where it is not possible to add the test, the requestor will be advised to collect a repeat sample.

SECTION 8	TRAINING & CONTINUING PROFESSIONAL DEVELOPMENT IN BLOOD SCIENCES

Consultants

The Consultant Clinical Scientists have post graduate qualifications and are Fellows of the Royal College of Pathologists. They participate in the College CPD process as required and are registered with the Health and Care Professions Council. They also meet regularly (weekly, fortnightly, or monthly) with hospital diabetes teams and with the endocrinologists to participate in case presentations, to discuss clinical cases and to discuss laboratory developments. They also participate in, and present to, the Medical Grand Round meetings.

All Consultant Haematologists are registered medical practitioners and Members of the Royal College of Pathologists. They all participate in the RCPath CPD programme and submit annual returns to the Royal College detailing their CPD activities which are subject to random audit.

Biomedical Scientists

Qualified BMS staff are registered with the Health and Care Professions Council.

BMS staff are required to participate in CPD to maintain State Registration. Some BMS staff participate in the voluntary IBMS scheme.

All BMS staff in Blood Sciences are in the process of being cross trained in the disciplines of Clinical Chemistry, Haematology, and in Immunology (Scunthorpe site only). Our aim is to produce an efficient Blood Sciences BMS team working in a lean environment with wider professional knowledge. Competency training in these disciplines is in place organised by the Consultant staff and Laboratory Managers.

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SECTION 9	LABORATORY HAEMATOLOGY HANDBOOK

9.1 Introduction

Haematology provides a range of tests to aid diagnosis and monitor certain treatments. The attention given to your request, and the scope of analyses performed is partially related to the amount of legible clinical information and test requests written on the form. If a specific abnormality is suspected, please indicate CLEARLY when making the requests.

The units and reference ranges have not been included in the test list. Many are age and / or sex related. The correct reference range for individual patients will be printed on the lab report and on enquiry mode via Trust IT systems if available. Please refer to INT-INS-92 on the intranet for full reference range data.

Important note: - The exception to this, are paediatric coagulation reference ranges which are not included on reports. Please refer to the paediatric reference table at the end of the Haematology section.

Please be cautious about making deductions from telephone calls to third parties. If in doubt seek advice.

9.2 Haematology Test List

The following chart gives information for use before collecting a sample including an indication of the correct Vacuette tube to use. Vacuette tubes automatically fill to the set volume. For multiple tests only one bottle, fully filled, of each colour will normally be required. Where additional tubes are required, this is indicated in the right-hand column. Paediatric samples can be collected using Microtainers, which may have a different colour code. For more information, please contact the laboratory. For further advice on the requesting of tests, please refer to the other pages in the handbook, contact the lab, or visit www.labtestsonline.org.uk

"Lab Tests Online" (https://labtestsonline.org.uk/) is a website that has been developed to provide patients and carers with information about medical laboratory tests. Posters and leaflets are available from the local laboratories.

Test	Container	Comment	Availability
A			
APTT	Blue Capped Tube	Heparin monitoring. Included in Coagulation screen	Daily 24 hrs
B			
Basophil Count	Lavender Capped Tube	Included in FBC	Daily 24hrs
Blood Film	Lavender Capped Tube		Daily 24hrs
Bone Marrow examination			Specialist request. Contact Consultant Haematologist
C			
Chromosome Analysis			Specialist request. Contact Consultant Haematologist
Coagulation factor assays	Blue Capped Tube x2	Investigation of bleeding disorders	Specialist request. Contact Laboratory. Centralised Path Links test. (Samples must be received and centrifuged with 4 hours of collection)
Coagulation screen	Blue Capped Tube	Investigation of coagulopathy	Daily 24hrs

Cold agglutinins	Pink Capped Tube		Contact laboratory before taking samples
Coombs test	Pink Capped Tube	See DCT	
Crossmatch	Pink Capped Tube	Blood group, antibody screen and compatibility studies prior to blood transfusion.	Daily 24hrs
Cryoprecipitate issue	Pink Capped Tube	Correction of coagulation defects	Specialist request. Contact Consultant Haematologist

D

DCT	Pink Capped Tube	Detection of red cell auto-antibodies (AIHA)	Daily 24hrs
D-Dimer (Age related)	Blue Capped Tube	For the exclusion of PE/DVT See XDP	Daily 24hrs

E

Eosinophil Count	Lavender Capped Tube	Included in FBC	Daily 24hrs
ESR	Lavender Capped Tube	Non-specific test elevated in inflammatory conditions and myeloma	Daily 24hrs
EMA binding test	Lavender Capped Tube	Investigation of haemolysis including hereditary spherocytosis	Specialist request. Contact Consultant Haematologist

F

Ferritin	Gel Tube.	Indicator of Iron Status	Daily
Fibrinogen	Blue Capped Tube	Must be requested –not included in coag screen.	Daily 24hrs
Folic Acid	Gel Tube.	Determination of Folate status. Measured together with Vitamin B12	Daily
Fresh frozen plasma (FFP) issue	Pink Capped Tube	For correcting coagulation defects	Specialist request. Contact Consultant Haematologist

Full Blood Count (FBC)	Lavender Capped Tube	Basic profile includes Hb, WBC, RBC and Platelets, plus WBC differential.	Daily 24hrs

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G

G6PD deficiency screen	Lavender Capped Tube	Assessment of deficiency causing haemolytic anaemia particularly in association with malaria drugs.	Specialist request. Contact Consultant Haematologist
Glandular Fever Test	Lavender Capped Tube	Screening test for Infectious Mononucleosis	As requested,
Group	Pink Capped Tube	ABO and Rhesus group	Daily 24hrs

H

Haematocrit (Hct)	Lavender Capped Tube	Included in FBC	Daily 24hrs
Haemoglobin (Hb)	Lavender Capped Tube	Included in FBC	Daily 24hrs
Haemoglobin electrophoresis	Lavender Capped Tube Minimum sample volume 1ml	Used to diagnose. Haemoglobinopathies	Centralised Path Links service Simple cases reported within 72 hours.
Haemosiderin (Urine)	Fresh early morning urine	Investigation of chronic haemolysis	As requested,
Haptoglobin	Gel Tube.	Investigation of haemolysis	Centralised Path Links service
HLA typing			Contact Blood Transfusion laboratory

I

Immunophenotyping white cells		Determination of white cell subsets and identification of neoplastic proliferation e.g., leukaemia.	Specialist request. Contact Consultant Haematologist
Infectious Mononucleosis	Lavender Capped Tube	See glandular fever.	
INR	Blue Capped Tube	Used to monitor Warfarin therapy	Available as requested. 24hrs

K

Kleihauer	Pink Capped Tube and Lavender Capped Tube	Rhesus prophylaxis. Special requirements dependent upon type of event and gestational age.	Contact Blood Transfusion laboratory

L

Leucocyte antibodies			Contact Blood Transfusion laboratory
Lupus anticoagulant	Blue Capped Tube x 3 & 1 white top	Investigation of suspected coagulation inhibitor	Centralised Path Links service (Samples must be received and centrifuged with 4 hours of collection)
Lymphocyte count	Lavender Capped Tube	Included in FBC	Daily 24hrs

M

Malaria Screen	Lavender Capped Tube		Daily 24hrs
Mean cell Hb (MCH)	Lavender Capped Tube	Included in FBC	Daily 24hrs
Mean cell volume (MCV)	Lavender Capped Tube	Included in FBC	Daily 24hrs
Monocyte count	Lavender Capped Tube	Included in FBC	Daily 24hrs
Monospot	Lavender Capped Tube	See Glandular Fever	

N

Neutrophil Count	Lavender Capped Tube	Included in FBC	Daily 24hrs

O

P

Packed cell volume		See haematocrit	
Paul Bunnell	Lavender Capped Tube	See Glandular Fever	
Platelet Function Analysis (PFA)	Blue Capped Tube		Specialist request. Contact Consultant Haematologist. Performed by the Royal Hallamshire Hospital Coagulation department. Samples must be tested within 1 hour of collection.

Plasma Viscosity	Lavender Capped Tube	ESR equivalent- request ESR unless clinical details of hyperviscosity states	Referred test.
Platelet Aggregation studies	Special Requirements		Specialist request. Contact Consultant Haematologist
Platelet antibodies			Contact Blood Transfusion laboratory
Platelet Count	Lavender Capped Tube	Included in FBC	Daily 24hrs
Platelet issue	Pink Capped Tube	Correction of thrombocytopenia	Specialist request. Contact Consultant Haematologist
Prothrombin Time (PT)	Blue Capped Tube	Monitoring liver dysfunction. Included in coagulation screen.	Daily 24hrs

R

Red Blood Cell (RBC) count	Lavender Capped Tube	Included in FBC	Daily 24hrs
Red Cell Enzymes			Specialist request. Contact Consultant Haematologist
Reticulocyte Count	Lavender Capped Tube	Measurement of juvenile red cells - indicator of marrow turnover.	Daily 24hrs
Rhesus prophylaxis		See Kleihauer	

S

Sickle screen	Lavender Capped Tube	Screening test for Sickle Haemoglobinopathy	Daily, 24hrs for urgent cases.

T

Thalassaemia screen	Lavender Capped Tube	Screening test for Thalassaemia or abnormal haemoglobins.	Centralised Path Links service

Thrombophilia investigations	Blue Capped Tube x 4 & 1 white top	See specific guidelines for the investigation of thrombophilia	Centralised Path Links service specialist request. Refer to guidelines. (Samples must be received and centrifuged with 4 hours of collection)
Transfusion reaction			Contact Blood Transfusion laboratory

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V

Vitamin B12	Gel Tube.	Determination of Vitamin B12 status. Measured together with folate.	Daily
Von Willebrand screen	Blue Capped Tube	Collect 2 tubes.	Centralised Path Links service (Samples must be received and centrifuged with 4 hours of collection)

W

WBC Differential	Lavender Capped Tube	Included in FBC	Daily 24hrs
White Blood Cells (WBC) count	Lavender Capped Tube	Included in FBC	Daily 24hrs

X

XDP	Blue Capped Tube	D-Dimer test performed as a substitute DIC	Daily 24hrs

Y

9.3 Full Blood Count

The Full Blood Count (FBC) comprises of the following: -

HB - Haemoglobin

RBC – Red Blood Cell count

WBC – White Blood Cell count

PLT – Platelet count

HCT – Haematocrit

MCV – Mean Cell Volume

MCH – Mean Cell Haemoglobin

MCHC – Mean Cell Haemoglobin Concentration

RDW – Red Cell Distribution Width

Plus, absolute counts of the five types of mature WBC which are present in descending order in a normal patient.

Neutrophils

Lymphocytes

Monocytes

Eosinophils

Basophils

In addition, the haematology analysers alert to the possibility of presence of immature or abnormal white cells. When this occurs a blood film is examined to ascertain the type of cells present and their morphology and we would offer a morphological opinion.

The FBC is mainly requested as a screening test but may be used to monitor treatment or progression of disease.
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The haemoglobin (HB) is used to detect anaemia or polycythaemia. The mean cell volume (MCV), mean cell haemoglobin (MCH) and to a lesser extent the mean cell haemoglobin concentration (MCHC) are useful in helping to classify the cause of anaemia (i.e., MCV low in iron deficiency but normally raised in B12 or Folate deficiency).

The White Blood Count (WBC) is useful in detecting infection, inflammation, leukaemia, and response to chemotherapy.

The Platelet (PLT) count detects potential bleeding problems due to thrombocytopenia; high counts may indicate occult bleeding or inflammation. Monitoring of the count is important during chemotherapy.

9.4 Coagulation Screening

Prothrombin Time (PT)

The Prothrombin Time (PT) is generally requested as part of a non -specific clotting screen or more specifically for the determination of the International Normalised Ratio (INR) for the monitoring of oral anticoagulants.

The PT measures the clotting time of plasma in the presence of an optimal concentration of tissue factor (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system. The test is sensitive to levels of factors II, V, VII, X and fibrinogen. The classical PT is performed by mixing equal volumes of citrated plasma, thromboplastin, and calcium chloride solution. The time taken for the plasma to clot when maintained at 37° C is recorded.

The PT may be used as: -

1. A rapid screening test to detect single or combined deficiencies of the extrinsic system including hereditary or acquired coagulation disorders, liver disease or Vitamin K deficiency.
2. A sensitive monitoring test for oral anticoagulant therapy
3. The basis for specific extrinsic pathway coagulation factor assays

Control of Oral Anticoagulant Therapy and the INR

It is now estimated that there are approximately 500 000 patients' currently prescribed oral anticoagulant drugs in the UK. The most prescribed drug is warfarin and less frequently acenocoumarol and phenindione. All these drugs need regular monitoring and dosage adjustment to maintain the desired therapeutic range.

Under anticoagulation may result in thrombosis which may be life threatening.

Over anticoagulation can result in haemorrhage which may be fatal.

The PT is the primary laboratory test for monitoring oral anticoagulant therapy. Different drugs used e.g. warfarin, acenocoumarin and phenindione can be monitored by the same test. Tests performed for monitoring of oral anticoagulation should be reported using the International Normalised Ratio (INR).

Plasma obtained from a healthy adult person is considered normal and can be used for calibration of a PT system. The mean normal prothrombin time (MNPT) is defined as the geometric mean of prothrombin times of the healthy adult population. The PT Ratio is the PT obtained with a test sample divided by the MNPT where all times have been determined using the same PT system. The ISI is a quantitative measure in terms of the first primary international reference preparation.

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The INR is defined for a given sample from a patient on long term oral anticoagulant therapy. The INR is calculated from the prothrombin time ratio using formula.

$$INR = (PT/MNPT)^{ISI}$$

Activated Partial Thromboplastin Time

The Activated Partial Thromboplastin Time (APTT) is generally requested as part of a non-specific clotting screen or in isolation for anticoagulant (heparin) control. The APTT measures the overall efficiency of the intrinsic and common coagulation pathways in citrated plasma.

The APTT is sensitive to deficiencies of factors in the intrinsic and common coagulation pathways i.e. XII, XI, IX, VIII, V, II and fibrinogen. It is also sensitive to the presence of inhibitors for example lupus type inhibitors and to the anticoagulant effect of heparin.

The APTT may be used as.

1. A screening test to detect single or combined deficiencies of the intrinsic system or common pathway including hereditary or acquired coagulation disorders, liver disease or Vitamin K deficiency.
2. A monitoring test for heparin anticoagulant therapy
3. The basis for specific intrinsic coagulation factor assays

Paediatric coagulation reference ranges – APTT reference ranges quoted as ratio's due to reagent lot variation.

INFANT AGE		1 Day		5 Days		1 Month		3 Months		6 Months	
Prem ~ Healthy premature infants 30 – 36 weeks gestation		Term	Prem	Term	Prem	Term	Prem	Term	Prem	Term	Prem
Prothrombin Time (secs)	Lower Limit	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3
	Upper Limit	15.2	15.7	14.5	15.0	14.5	14.5	14.5	14.5	14.5	14.5
APTT (Ratio)	Lower Limit	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
	Upper Limit	1.2	1.8	1.4	1.7	1.3	1.5	1.3	1.3	1.1	1.6
Fibrinogen values in healthy premature & term infants are not significantly different from those of adults											

Table adapted from Sheffield paediatric coagulation – Sheffield Children's Hospital

D-Dimer (age-related)

Rationale and impact of Age adjusted D-dimer testing within NG158: In people aged over 50, there was limited prospective evidence available for DVT and only retrospective evidence available for PE. This evidence suggested that adjusting D-dimer test thresholds for age improves the usefulness of these tests for ruling out venous thromboembolism (VTE) in this age group. The evidence also suggested that age adjustment does not reduce the accuracy of the tests in identifying VTE. The committee noted that adjusting test thresholds for age could be beneficial in reducing anxiety and unnecessary imaging for people with suspected DVT or PE. Although the evidence was not plentiful, the committee agreed that, taken together with the potential benefits, it was sufficient to support a recommendation suggesting age adjustment in D-dimer test thresholds for people aged over 50.

Requests for D-dimer investigation to exclude DVT/PE will now be reported as a quantitative value against a normal range 0-230 ng/ml DDU.

Requests for XDPs in cases of DIC will remain the same, we report a quantitative value against a normal range of 0-250 ng/ml in D-Dimer Units (DDU).

For all requests, any value under 150 ng/ml DDU will be reported as <150 ng/ml DDU due to the sensitivity/specificity of the method at the lower values.

The following automatic comment will be attached for age related D-dimer (> 50 years), allowing the clinician to interpret:

"Please note: NICE guideline NG158 (March 2020) recommends that the use of an age adjusted d-dimer threshold* should be considered in patients over the age of 50 being investigated for a possible DVT or PE with a low-risk Wells score.

"*Age adjusted d-dimer threshold = Age (years) x 5 e.g., patient age 75 x 5 = cut off 375 ng/ml DDU"

The cut-offs for each test are different based on the XDPs being calculated on a normal population and the D-dimer for VTE/PE being calculated on a mixed group of low/moderate/high pre-test probability patients (wells score). This prevents us having a single test because of the risk it would be interpreted incorrectly.

Be aware D-dimer requests are <u>not appropriate for patients on anticoagulants</u>.

<u>Links</u>

- **NICE Guidance**

Overview | Venous thromboembolic diseases: diagnosis, management, and thrombophilia testing | Guidance | NICE

9.5 Infectious Mononucleosis Screening Test

Infectious mononucleosis (IM) is an acute herpes virus infection caused by the Epstein-Barr virus (EBV). EBV enters B lymphocytes via CD21, a surface receptor for the C3d component of complement and after the acute infection has been resolved, a lifelong sub clinical infection is maintained, with a low frequency of infected B cells and detectable virus in the saliva, the main vehicle of contagion.

EBV infection of children usually results in immunity without the development of clinical symptoms. This immunity is generally detectable serologically and protects for life. Usually only after the age of 10 years is infection by EBV associated with the clinical symptoms of IM.

It is a disease of variable severity being characterised by a range of symptoms that can include lethargy, sore throat, lymphadenopathy, splenomegaly, hepatitis, and jaundice. Unusual consequences of the disease are autoimmune haemolytic anaemia, spontaneous splenic rupture, aplastic anaemia, and thrombocytopenia.

Treatment of IM is primarily symptomatic with bed rest to prevent serious complications of the liver or spleen and analgesics to control pain. Steroids may be indicated in the management of associated haemolytic anaemia, thrombocytopenia, progressive neurological complications, and incipient airways obstruction.

During the acute phase of the illness, a variety of antibodies are produced including certain heterophile antibodies which appear in 85-90% of IM cases. These antibodies are known as IM heterophile antibodies and are primarily of the IgM class. The IM heterophile antibodies are usually demonstrable one week after the onset of the illness, peak at two to four weeks and decline to low levels after 12 weeks. One year after the onset of the illness it is not unusual for heterophile antibodies to still be detected in patient's serum.

The Rapid Glandular test will only indicate the presence of Infectious Mononucleosis antibodies in the specimen and should not be used as the sole criteria for the diagnosis of Infectious Mononucleosis infection. All results must be interpreted together with other clinical information available to the physician.

9.6 Investigation of Malaria

Malaria continues to be a great burden globally with >300 million acute cases per annum, accounting for >1 million deaths; it is the most important parasitic disease worldwide.

In 2015, 1400 cases of malaria were reported in the UK, including 6 deaths. Most should be preventable. The largest proportion of travellers returning to the UK with malaria is those who have been visiting friends or relatives.

Suspected malaria is a medical emergency – sampling and processing should not be delayed.

Malarial parasites may be present in the peripheral blood, even in the absence of fever. Therefore, if a clinical suspicion of malaria exists, blood sampling is necessary whether the patient shows fever or not.

The recommended method and current gold standard used for the routine laboratory diagnosis of malaria is the microscopic examination of stained thick and thin blood films. Thick films offer additional sensitivity due to a larger volume of blood being examined at any one time. If thick films are positive, the species should be determined by the examination of a thin film. Identification of the different malaria parasites is important, as the treatment varies accordingly. *Plasmodium falciparum* can be rapidly fatal, and the percentage of parasitized cells should be estimated as an indicator of severity and an aid to drug therapy required to eradicate.

9.7 Erythrocyte Sedimentation Rate

The Erythrocyte Sedimentation Rate (ESR) determination is a commonly performed screening test as a measure of the inflammatory response. Whilst routinely used for many years, the usefulness of this test has decreased as new methods of evaluating disease have been developed. Numerous studies have been undertaken to determine whether other tests, such as measurement of C - Reactive Protein (CRP), may perform better than the ESR.

Repeatedly, the ESR has been shown a satisfactory monitor of acute-phase response to disease after the first 24 hours. During the first 24 hours in an inflammatory process, CRP may be a better indicator of the acute phase response.

The ESR remains helpful in the specific diagnosis of a few conditions, including temporal arteritis, polymyalgia rheumatica and, possibly, rheumatoid arthritis. It is useful in monitoring these conditions and may predict relapse in patients with Hodgkin's disease. Use of the ESR as a screening test to identify patients who have serious disease is not supported due to its non-specificity.

9.8 Haemoglobinopathy Screening

Haemoglobins (Hb) are a group of proteins whose chief functions are to transport oxygen from the lungs to the

tissues and carbon dioxide in the reverse direction. They are composed of polypeptide chains called globin and iron. protoporphyrin haem groups. Human haemoglobin is formed of two pairs of globin chains to each of which is attached one molecule of haem. The α chains comprise of 141 amino acids and the β, γ and δ chains of 146.

Hb F (α2 γ2) is the predominant haemoglobin in the foetus and neonate. After birth the level of Hb F falls and is replaced by Hb A (α2 β2) in normal individuals. Within 1 year the Hb F falls to <1%. In some individuals the level of Hb F does not fall e.g., Hereditary Persistence of Foetal Haemoglobin, β-thalassaemia major, δβ- thalassaemia and during pregnancy where the level of Hb F can often be 1-4%. There are over 1000 haemoglobin variants and although many are harmless some have serious clinical effects. The most clinically relevant and frequent encountered haemoglobin variants are Hb S, C, E, D, and O-Arab.

Abnormal Hb's are of importance for diagnosing sickle cell anaemia or the interaction of the abnormal Hb with a concurrent thalassaemia syndrome. The level of Hb A2 (α2δ2) is measured and of importance for the diagnosis of β thalassaemia trait.

The clinical significance of abnormal haemoglobin can range from symptom free to life threatening disease. Haemoglobinopathy screening is centralised onto the Scunthorpe site. Simple cases can be fully diagnosed, i.e., Sickle cell trait. Complex cases may require referral to an external reference laboratory for confirmation and further investigation. For pregnant women partner screening may be recommended and a normal partner result may negate the requirement for complex genetic screening of the maternal sample as the child is not classed as "at risk" of a Haemoglobinopathy with serious clinical implications.

9.9 Ante Natal Screening Service

The Ante Natal testing screen includes Full Blood Count (FBC), Blood Grouping & Antibody Screening, and Infectious Disease Screening (HIV, Hepatitis B, Rubella and Syphilis), and Haemoglobinopathy screen.

FBC's are tested in all Path Links laboratories, the blood group and infectious diseases screening is centred in Grimsby. Haemoglobinopathy screening is centralised at Scunthorpe site.

The Path Links Ante Natal Screening form should be used in all instances. The request form should be completed legibly and as comprehensively as possible. The following patient information must be provided:

- NHS Number (mandatory) — On form & sample
- Hospital Number — On form & sample
- Surname — On form & sample
- Forename — On form & sample
- Date of Birth — On form & sample
- Full Address and Post Code — On form only

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Samples must be dated and signed by the person taking them. Addressograph labels must not be applied to samples. Failure to meet these requirements will lead to sample rejection and a requirement for repeat sampling. The appropriate sample types are:

Blood Grouping, Antibody Screening, Antibody Investigations: 1 x 6ml EDTA sample (pink cap)

HIV, Hepatitis, Rubella, Syphilis: 1 x 4ml Clotted sample (Yellow cap)

FBC, Sickle & Thalassaemia: 1 x ml EDTA sample (Purple cap)

In all instances of a positive antibody screen finding, the primary sample will be referred to the NBS laboratory for further investigations. Repeat sampling will only be necessary in the event of sample spoilage or insufficiency.

Positive findings of infectious disease screening may require repeat sampling in accordance with National Guidelines.

- Testing protocols are aligned with the NHS Screening and UK National Screening Committee, Infectious Diseases in Pregnancy Screening Programme.

- For three of the four clinical conditions (Hep B, HIV, and syphilis) there is a requirement for confirmatory testing using different analytical methods on the initial screening specimen before a report is issued.

- Subsequently, a second specimen should be taken after referral to clinical services to confirm the initial results and the woman's identity and perform additional diagnostic tests. Two positive results from separate specimens are required to establish positive Hepatitis B, HIV and Syphilis status.

- Confirmation of an initial Rubella screening result of <10 IU/ml by an alternative analytical method is not considered necessary.

Positive findings for Haemoglobinopathy screen will necessitate partner screening to identify any "at-risk" child. This provides the opportunity for the parents to receive appropriate counselling in order that they can make an informed choice regarding any action they may wish to take or support that the child may require in both the ante-natal and post-natal period.

It is imperative that an accurately completed Family Origin Questionnaire (FOQ) is received with all "booking" samples as we do not have consent to test without this document. The ethnic origins are important as different haemoglobinopathies are more common in certain populations so the action required may vary dependent on the ethnic origins of both partners.

Ethnicity of at least 2 generations should be considered.

9.10 Guidelines for the investigation and detection of heritable thrombophilia

Haematology Laboratories are receiving an increasing number of requests for "Thrombophilia Testing". This reflects a growing interest among General Practitioners and Hospital Specialists in the role of heritable abnormalities in predicting an increased risk of thrombo-embolic disease in some individuals and their kindred. Until recently Haematologists have been undecided how to advise patients and their doctors in the light of the results of these tests. There has been no consensus on how the results of thrombophilia tests should influence the patient's treatment.

The latest "Clinical Guidelines for testing for Heritable Thrombophilia" were published in 2010 British Journal of Haematology Volume 149, Issue 2, pages 209–220, April 2010

Based on these guidelines we have drawn up a series of recommendations of who should, and should not, have thrombophilia testing. These recommendations are not meant to be prescriptive. Please contact Dr. Pavel Chudakou consultant Haematologist with particular interest in coagulation for advice when requesting this test. pavel.chudakou@nhs.net Tel: 01522 512512.

Thrombophilia testing is recommended in the following cases:

- those who have had a first proven venous thromboembolism (VTE) before age 50.
- those who have personal history of unprovoked (spontaneous) multiple VTE.
- those who have a personal history of VTE and a family history of VTE in one or more first-degree relatives.
- asymptomatic women being considered for the combined oral contraceptive pill or hormone replacement therapy who have at least two first or second-degree relatives with VTE linked to a known genetic thrombophilic abnormality.

Thrombophilia testing is NOT recommended in the following:

- patients who have suffered VTE in the previous month.
- patients who are receiving anticoagulant drugs such as Warfarin or heparin

- unselected asymptomatic individuals
- those who have a single VTE after age 50.
- asymptomatic relatives of persons with VTE in whom no specific thrombophilic abnormality has been reported.
- children with relatives who have VTE or thrombophilic abnormalities.
- those who have cancer.
- cases of arterial thromboembolism.
- asymptomatic relatives of asymptomatic individuals with thrombophilic abnormalities.
- women who are pregnant or taking the oral contraceptive pill.

Requesting Thrombophilia Testing

Persons in whom thrombophilia testing is recommended will normally need explanation, counselling, and sometimes treatment, whether a thrombophilic abnormality is detected or not. The referring doctor should write a letter to the Consultant Haematologist of the appropriate Path Links laboratory.

Referral letters should contain the following information.

- Personal medical history including VTE.
- Personal history of previous surgery, pregnancy, or contraceptive pill use.
- Family history of VTE and any thrombophilic abnormality.
- Current and recent medication including anticoagulants, contraceptive pill, and HRT.

Blood Samples for thrombophilia testing

Four coagulation samples containing citrate anticoagulant are normally used for thrombophilia testing. Samples can be obtained from the patient when she/he attends the Haemostasis Clinic. Consultant Haematologists welcome discussion on individual cases even when they do not meet the guideline recommendations for thrombophilia testing.

9.11 Blood Transfusion Guidelines

General Advice for Adults

In medical cases where there is no evidence of blood loss, avoid transfusion if possible until the cause of the anaemia is established. Often in such cases, the need for transfusion may be avoided altogether. For example, transfusion in pernicious anaemia, especially when markedly anaemic, may be particularly hazardous and is usually better treated by B12 replacement therapy alone. If the patient is symptomatic, a single unit transfusion maybe appropriate to alleviate the symptoms prior to commencement of B12 replacement therapy. Transfusion in such instances must be administered with extreme caution and the patient monitored appropriately. It is recommended that advice from the Consultant Haematologist is sought for such patients.

Transfusion should only be considered when the benefits of the transfusion are deemed to outweigh the risks associated with transfusion and when available alternatives to transfusion have been considered but determined not to be appropriate. Transfusion should aim to relieve symptoms of anaemia and be discontinued once this has been achieved. Evaluation of the transfusion is paramount and should be documented within the health care records. Refer to Overview | Blood transfusion | Quality standards | NICE for full guidance.

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In general, transfusion is only indicated for blood loss or true refractory anaemia. Well compensated chronic anaemia, even when severe, should seldom be an indication for emergency transfusion.

The 2-sample rule and timing of sample in relation to transfusion:

In line with the BCSH guideline two confirmed blood groups are required prior to the release of group specific/cross matched blood components. A second sample, with request form, is required due to the possibility of inadequate patient identification and labelling errors which lead to an unacceptable risk of Wrong Blood In Tube (WBIT).

First Sample:

Can be historical or taken independently on the same day as the 2nd sample.

Second Sample:

Must be a separate venepuncture event < 5 days prior to surgery/transfusion ideally performed by a different staff member, ensuring 2 separate positive patient identifications have been carried out. A separate request form must be sent with this second sample.

Patients requiring red cells that do not comply with BCSH guideline of 'one sample on two separate occasions' must be provided with Group O, until the blood group check sample can be established.

Timing of sample collection in relation to previous transfusions:

Previous blood transfusion or pregnancy may cause a primary or secondary immune response and samples selected for crossmatching or antibody screening must take account of this, so that any newly developed antibodies are detected. It is also important to note that any blood component containing residual red cells can elicit an immune response.

Group and Save samples will be stored for 7 days except for patients who have received a transfusion more than 72 hours previously or in women who have been pregnant within the last 3 months. Samples collected no more than 72 hours in advance of the actual transfusion should be used when the patient has received a blood transfusion or has been pregnant in the last three months.

Blood products

Fresh Frozen Plasma / Octaplas

Fresh frozen plasma / Octaplas is specifically used for the correction of coagulation defects in patients who are having blood loss, based on the results of a coagulation screen or during a massive transfusion of red cells in a single transfusion episode. Requests for Fresh frozen plasma or Octaplas initially should be made to the laboratory by phone in normal working hours or to the on-call haematology BMS outside normal working hours.

Please be aware that once Fresh frozen plasma / Octaplas is thawed it must be transfused within 24 hours and cannot be refrozen if not used. Please only request Fresh frozen plasma / Octaplas if it is going to be transfused; it should not be requested as a 'standby'.

Platelet concentrate

Demand for platelets nationally, has seen a significant rise over the last few years. The supply of platelets is often less than the demand making platelets an extremely precious resource. Platelet requests are provided on clinical basis using BSH guidance and NICE NG24 Overview | Blood transfusion | Guidance | NICE. Requests that do not conform within this framework will be referred to the Consultant Haematologist.

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Cryoprecipitate

Cryoprecipitate use is indicated by results of the coagulation screen in conjunction with the

Consultant Haematologist

Please be aware that once Cryoprecipitate is thawed it must be transfused within 4 hours and cannot be refrozen if not used. Please only request Cryoprecipitate if it is going to be transfused; it should not be requested as a 'standby'.

Neonate Transfusions

Exchange transfusion

Graft Versus Host Disease (GVHD) has been a complication of intrauterine transfusions and neonatal transfusions British Committee for Standards in Haematology (BCSH) guidelines recommend that all blood for exchange transfusions be irradiated. Blood must be compatible with maternal plasma and ABO compatible with the neonate.

Group O RhD Negative (RhD Positive may be required dependent upon the specificity of maternal antibody. Paediatric red cells for exchange transfusion are available from National Health Service Blood & Transplant Service (NHSBT) Sheffield. These units are less than 5 days old and free from irregular antibodies, including high titre anti - A and anti - B. They are also CMV negative, HEV negative, irradiated and sickle haemoglobin (HbS) negative with a haematocrit of 0.50 - 0.60. These units are identified by a small over stick label – 'Suitable for Exchange Transfusion'. A serological crossmatch of maternal plasma against donor red cells is required. Because of the accelerated potassium leak, irradiated blood should be used within 24 hours of irradiation for exchange transfusion.

Top - up transfusion of Neonates

Small volume red cell split units are provided for neonatal top-up transfusion. These should be group O, CMV negative, HEV negative, irradiated, sickle haemoglobin (HbS) negative and negative for high titre anti - A and anti – B, available as a paediatric pack. These units are identified as 'Suitable for Neonatal Use'.

Older children's transfusions

Group and antibody screen testing prior to transfusion will be required on all children over four months old.

(REFERENCE: BCSH guidelines for fetuses, neonates, and older children: British Journal of Haematology 2016).

Irradiated, CMV & HEV negative requirements. Note the following when CMV negative, HEV. negative or irradiated products (red cells or platelets) are requested for a patient on the first occasion:

- There should be a written request from the Clinician (a transfusion request form which should be bar-coded and DART scanned). Requests to mark new patients as requiring. irradiated products may come from either the Consultant Haematologist or Pharmacist, dependent upon local protocol.

- On iLAB ensure the appropriate requirement is flagged for the patient in their SR pad. It may also be useful to write into the SRPAD the date for commencement of the special requirements, and finish date (if appropriate).

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- Please check for any previous issues prior to the first request for special requirements – if any red cells or platelets have been issued pre-special requirements and they are still available, then please make sure that they are withdrawn.

Recipients of bone marrow transplants.

Following bone marrow transplants patients require blood products that are irradiated & HEV negative with possible CMV negative. Requirements for each patient are displayed in the Special Requirements Pad at both Order Entry and stock allocation in iLAB. If in doubt, please refer to Consultant Haematologist.

Some patients that have received allogeneic transplants at hospitals outside Path Links present unusual. grouping and crossmatching problems. Please refer to centre that performed the transplant for advice on product requirements.

Potential recipients of bone marrow or solid organ transplants.

Newly diagnosed patients with leukaemia, aplastic anaemia etc. will require HEV negative units & may require special products such as C.M.V. negative and or irradiated. Discuss requirements with Consultant Haematologist.

Other patient groups requiring Irradiated products.

It is recommended that all patients with Hodgkin's disease have irradiated red cells and platelets. Patients treated with purine analogue drugs (Fludarabine, Cladribine, Alemtuzumab anti - CD52 (Campath), Deoxycoformycin, Clofarabine and Bendamustine) should have irradiated cellular products. Irradiated products are also recommended for patients being treated with rabbit anti-thymocyte globulin (ATG). Recipients of allogenic haemopoietic stem cell transplantation (SCT) must receive irradiated blood components during their treatment. Existing patients requiring irradiated blood will be identified at both Order Entry and stock allocation in iLAB.

Sickle cell anaemia patients- HbS-negative, preferably matched for D, K, C, c, E and e antigens should be. selected for transfusion. Many patients are phenotypically cDe and D-positive blood negative for C and E antigens may be difficult to find, particularly if there are other atypical antibodies. D-negative blood should be. selected in these circumstances. If time allows a full phenotype should be obtained & matched accordingly.

Thalassaemia patients – Matched for D, K, C, c E & e antigens should be selected for transfusion. If time allows a full phenotype should be obtained & matched accordingly.

Pregnancy. CMV negative & HEV negative red cells are required for all planned transfusions in pregnancy. For transfusions during labour and delivery CMV & HEV negative products are not required.

Satellite Blood Banks

Storage of blood and collection from a satellite blood bank

Units of blood for transfusion must always be stored in the appropriate blood bank refrigerators that are always monitored. Blood which is removed from the blood bank should be selected for the correct patient by comparing the patient's details form the clinical area, to the patient details on the blood bag compatibility label. The date and time the unit is removed from the blood bank must be recorded by the individual removing the unit.

Only 1 unit of blood should be removed at any time, as the correct storage of blood for clinical use is of paramount importance. In addition, blood must only be collected for one patient at a time to reduce the risk of patients receiving the wrong blood.

If a unit is returned unused, the date and time the unit was returned to the blood bank must be recorded.

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Units must be returned within 30 minutes and must never be replaced into the blood bank after the 30-minute period.

If the unit is unable to be returned to the blood bank, contact the transfusion laboratory for advice. Blood must be transfused within 4 hours of being removed from the blood bank.

Blood is routinely returned to the laboratory stock from the satellite bank 24-48 hours (dependant on patient's clinical details) after date of issue. If a patient's operation date is postponed, please inform the department so that blood can be made available when required.

Checking blood before transfusion

The removal of blood from the satellite blood bank and subsequent checking of the unit should be performed at the patient's bedside and in line with Trust policy. ALL patient details on the unit of blood should be checked against the patient's identity band to ensure that the blood is being administered to the patient it is intended for. The expiry date of the unit should be checked and transfused before midnight on the quoted day of expiry. If there are any discrepancies, inform the Transfusion Department or, if out of hours the on-call Haematology BMS.

Transfer of blood to other hospitals

If blood is being transported to a location on another site, the blood must be sent in an appropriate transport pack with a cool pack. Also, without documentary evidence of prior storage and records of time the blood has been out of a blood bank, the destination hospital will not accept the blood. If a patient is being transferred to another hospital and blood is required to be transported with them, contact the transfusion department or the on-call haematology BMS if out of hours who will supply the appropriate transport box and necessary documentation. On arrival at the new location any unused blood should be placed immediately in an approved blood bank refrigerator.

Transfusion Incidents

Suspected Transfusion Reaction

In the case of a suspected transfusion reaction, the transfusion should be stopped immediately, and the laboratory contacted. The remainder of the unit being transfused at the time of the reaction should be returned directly to the laboratory, partly used units should NOT be returned to a satellite blood bank. A transfusion reaction request form will be issued which is to be completed and returned to the laboratory with the appropriate samples. The patient should not be further transfused until the full investigation has been performed which is usually the next routine working day. Advice from the consultant Haematologist can be sought if required.

Transfusion Incidents

The Hospital Transfusion Committee (HTC) reviews all transfusion incidents. All serious adverse incidents are reported to the Medicine and Healthcare Products Regulatory Agency (MHRA) via the Serious Adverse Blood Reactions & Events (SABRE) reporting system and to Serious Hazards of Transfusion (SHOT).

Hospital Transfusion Committee (HTC)

The principal functions of the committee are to implement national policy and guidelines on the clinical use of blood and blood products at local level and to monitor usage of blood and blood products. The committee will also review incidents of severe adverse effects or errors associated with transfusion, identify any corrective action required and refer them to the National Committee on the Clinical Use of Blood.

For further detailed transfusion information, please refer to the relevant Trust Blood Transfusion Policy.
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SECTION 10	CHEMICAL PATHOLOGY TEST LIST & INVESTIGATIONS HANDBOOK

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10.1 Introduction to blood sample requesting, collection, handling, storage, transport, and separation.

Chemical Pathology provides a range of tests to aid diagnosis and monitor certain treatments. The attention given to your request, and the scope of analyses performed is partially related to the amount of clinical information and test requests typed (electronic requesting) or written (manual requesting) on the form. If a specific abnormality is suspected, please indicate this CLEARLY when making the requests.

The comments below are NOT intended to be comprehensive, and do not necessarily apply to children. If in doubt, please seek advice BEFORE sending a sample or a patient to the laboratory.

Sample collection, handling, storage, and transport, particularly in primary care, is a process which needs strict control to preserve sample integrity prior to any centrifugation. It has long been recognised that special attention needs to be given to ensuring that optimum conditions are applied to whole blood samples where it is intended to separate the serum or plasma for biochemical analyses. Standard textbooks in this field[1] have offered advice covering the various steps involved in this "pre-analytical" phase, including avoidance of haemolysis. It is widely recognised that even when not detectable by visual inspection, haemolysis may be detected by spectroscopic examination. This latter fact is in use in many modern laboratories when pre-analytical, on-board spectroscopic examination is used to determine the presence of haemolysis in separated serum or plasma (this is also used to definitively detect and assess the presence of icterus & lipaemia). Manufacturers routinely offer data covering this area for routine use with their own analysers[2]. The following advice is intended to apply to each step of the process of blood sample collection, handling, storage, and transport to avoid sample degradation (see below) prior to centrifugation and analysis.

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10.2 Pre-analytical considerations and guidance

10.2.1 Sample degradation: definitions

"Degradation" refers to those processes which lead to degradation of the cellular components of the blood with consequent leakage or release of intra-cellular contents into the serum / plasma compartment. This may occur secondary to poor venepuncture, rough sample handling (including mechanical agitation prior to and during transport), and adverse temperature conditions (extremes of cold or heat). This can lead to both frank haemolysis and trans-membrane leakage of intra-cellular components into the blood serum or plasma. The most important analytes of clinical interest which are affected if there is **frank haemolysis** are AST, folate, iron, LDH, magnesium, phosphate, potassium, PTH and Troponin-T. Some or all these analytes may also rapidly change through **cellular leakage** in unseparated whole blood *not* affected by haemolysis (within 0.5-4 hours)[3]. These processes are further discussed below.

10.2.2 Request form completion and pre-analytical considerations relating to sample collection and preservation.

Please ensure that every part of the request form is completed with all patients, test, and clinical details. This is essential for transmission of the completed report to its correct destination. Please see Trust Labelling Policy for full details.

Completion of the form with relevant, legible clinical and, where applicable, medication details is essential for interpretation by laboratory and Consultant staff. It allows extra tests to be added where appropriate, corrections to be made if an incorrect test request has been made and saves patient time and phlebotomy episodes.

· **Timing of blood sampling**

The ideal time for taking blood for many analytes is after fasting overnight **(8 hours minimum),** although this is not always possible or even desirable in hospitalised patients[1]. Some analytes are significantly higher in the non-fasting state, e.g., serum / plasma creatinine, glucose, iron, and triglycerides. Others show very significant circadian variation and sample collection time needs to be recorded to optimise the usefulness of the result, e.g., cortisol, iron, testosterone (males). Many drugs interfere with assays either by physiological action or by direct interference with the assay (e.g., anti-epileptics inducing certain liver enzymes, cortisol analogue medications such as hydrocortisone,

prednisone and prednisolone directly cross-reacting in cortisol immunoassays). Samples for drug assays should be either trough or >6 hr post-dose (digoxin), *except* if toxicity is suspected when a random sample is acceptable.

· **Special sample collection and preservatives**

o **Glucose**

Glucose is the commonest test requested requiring special preservative to avoid loss post-venepuncture. In primary care locations it **must** be taken into the grey-topped blood bottles which contain sodium fluoride and potassium EDTA preservative & anticoagulant. Stability of glucose in these containers is up to 7 days at room temperature (20 – 25̊C)[3]. Samples from primary care taken into ochre-topped blood bottles will **not** be analysed for glucose.

o **other analytes**

There are other less common tests which also require a specialised approach to sample bottle type, preservative, and promptness of serum separation. Examples include:

Lactate	: use a grey-topped fluoride EDTA tube and send immediately to the laboratory
Parathyroid hormone (PTH)	: separate within 6 hours
Insulin & ACTH	: separate *& freeze* immediately.
Renin / Aldosterone	: special patient preparation and immediate separation of sample to freeze.
Trace elements	: exclusive trace element sample tubes must be used. Contact the local laboratory before you request these tests (selenium, zinc, copper)

There are many other instances – please consult your local laboratory for advice. before patient time and samples are wasted.

· **Sample collection – venepuncture**

During the venepuncture / collection process haemolysis is minimised by avoiding mechanical breakdown of red cells and movement of water into or out of the cells. The Greiner system in use throughout the Path Links area features automatic blood uptake via the vacuum in the blood tube. In this system, a volume of 4 mL is withdrawn under vacuum pressure. After the 21-gauge needle is inserted into the vein, a blood bottle is placed on to the other end of the needle and within a plastic collection vessel. In this manner blood is extracted in conditions designed to minimise the mechanical stress on red cells and to minimise the possibility of haemolysis. It is vital that practitioners do not takes sample into non-standard syringes with needle attached and then proceed to inject the blood through the rubber membrane of the blood bottle. This involves use of undue pressure because of the vacuum within the blood tube and **always** results in haemolysis. This practice is impossible to detect in the laboratory (apart from the result of a haemolysed sample) as the rubber membrane of the blood bottle is also pierced by the needle during the correct venepuncture process.

· **Venous stasis during venepuncture**

Avoid prolonged stasis during venepuncture. Extreme stasis of 3 minutes duration can increase serum potassium by 10-20% as well as altering serum protein concentrations[4]. This can also alter concentrations of protein-bound analytes such as calcium and some drugs. Extensive fist-clenching to localise veins can also increase serum potassium and if multiple sample types are to be taken, the sample for electrolyte analysis should be taken first, *unless* routine coagulation (PT and aPTT) is also required. Currently, the recommended order of draw is:

Blood culture
Routine coagulation
Serum tubes (ochre or white tops)
Heparin tubes
EDTA tubes
Glucose tubes
All other tubes

10.2.3 Significant pre-analytical factors affecting test results and their interpretation.

Many factors can affect test results: the time of day the sample taken, diet, fasting or non-fasting, stress or anxiety, pregnancy, posture when the sample is taken, recent heavy exertion can affect some results. For example, albumin and calcium levels can increase a little when moving from lying down to an upright position. Vigorous exercise can affect levels of creatinine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH). These examples demonstrate the significance of taking blood or urine samples in a standardised fashion for performing and interpreting laboratory tests. It should be recognised that significant intra-individual biological variability occurs with all analytes (e.g., potassium can vary as much as 0.5mmol/L in any individual on a day-to-day basis) therefore this should be considered when considering the significance of any result changes. Moreover, each test result is subject to analytical variability which can also influence interpretation of results. The laboratory can advise further if in doubt.

Potassium is significantly affected by in transit storage temperature before separation (see table 2 and section 10.2.4, below). In cold weather, potassium leaks out of the cells and causes factitious hyperkalaemia. The reverse happens in hot weather. Samples collected from GP surgeries are transported in insulated containers to minimise this effect, surgeries should do the same to avoid unnecessary temperature variation prior to sample pick-up.

Table 1, below, though by no means exhaustive, illustrates some of the common interferences encountered in tests requested in the department. Drug effects may be biological or analytical. In the former, a real *in vivo* change in the analyte occurs, not usually directly related to the therapeutic effect of the drug. Patient status (stress, fasting) can have very significant effects on some test results.

Table 1: Common biochemistry tests affected by drug therapies or patient status.

Biotin has been identified as a possible source of interference in some immunoassays provided by the laboratory, the degree of interference is dependent on the dose which the patient is taking. 5-10mg tablets are available over the counter, high dose therapy (100mg tablets) is prescribed for certain metabolic diseases, and there are ongoing trials in patients with multiple sclerosis using up to 300mg. The degree of interference seen is dependent on the concentration of circulating biotin and is therefore difficult to predict accurately, however the following is a guide to what might be seen. Any concerns regarding results should be addressed to the Consultant Clinical Biochemist.

Positive Interference: Cortisol, B12, DHAS, Digoxin, Folate, fT3, fT4, Oestradiol, Progesterone, Testosterone, vitamin D, TPO antibodies. Anti-Tg antibodies

Negative interference: AFP, CEA, Ferritin, FSH, Growth Hormone, HCG, LH, NT-Pro-BNP, Prolactin, PSA, PTH, SHBG, Troponin T, TSH, Ca 125, Ca 15-3, Ca 19-9, Thyroglobulin

Apparently Unaffected: ACTH, Androstenedione, Carbamazepine, Gentamycin, IGF-1, Phenytoin, Theophylline, Vancomycin

Test	Interference	Effect	Analytical (A) Biological (B)
Alkaline phosphatase	Anticonvulsants, barbiturates, oral contraceptives EDTA contamination	Increase Decrease	B A
Calcium	Venous stasis, Vitamin D therapy Pregnancy Citrate EDTA contamination	Increase Decrease Decrease Decrease	B B B/A A
Cholesterol	Oestrogens	Decrease	B
Cortisol	Prednisolone cross-reacts (12%) in the assay. Very significant circadian variation	Increase Variable	A B
Creatinine	Meat/high protein meal	Increase	B
Glucose	Furosemide, thiazides, corticosteroids, Oestrogens, stress Vitamin C, storage	Increase Decrease	B A
Gamma-GT	Anticonvulsants, barbiturates, alcohol	Increase	B
Potassium	Insulin, corticosteroids, diuretics Amiloride, anti-neoplastic agents Haemolysis, EDTA contamination, storage	Decrease Increase Increase	B B A

Prolactin	Oestrogens, MAO inhibitors, cimetidine	Increase	B
Sodium	Lithium Diuretics, carbamazepine Lipaemia	Increase Decrease Decrease	B B A
Testosterone (males)	Very significant circadian variation – sample between 010.00-010.00hr	Decrease	B
Thyroxine	Amiodarone, pregnancy, oestrogens Phenytoin, corticosteroids Heterophile antibodies (antibodies induced by external antigens which cross-react with self-antigens.)	Increase Decrease Variable	B B A

Table 2: below, though by no means exhaustive, illustrates some of the common problems encountered with tests when samples have been inappropriately collected or stored. In the former, a real *in vivo* change in the analyte occurs, not usually directly related to the therapeutic effect of the drug. Patient status (stress, fasting) can have very significant effects on some test results.

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Table 2: Spurious results due to inappropriate sample collection and/or storage

Problem	Common Causes	Consequences
Delays in separation of serum	Overnight storage Delay in transit	Increased K^+, PO_4, AST, LDH Decreased HCO_3, (Na^+ occasionally)
Storage (1)	Storing unseparated sample at 4^0C	Increased K^+ Decreased HCO_3
Storage (2)	Storing unseparated sample at >25 ^{0}C	Decreased K^+
Haemolysis	Expelling blood through needle into tube Overly vigorous mixing of specimen Storing specimen in freezer (-20^0C) Excessive delay in transit	Increased K^+, PO_4, Bilirubin, LDH, Iron, Mg^{2+}, CK, AST, LDH Decreased Na^+, Cl^-, Glucose
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Inappropriate sampling site	Specimen taken from drip arm	Increased drip analyte, e.g., glucose, K^+, Mg^{2+} Dilution effect
Incorrect container or anticoagulant	No enzyme inhibitor EDTA tube (pink or purple) or Transferring blood from one tube to another	Low glucose Increased K^+ Decreased Ca^{2+}, ALP, Mg^{2+}
Lipaemia	Specimen taken after a fatty meal	Decreased Na^+

10.2.4 Post-venepuncture sample handling, storage, and transport

After venepuncture samples should be kept at ambient room temperature (definition: 20-25^{0}C). This temperature condition should be always adhered to during the post-collection, pre-analytical phase *for as long as the sample remains un-centrifuged*.

The samples should be transferred to Path Links sample carriers as soon as possible and be stored at room temperature while they await collection by a Path Links courier. Whole blood samples for Chemical Pathology should *never* be refrigerated.

Whole blood stability pre-centrifugation – the example of potassium

· **Delay in sample separation and cellular leakage of constituents**

o **Hyperkalaemia**

The most frequently identified pre-analytical artefact is mild hyperkalaemia resulting from prolonged transit times post-collection, with or without some degree of haemolysis (see next section). The transportation of whole blood for 3-4 hours at room temperature raises the concentration of potassium by approximately 10% due to cellular leakage[3]. A sample normally having a serum potassium of 4.2 mmol/L would experience an increase to 4.6 mmol /L. NOTE: This example relates solely to **cellular leakage** secondary to delayed separation of the serum from cells.

o **Masking of hypokalaemia**

This facet of sample deterioration is much less considered than hyperkalaemia and it is far less easily detected. In such cases, it is reasonable to assume that there are patients whose normal serum potassium is low, but when *in vitro* sample degradation or haemolysis occurs, the serum potassium is elevated into the reference range. Given the number of elderly patients in the community on a medication (or combination of medications) tending to cause potassium wasting this cannot be ignored in any discussions of sample quality. The same standards of post-venepuncture sample handling should be applied (see below)

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· **Haemolysis**

Red blood cells contain potassium at a concentration of approximately 100 mmol/L. Haemolysis (rupture of red cells) will increase the concentration of potassium in serum and rupture of as few as 0.5% of the red cells will increase serum potassium by 0.5 mmol/L so it is essential to avoid this[5]. From a different viewpoint, a serum haemoglobin concentration of 0.5 g/L will cause an increase of about 10% in serum potassium. Mechanical damage to samples from any cause (poor venepuncture, poor sampling handling and transport conditions) will cause haemolysis in proportion to the severity of the insult.

· **Ambient temperature and its effect on whole blood stability**

Cellular leakage is accentuated at extremes of temperature, whether cold or heat, occurring due to seasonal climate variation. Thus, high ambient temperatures cause spurious **hypokalaemia[6]** and low ambient temperatures cause spurious **hyperkalaemia[7]**.

Other analytes affected in this manner include phosphate, magnesium, LD and iron. The WHO review of the stability of whole blood[3] states that serum / plasma potassium will increase from 1 hour after venepuncture (page 39) and they suggest that an increase of about 10% will occur after a period of "3-4 hours" (page 10). A recent review of best practice in primary care pathology[4], suggests (in the case of suspected factitious hyperkalaemia) taking a second specimen "to arrive within 4 hours of venepuncture". Although no data or reference is cited in support of this, most experienced laboratorians would agree with this pragmatic view, supported as it is by the WHO recommendation[3].

Preferred minimum standard for delay between venepuncture and separation for whole blood samples is thus 4 hours.

However, internal sample audits have showed that this standard is only achieved in ~30% for primary care samples. Where surgeries are aware that samples may take >4 hours, and where potassium and other analytes sensitive to cellular leakage or haemolysis are required, it is suggested that alternative approaches are considered – either ask the patient to book an appointment in the local pathology department or consider the use of an in-surgery centrifuge.

NOTE: when Greiner serum tubes have been centrifuged, resulting in separation of the serum and red cells by the gel plug, the serum remains patent for several days and the samples can be safely refrigerated prior to transportation.

· **Other factors and conditions causing hyperkalaemia.**

o **High cell counts – pseudohyperkalaemia**

Severe leucocytosis (>20 x 10. /L) and thrombocythaemia (platelets > 1000 x 10^9/L) will cause pseudohyperkalaemia – i.e., hyperkalaemia occurring post-venepuncture and occurring *in vitro* as the sample clots[4]. Familial pseudohyperkalaemia is also a recognised condition to be aware of[10.]

- **In vivo haemolysis**

  Congenital or acquired haemolytic disease, embolism and extensive tissue breakdown will promote *in vivo* haemolysis[4]. It is important to recognise that this represents true hyperkalaemia requiring treatment. This type of haemolysis can be mistaken by laboratories for *in vitro* haemolysis as it is often detectable by visual inspection. It is vital that clinicians recognise this possibility and liaise closely with local laboratory staff to perform repeat analyses to definitively rule in or rule out this condition.

Sample separation (centrifugation)

Whole blood samples require centrifugation to separate the serum from the cellular components of whole blood prior to analysis in Chemical pathology laboratories. Centrifugation may be carried out in either primary care, using trained and competent staff, or in the laboratory. Centrifuge settings will vary according to manufacturer and site, but laboratory samples in general are spun for 10 minutes at 1600g. When separating routine serum samples, the temperature should not drop below 15^0C or exceed 24^0C[3].

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Time limits for requesting additional examinations.

Users can normally add on additional tests by telephoning the laboratory as necessary. In general, samples are stored post analysis for around a week. Laboratory staff will advise if the sample is still patent in relation to the test(s) being requested.

10.2.5 Referral of samples to other laboratories for analysis

A small proportion of low-volume and esoteric tests are referred to laboratories elsewhere in the United Kingdom. A full updated list of these tests can be found in the Path Links Sendaway Guide. The turnaround time for these test results is very variable, from 5 working days after receipt of sample, to several months for some of the molecular genetics tests. Please direct enquiries to the laboratory Head of Department if you need to check the progress of any "sendaway" test results.

10.2.6 Laboratory test repertoire and reference ranges

Please see section 10.3 for the full test repertoire. If the test(s) you require is/are not listed, please direct enquiries to the laboratory Head of Department.
The laboratory reference ranges are given on all printed and electronic reports and are tailored for age and sex where appropriate. They are taken either from manufacturer test data appropriate for the method and analyser used; from internally assayed patient material where appropriate or where reagent changes necessitate; or from UK or international guidelines.
When new ranges are introduced, an advisory note (including date of application) is included with the electronic record (Web View and pathology computer) and on printed reports.
Interpretative comments, including cut-off targets, are appended by consultant staff during clinical authorisation. Reference ranges and interpretative comments on test results from referral laboratories are similarly reproduced on all printed reports and within the electronic record. These are amended when necessary. Access to all this information is available to any authorised clinical user having password access to the electronic patient results, as well as on receipt of printed reports.

10.2.7 Uncertainty of results

Every laboratory result is subject to "uncertainty" or variability. The main components contributing to this uncertainty are analytical variability (or imprecision), which arises when the test is performed in the laboratory using the analytical procedure, and the patients' intra-individual biological variability (which is often much greater than the analytical imprecision). These concepts are generally poorly understood by clinicians, but a basic understanding of these issues is vital when deciding whether a particular result has changed significantly or not. The laboratory has comprehensive data on these topics and users of our service are encouraged to contact the laboratory if they would like more details.

10.2.8 References

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10.3 Chemical Pathology Test List Return to Contents

The following table gives information about **Chemical Pathology tests**, sample tubes, and site of analysis. As a rule, a minimum of 3mL of blood will be needed for individual tests. Vacuette tubes automatically fill to the set volume. For multiple tests only one bottle, fully filled, of each colour will normally be required. Where additional tubes are required, this is indicated in the right-hand column. Paediatric samples can be collected using Microtainers, which may have a different colour code. For more information, please contact the laboratory or refer to the other pages in the handbook. **NB**: A separate, white-topped serum bottle is required for all Immunology tests in addition to any Chemical Pathology tests.

Test	**Tube type and special instructions**	**Where analysed / frequency of analysis**
Alpha 1 Acid Glycoprotein	Gel Tube.	Analysed by reference laboratory.
ACE	Gel Tube	Centralised Path Links test (daily)
Acid Base / Blood gases	Heparinised syringe. Refer to laboratory for further information.	on all Sites. Please contact local lab for more details.
Adrenaline (Plasma)	Green Capped Tube – Special collection conditions. **Contact laboratory before sampling**	Analysed by reference laboratory.
Adrenocorticotrophin (ACTH)	Lavender Capped Tube – **Send immediately (on ice preferably) for centrifugation and freezing**	Centralised Path Links test (weekly)
Alanine-transaminase (ALT)	Gel Tube.	Daily. 24 hrs
Albumin	Gel Tube.	Daily. 24 hrs
Alcohol (ethanol)	Grey Capped Tube	Daily
Aldosterone (Plasma)	Green Capped Tube – **Send immediately for centrifugation and freezing. Special patient preparation needed, contact laboratory. GP samples must be collected at the laboratory**	Analysed by reference laboratory. Can be measured concurrently with plasma renin.
Alkaline Phosphatase (ALP)	Gel Tube.	Daily. 24 hrs
Alpha Fetoprotein (AFP)	Gel Tube.	Centralised Path Links test (daily)
Alpha 1 antitrypsin	Gel Tube.	Centralised Path Links test (daily)
17 Alpha Hydroxyprogesterone	Gel Tube.	Analysed by reference laboratory.
Aluminium	Dark Blue Capped Tube	Analysed by reference laboratory.

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Amino acids (Urine)	Fresh early morning urine sample. Plain container	Analysed by reference laboratory.
Ammonia	Lavendar Capped Tube **Transport to lab immediately on ice**	As required. (<24hrs) Please contact the laboratory before collection.
Amphetamines	Fresh Urine. Plain container Contact laboratory for further details if required	Analysed by reference Laboratory.
Amylase	Gel Tube.	Daily. 24 hrs
Amylase (Urine)	Fresh early morning urine collection Plain container	Daily
Androstenedione	Gel Tube.	Analysed by reference Laboratory.
Angiotensin Converting Enzyme (ACE)	Gel Tube.	Analysed by reference laboratory.
Aspartate Transaminase (AST)	Gel Tube.	Daily

B

Bence Jones Protein [free light chains] (Urine)	Early morning fresh urine. Plain container	Centralised Path Links test.
Beta 2 Microglobulin	Gel Tube.	Centralised Path Links test (daily)
Beta Carotene	Gel Tube.	Analysed by reference laboratory.
Bicarbonate (HCO_3^-)	Gel Tube.	As required (24hrs)
B-HCG (Human Chorionic Gonadotrophin)	Gel Tube.	Daily. Available 24hrs.
BNP (NT-pro-BNP)	Gel Tube.	Daily
Bile Acids	Gel Tube	Centralised Path Links test (daily)
Bilirubin	Gel Tube.	Daily. 24hrs

C

C Peptide	Serum (ochre top) or Green Capped Tube. Requires simultaneous glucose sample, **send immediately to the laboratory for centrifugation and freezing**	Analysed by reference laboratory.
CA15-3	Gel Tube.	Centralised Path Links test (daily)

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CA19-9	Gel Tube.	Centralised Path Links test (daily) Return to Contents
CA125	Gel Tube.	Centralised Path Links test (daily)
C 1 Esterase Inhibitor	Gel Tube.	Analysed by reference laboratory.
C-Reactive Protein	Gel Tube.	Daily. 24hrs
Caeruloplasmin	Gel Tube.	Centralised Path Links test (daily)
Caffeine	Gel Tube.	Analysed by reference laboratory.
Calcitonin	Serum Gel tube, **send immediately to the laboratory for centrifugation and freezing**	Analysed by reference laboratory.
Calcium	Gel Tube.	Daily. 24hrs
Calcium (Urine)	24-hour collection with Acid preservative. Care should be taken when collecting.	Daily
Calcium Adjusted	See calcium	Daily. 24hrs
Calprotectin (faeces)	Pellet of faeces	Centralised Path Links test (4 x weekly)
Cannabinoids (Urine)	Fresh Urine. Plain container Contact laboratory for further details if required	Analysed by reference laboratory.
Carbamazepine (Tegretol)	Gel Tube.	Centralised Path Links test. Available 24hrs by directly contacting 'on-call' BMS if suspected overdose.
Carboxy-haemoglobin	Green (lithium heparin) tube	As required. Available 24hrs by directly contacting 'on-call' BMS if suspected exposure.
Cardiac Markers (CK, hs troponin T)	Gel Tube.	Daily. 24hrs
Carcinoembryonic Antigen (CEA)	Gel Tube.	Daily.
Catecholamine metabolites (Urine) – metadrenaline, normetadrenaline and 3-methoxytyramine.	24-hour collection with Acid preservative. Care should be taken when collecting.	Analysed by reference laboratory.
Chloride (Cl)	Gel Tube.	Daily. 24hrs
Cholesterol	Gel Tube.	Daily. 24hrs
Cholinesterase (anaesthetic sensitivity)	2x EDTA Lavender Capped Tube	Analysed by reference laboratory.

Cholinesterase (agricultural sprayers)	2x EDTA Lavender Capped Tube	Analysed by reference laboratory.
Chromogranin B	2x EDTA Lavender Capped Tube **Send immediately to the laboratory for centrifugation and freezing**	Analysed by reference laboratory.
Chromosomes	Green Capped Tube	Analysed by reference laboratory
Chromogranin A	2x EDTA Lavender Capped Tube **Send immediately to the laboratory for centrifugation and freezing**	Analysed by reference laboratory
Cocaine Metabolites (Urine)	Fresh Urine. Plain container Contact laboratory for further details if required	Analysed by reference laboratory
Conjugated Bilirubin	Gel Tube.	Daily.
Copper	Red Capped Tube	Analysed by reference laboratory
Copper (Urine)	24-hour collection –special acid washed container obtainable from Laboratory	Analysed by reference laboratory.
Cortisol	Gel Tube.	Frequency of analysis is site dependent.
Cortisol (Urine)	24-hour collection with NO preservative.	Analysed by reference laboratory.
Creatine Kinase	Gel Tube.	Daily. 24hrs
Creatinine	Gel Tube.	Daily. 24hrs
Creatinine Clearance (Urine) NOTE: This test has been largely replaced by the automatic calculation of eGFR per Renal Association Guidelines.	24-hour collection with NO preservative. Ensure blood sample is collected in conjunction with urine.	Daily.
Cryoglobulin/ cryofibrinogen	**Plain red top tube and purple EDTA. Please contact the laboratory before collection.**	As required.
Ciclosporin	Lavender Capped Tube	Analysed by reference laboratory.
Cystine (Urine)	Fresh early morning sample or 24hour collection for known positives.	Analysed by reference laboratory.

D

Dehydroepiandrosterone sulphate (DHAS)	Gel Tube. laboratory.	Analysed by reference test (weekly)
Digoxin	Gel Tube. **Take sample at least 6 hrs post dose**	Daily. Available 24hrs by directly contacting 'On-call' BMS if suspected overdose.
Drugs of abuse (Urine)	Fresh Urine. Plain container Contact laboratory for further details if required	Analysed by reference laboratory.

E

Electrolytes [Na, K, Urea] (Urine)	Random Fresh Urine or 24 hours collection with NO preservative.	Daily
Erythropoietin (EPO)	Gel Tube.	Analysed by reference laboratory.
Ethanol	Grey Capped Tube	Daily
Ethosuximide	Gel Tube.	Analysed by reference laboratory.
Ethylene Glycol	Grey Capped Tube **Please contact laboratory prior to requesting if urgent analysis required.**	Analysed by reference laboratory.

F

Follicle Stimulating Hormone (FSH)	Gel Tube.	Centralised Path Links test
Fragile X	Lavender Capped Tube	Analysed by reference. laboratory.
FT_3 (Free tri-iodothyronine)	Gel Tube.	Daily
Faecal Calprotectin	Peller of faeces	Centralised Path Links test
Faecal Immuno testing (FIT)	Specialised FIT tube	Daily
Faecal elastase (faeces)	Pellet of faeces	Centralised Path Links test
Free T_4 (FT_4)	Gel Tube.	Centralised Path Links test

G

Gamma Glutamyl Transferase (GGT)	Gel Tube.	Daily. 24 hours
Gastrin	2x EDTA Lavender Capped Tube **Send immediately to the laboratory for**	Analysed by reference laboratory.

	centrifugation and freezing. Ranitidine and omeprazole Rx will invalidate the test	
Gene probe Return to Contents	Lavender Capped Tube	Analysed by reference laboratory.
Gentomycin	Gel Tube	Daily
Globulins	Gel Tube.	Daily. 24hours
Glucagon	2x Lavender Capped Tubes (EDTA) **sample and immediate centrifugation and freezing. Please contact laboratory before collection**	Analysed by reference laboratory.
Glucose	Grey Capped Tube (gel serum sample is suitable only if < 2hours old on receipt in lab). This test is also available in the Radiometer ABL90 FLEX PLUS blood gas analysers - local data indicates no clinically significant difference between results. If more information is required, please contact your local Consultant Biochemist.	Daily. 24hours
Glycated Haemoglobin (HbA_{1c})	Lavender Capped Tube	Daily
Growth hormone	Gel Tube.	Centralised Path Links test (weekly)
Gut hormones	4x Lavender Capped Tubes (EDTA) **Fasting sample and immediate centrifugation and freezing. Please contact laboratory before collection**	Analysed by reference laboratory.

H

Haemoglobin (Urine)	Random fresh urine. Plain container	As requested
Haemoglobin A_{1c}	Lavender Capped Tube	Daily
HDL cholesterol	Gel Tube.	Daily
Hydroxyindole acetic acid [5HIAA] (Urine)	24 hr urine collection containing Hydrochloric acid (HCl) as preservative	Analysed by reference laboratory.
17-alpha Hydroxy-progesterone	Gel Tube.	Analysed by reference laboratory.
Homocysteine	Lavender Capped Tube. **Fasting sample. Send immediately to laboratory for centrifugation and freezing.**	Centralised Path Links test (Weekly)

I

IGF-1	Gel Tube.	Centralised Path Links test (daily)

Immunoglobulin subclasses	Gel Tube.	Referred to reference laboratory.
Immunoglobulins Return to Contents	Gel Tube.	Centralised Path Links test (daily)
Insulin	Yellow Capped Tube. Requires simultaneous glucose sample, **send immediately to the laboratory for centrifugation and freezing**	Analysed by reference laboratory.
Iron & transferrin saturation	Gel Tube.	Daily

J

K

L

Lactate	Grey capped tube. Send immediately to the laboratory for centrifugation. This test is also available in the Radiometer ABL90 FLEX PLUS blood gas analysers - local data indicates no clinically significant difference between results. If more information is required, please contact your local Consultant Biochemist.	As requested
Lactate dehydrogenase (LDH)	Gel Tube.	Frequency of analysis is site dependent.
LDL cholesterol	See Lipids	Daily
Lead (blood)	Lavender Capped Tube	Analysed by reference laboratory.
Lipids	Gel Tube.	Daily. 24 hours
Lithium	Gel Tube. Please refer to site specific tube guides. Samples should be taken 12hrs post dose	Centralised Path Links test. Available 24hrs by directly contacting 'on-call' BMS if suspected overdose.
Liver Function tests (LFT)	Gel Tube.	Daily. 24 hours
Luteinising Hormone (LH)	Gel Tube.	Daily

M

Macroprolactin screen	Gel Tube.	Automatically added to all newly found high prolactins. Centralized Path Links Test
Magnesium	Gel Tube.	Daily
Mercury (Blood) Return to Contents	Lavender Capped Tube	Analysed by reference laboratory.

Mercury (Urine)	Early morning fresh urine. Plain container.	Analysed by reference laboratory.
Metanephrine (24 hr urine)	See catecholamine metabolites	Analysed by reference laboratory
Metanephrine (plasma)	Lavender capped (EDTA) sample x2, taken after 30 minutes at rest then sent immediately to laboratory for separation and freezing.	Analysed by reference laboratory
Methaemoglobin	Green Capped Tube	As requested
Methanol	Grey Capped Tube **Please contact laboratory prior to collection if urgent analysis required.**	Analysed by reference laboratory.
Microalbumin (Urine)	Early morning fresh urine. Plain container	Frequency of analysis is site dependent.
Mucopolysaccharides (Urine)	Early morning fresh urine. Plain container.	Analysed by reference laboratory.
Myoglobin (Urine)	Not available. Assay of serum CK recommended.	Contact laboratory for further advice

N

Normetanephrines (Plasma)	2 x 1 mL EDTA Plasma sample, taken after 230 minutes at rest then sent immediately to laboratory for separation and freezing.	Analysed by reference laboratory
Normetanephrine (Urine)	See catecholamine metabolites	Analysed by reference laboratory.

O

Oestradiol	Gel Tube.	Centralised Path Links test
Opiates (Urine)	Fresh Urine. Plain container Contact laboratory for further details if required	Analysed by reference laboratory.
Organic Acids (Urine)	Fresh random urine. Plain container.	Analysed by reference laboratory.
Osmolality	Gel Tube.	Daily
Osmolality (Urine)	Fresh random urine. Plain container.	Daily
Oxalate (Urine)	24 hr urine collection containing Hydrochloric acid (HCl) as preservative	Analysed by reference laboratory.

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P

Pancreatic Polypeptide	**2x Lavender capped (EDTA) samples**	Analysed by reference

	Fasting sample and immediate centrifugation and freezing. Please contact laboratory before collection.	laboratory.
Paracetamol	Gel Tube.	As requested,. 24 hours
Parathormone (PTH)	Gel Tube.	Daily. 24hours
Paraquat (Urine)	Fresh random sample (white topped bottle).	Daily. ONLY Available 24hrs Please directly contact Local Consultant Biochemist To discuss.
Phenobarbitone	Gel Tube.	Analysed by reference laboratory.
Phenytoin	Gel Tube.	Centralised Path Links test
Phosphate	Gel Tube.	Daily. 24hours
Placental Alkaline Phosphatase	Gel Tube.	Analysed by reference laboratory.
Pleural aspirate	Plain container, NB aliquot in Grey topped tube needed if glucose required and **pH a blood gas syringe**	As requested
Porphobilinogen screen (Urine)	Fresh random sample (plain bottle). **Must protect from light**	Analysed by reference. laboratory. Contact laboratory Prior to collecting sample If urgent.
Porphyrins (Blood)	Lavender Capped Tube **Must be fresh sample, protected from light**	Analysed by reference laboratory.
Porphyrins (Faeces)	Fresh stool sample. **Must be protected from light**	Analysed by reference laboratory.
Porphyrins (Urine)	Fresh random sample (plain bottle). **Must be protected from light**	Analysed by reference laboratory
Posaconazole Return to Contents	**Pre-dose**, serum sample	Analysed by reference laboratory
Potassium (K)	Gel Tube. This test is also available in the Radiometer ABL90 FLEX PLUS blood gas analysers - local data indicates no clinically significant difference between results in non-haemolysed samples. Potassium is released from platelets during clotting. Literature suggests that plasma and whole blood potassium concentrations are 0.1-0.7 mmol/L lower than those in serum.	Daily. 24hrs

	http://www.acb.org.uk/Nat%20Lab%20Med%20Hbk/Potassium.pdf If more information is required, then please contact your local Consultant Biochemist.	
Pregnancy test (serum)	Stat B-HCG. Gel Tube. Please refer to site specific tube guides.	Daily
Progesterone	Gel Tube. Please refer to site specific tube guides. Samples should be taken in mid-luteal phase	Centralised Path Links test
Prolactin	Gel Tube.	Centralised Path Links test
Prostate Specific Antigen (PSA)	Gel Tube.	Daily
Prostate Specific Antigen (PSA), free	Gel Tube.	Analysed by reference laboratory
Protein (Urine)	Random sample in plain container or 24 hour collection with NO preservative.	Daily
Protein, total (serum)	Gel Tube.	Daily. 24hrs
Pyruvate (plasma or CSF)	Special collection required, **please contact the laboratory**.	Analysed by reference laboratory

Q

R

Renal Calculi	Calculi retrieved at surgery	Analysed by reference laboratory
Renin	Green Capped Tube, **send immediately to the laboratory for centrifugation/freezing. GP samples must be collected at the laboratory**	Analysed by reference laboratory.

S

Salicylate	Gel Tube.	Daily. 24hrs.
Sex Hormone Binding Globulin (SHBG)	Gel Tube.	Centralised Path Links test (weekly)
SFLT/PLGF	Gel Tube.	Centralised Path Links Test, contact local laboratory when sending sample.
Sodium (Na)	Gel Tube. This test is also available in the Radiometer ABL90 FLEX PLUS blood gas analysers - local data indicates no clinically significant difference between results. If more information is required, please contact your local Consultant Biochemist.	Daily. 24hrs Note

Sweat test	Special collection required, by appointment, **please contact the laboratory.**	As requested

T

Tegretol (Carbamazepine)	Gel Tube.	Centralised Path Links test. Available 24hrs by directly contacting 'on-call' BMS if suspected overdose.
Tecioplainin	Gel Tube	Daily - Centralised Path links test
Testosterone	Gel Tube. In males, collect sample between 010.00-010.00h	Daily
Theophylline	Gel Tube.	Daily. Available 24hrs by directly contacting 'On-call' BMS if suspected overdose.
Thyroglobulin (including anti-thyroglobulin antibodies)	Gel Tube.	Centralised Path Links test (weekly)
Thyroid Function Test (TFT)	Gel Tube.	Daily
Thyroid Peroxidase Antibody	Gel Tube.	Centralised Path Links test (daily)
Thyroid receptor Antibody	Gel Tube.	Centralised Path Links – once a week (Wednesday)
Tobramycin	Gel Tube	Centralised Path Links test (daily)
Total Protein	Gel Tube.	Daily. 24 hours
Toxicology (Blood)	Gel Tube. **Please contact laboratory prior to collection**.	Analysed by reference laboratory.
Toxicology (Urine)	Fresh Urine. Plain container Contact laboratory for further details if required	Analysed by reference laboratory.
Trace metals	Type of sample depends on analysis required. Please contact the laboratory for more information.	Analysed by reference laboratory.
Triglyceride	Gel Tube.	Daily
Hs Troponin T	Gel Tube.	Daily. 24 hours
Trypsin	Green Capped Tube	Analysed by reference laboratory.

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U

Urea and Electrolytes (serum)	Gel Tube.	Daily. 24hrs
Urea and Electrolytes (Urine)	Random Fresh Urine in plain container, or 24-hour collection with NO preservative.	Daily
Uric acid (serum)	Gel Tube.	Daily. 24hrs
Uric acid (Urine)	24hr Collection containing NO preservative.	As requested.

V

Valproate (Epilim)	Gel Tube.	Analysed by reference laboratory.
Vancomycin	Gel Tube	Daily
Vasoactive Intestinal Peptide	**Lavender capped (EDTA) sample Fasting sample and immediate centrifugation and freezing. Please contact laboratory before collection**	Analysed by reference laboratory.
Vitamins, A, B, C, E.	Type of sample depends on analysis required. Please contact the laboratory for more information. E.g., Vitamins A & E require lithium heparin samples protected from light.	Analysed by reference laboratory.
Vitamin D	Gel Serum or Lithium Heparin Plasma Tube.	Analysed daily by Grimsby and Grantham laboratories.

W

X

Y

Z

Zinc	Red Capped Tube	Analysed by reference laboratory

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10.4 Adult and Paediatric Investigations

10.4.1 Adrenal Cortex

Primary (Addison's) and secondary (pituitary) adrenal failure

Patients with adrenal failure may present acutely with hypoglycaemia or hyponatraemia and dehydration or chronically with general malaise, anorexia, vomiting, intermittent abdominal pain and weight.
loss. Signs of dehydration include hypotension, often postural, and tachycardia. In primary adrenal failure, pigmentation is seen on sun exposed areas and sites of friction such as the palmar creases and buccal mucosa.

If the diagnosis is strongly suspected, there should be no delay in administering glucocorticoids as soon as blood has been taken for plasma cortisol and ACTH; the definitive diagnosis can wait. If dexamethasone is given, the short Synacthen test can be performed the next day as dexamethasone does not cross-react with cortisol assays. Random cortisol results are of limited value because of wide circadian variation. A short synacthen test should always be performed if there is a high index of suspicion of adrenal insufficiency.

Care should be taken in interpreting the short Synacthen test in women taking the oral contraceptives or HRT since elevations in cortisol-binding globulin will result in high basal and stimulated cortisol concentrations (Clark et al 1998, Ostlere et al 1998). NOTE: Our cut-off for an acceptable adrenal response to synacthen is method-specific and full interpretation of results is provided (El-Farhan N, Pickett A, et al (2010).

References

Clarke P, Neylon I et al (1998): Defining the normal cortisol response to the short Synacthen test: implications for the investigation of hypothalamic-pituitary disorders. Clin Endocrinol 49(3): 287-92.

Ostlere LS, Rumsby G, Holownia P, Jacobs HS, Rustin HA, Honour JW. Carrier status for steroid 21-hydroxylase deficiency is only one factor in the variable phenotype of acne. Clin Endocrinol (Oxf) 1998; 48:209-215.

El-Farhan N, Pickett A, et al (2010): Determination of method-specific normal cortisol response to the short Synacthen test, ACB Focus 2010, poster paper.

Hypercortisolism - Cushing's syndrome

Cushing's syndrome may be ACTH-dependent, and due to a pituitary tumour or an ectopic source, or ACTH-independent and due to an adrenal tumour (in which case the ACTH will be suppressed) and can also be observed in patients taking long term corticosteroids.

There are two different patterns of ACTH-dependent Cushing's syndrome:

(1) Cushing's disease is due to a pituitary adenoma secreting ACTH. It is an indolent process which presents with a plethora of signs: typical facies, obesity, proximal myopathy, secondary diabetes, hypertension, hypogonadism, osteoporosis, purple striae, hirsuties and acne, ankle oedema and buffalo hump. Following the diagnosis, the disease can often be retrospectively detected on old photographs where clinical signs may have been present for many years.

(2) Ectopic ACTH can be secondary to tiny (and often unlocalisable) benign tumours which mimic the natural history of a pituitary adenoma. Alternatively, ACTH may be produced by malignant tumours and resulting in excessive mineralocorticoid action causing muscle weakness due to hypokalaemic alkalosis with plasma potassium often < 2.0 mmol/L. These patients are usually thin and deeply pigmented and have an extremely poor prognosis with a 50% survival of only 6 weeks.

Cushing's disease is one of the most difficult problems in clinical endocrinology. It is frequently suspected but rarely diagnosed. There are two phases (1) the diagnosis of hypercortisolism and (2) the localisation of the source of excess ACTH.

The recommended first-line screen for hypercortisolism is an **overnight dexamethasone suppression test** with/without 24-hour urinary cortisol. This has a relatively high false positive rate but very low false negative. The localisation of the source of ACTH is extremely difficult and it is best to refer patients to an endocrinologist when non-suppressible cortisol has been demonstrated. Follow-up extensions of the dexamethasone suppression test may need to be employed (both low and high dose dexamethasone suppression test) in conjunction with imaging techniques.

If the diagnosis is strongly suspected and the screening tests are negative, the diagnosis should not be discounted as there are well recorded cases of cyclical Cushing's disease with episodes of clinical and biochemical normality between episodes of typical clinical and biochemical disease.
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Hyperaldosteronism - Conn's syndrome

See section 10.5.1 page 73.

10.4.2 Adrenal medulla

Phaeochromocytoma

Catecholamine secreting tumours are rare and as many as 50% are diagnosed postmortem. The clinical features described are of episodic or uncontrollable hypertension and/or symptoms of sympathetic overactivity e.g., sweating, palpitations, pallor, and headache. However, many patients with phaeochromocytoma diagnosed during work-up or follow-up of Multiple Endocrine Neoplastic (MEN) syndromes are asymptomatic.

The diagnosis is based on increased urinary catecholamine metabolite (metanephrines) excretion. We recommend at least two consecutive 24 hour urine collections made into containers containing acid to ensure preservation of the catecholamine metabolites. Plasma catecholamine measurement is not routinely recommended in low-risk patients. We suggest the following approach:

24 hr. urine metanephrines in patients with relatively low risk such as hypertension

Plasma metanephrines are the test of choice in patients with a high probability of developing a phaeochromocytoma (genetic syndromes, history or family history)

When interpreting the results of urinary Metanephrines consider:

A single 24hr. urinary metanephrine result slightly above the upper reference range will only marginally increase the pre-test probability of a tumour being present.

An elevation of four-fold above the intervals can almost provide 100% probability of a phaeochromocytoma.

Some antihypertensive agents may interfere with the analytical methods and the laboratory should be contacted for advice:

Source	Outcome
Tricyclic antidepressants Phenoxybenzamine Calcium-channel blockers Stimulants (e.g., caffeine, nicotine) Monoamine oxidase inhibitors Paracetamol	Increases in plasma and urinary catecholamines and/or metanephrines
Caffeine, a-Methyldopa & labetalol	Variable analytical interferences with HPLC assays
Levodopa	Can cause analytical interferences and increases in catecholamines and metanephrines

Reference

Peaston R T and Ball S: Biochemical detection of phaeochromocytoma: why are we continuing to ignore the evidence? Ann Clin Biochem 2008; 45: 6–10.
Barron J. J. Clin. Pathol. 2010 Phaeochromocytoma: Biochemical Challenges for Screening and Diagnosis. Pub online June 14, 2010, doi: 10.1136/jcp.20010.071647

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10.4.3 Anterior Pituitary

Baseline tests for the patient suspected of having hypopituitarism include TSH, FT4, FT3, cortisol, prolactin, FSH, LH, oestradiol (females) and testosterone (males). In males with borderline-low total testosterone results, free testosterone may be calculated (via the calculator at www.issam.ch) at the discretion of the local site consultant. If these suggest pituitary dysfunction, second line follow up tests include various dynamic function tests the majority of which are best conducted under the auspices of one of the local Endocrine Clinics. Please refer to Dynamic Function Tests, section 10.6 for specific investigations into possible acromegaly (GTT for acromegaly) and hypopituitarism (Glucagon Stimulation test and Insulin Stress test). See also sections on Adrenal Cortex, Gonadal and Thyroid Function.

10.4.4 Bone and Calcium

The laboratory bone profile consists of serum calcium, phosphate, albumin, adjusted calcium, and alkaline phosphatase.

Hypercalcaemia

If serum calcium is high, parathyroid hormone (PTH) is the essential co-investigation in a newly presenting patient. This should differentiate those having primary hyperparathyroidism from those with malignancy (or other causes). These 2 categories account for >95 % of hypercalcaemia. Note that Familial Hypocalciuric Hypercalcaemia (FHH) may be confused with primary hyperparathyroidism. In FHH the patient will have hypercalcaemia and a PTH usually within the reference range or only slightly raised. In such cases it is essential to assess urinary calcium excretion.

Please contact the laboratory Consultant for further advice if this is suspected.

Hypocalcaemia

For those found to be hypocalcaemic, measurement of serum magnesium is mandatory and is added by a reflex laboratory computer rule when the measured calcium is <1.8 mmol/L. If hypomagnesaemia is ruled out as a cause, phosphate, urea, and electrolytes, and PTH must also be measured. Further tests will be dependent upon the outcome of the first line tests.

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10.4.5 Diabetes mellitus

Classically, diagnosis is by means of blood glucose measurement, either in random or fasting samples, or by means of a formal oral glucose tolerance test (OGTT).

Random Blood Glucose

	Venous Plasma mmol/L
Diabetes likely	≥ 11.1

Fasting Plasma Glucose

	Venous Plasma mmol/L
Diabetes likely	≥ 7.0
Impaired Fasting Glycaemia (IFG)	6.1 – 6.9

> If a random plasma glucose is ≥11.1 or fasting plasma glucose ≥ 7.0 and the patient has symptoms (polyuria, polydipsia, or unexplained weight loss), then further tests are unnecessary, and DM is confirmed.
> If symptoms are not present, another raised fasting or random plasma glucose concentration is needed to diagnose DM.

Individuals with IFG should have their fasting plasma glucose checked annually. All those with a repeat non-diagnostic fasting plasma glucose (in the IFG range) should have oral glucose tolerance test (OGTT) to exclude/diagnose DM.
If in doubt an oral glucose tolerance test should be performed. A fasting glucose alone may not be diagnostic.

Glycated Haemoglobin (HbA_{1c})

This test is used as an aid to monitor the control of patients with Diabetes Mellitus (DM). The life of the average healthy red cell is four months. Glucose is bound to haemoglobin to form HbA_{1c}. Thus, the concentration of HbA_{1c} is proportional to the integrated values for blood glucose concentration over this period. However, changes in the immediate preceding month have a much greater effect than those several weeks previously.

Worldwide standardisation of the reference method used to measure HbA_{1c} was endorsed at a meeting of 18 expert bodies including leading health charity Diabetes UK and the Association for Clinical Biochemistry (ACB) in April 20010. The meeting was convened by the Department of Health's National Director for Diabetes, to discuss a consensus paper recently published by the International Federation of Clinical Chemistry (IFCC), American Diabetes Association and European Association for the Study of Diabetes. The IFCC put forward to the meeting its Reference Measurement method, which would, for the first time, allow healthcare professionals across the world to easily report HbA1c results uniformly. We switched to dual reporting of results as % and as mmol/mol Hb thereafter, but from June 2011 we followed the UK guidelines and switched to reporting HbA_{1c} test results in new units only, i.e., mmol/mol Hb.

A "non-diabetic" reference range of 23 – 43 mmol HbA_{1c} / mol Hb is quoted on our reports, but this is very rarely achieved in patients with diabetes. The reports indicate that the target range for good diabetic control as <53 mmol/mol (per WHO guidelines).

The WHO recommends that where suitable assays exist, HbA_{1c} may be used for diagnosis of diabetes, with a cut off of 48 mmol/mol Hb. A UK expert group has met and agrees that the WHO requirements are met within the UK. However, HbA_{1c} is not suitable for use in everyone. It should not be used to make a diagnosis of diabetes in pregnancy **or where Type 1 diabetes mellitus is suspected**. Please contact your local Consultant Clinical Scientist if further information is required.

If the results obtained do not fit your clinical judgement of the condition of the patient it maybe helpful to request some appropriate haematological tests and check renal function. The technique in use does detect **some** abnormal haemoglobins and as such they would be reported if observed. Caution is necessary in interpretation in the following conditions.

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	Result		Result
Haemolytic anaemia & acute blood loss in general	Lower (Decreased RBC survival)	Uraemia	Current method is not affected by Carbamylated Hb
Aspirin Therapy	Higher	Alcohol use	Lower (Hypoglycaemic effect)
Iron Deficiency	Higher (extended RBC life)	Pregnancy	Lower
Haemoglobinopathies	Results are variable. Suggest discuss with laboratory		

Reference

Guidance for the change in HbA1c standardisation and reporting units can be found at:
http://www.acb.org.uk/docs/Article%20Summary%20Use%20of%20HbA1c%20WHO%20guidance%2025 1111[1].pdf
Guidance on haemoglobin methods interfered with by variants can be found at:
http://www.ngsp.org/interf.asp

10.4.6 Gastro-Intestinal

Amylase

Serum Amylase is a first line test for acute pancreatitis (AP). A serum amylase activity greater than three times the URL remains the primary diagnostic test for AP in most centres and the evidence generally supports this (Harper and Cheslyn-Curtis, 2011). It is also commonly raised in diabetic ketoacidosis and renal failure, when results like those seen in acute pancreatitis may be encountered. Lesser elevations are seen in mumps infection, constriction of the sphincter of Oddi by opiate drugs and tumour, lesions of the fallopian tubes and many other acute abdominal conditions.
Serum lipase is another potential marker of AP, but most studies indicate similar specificities for amylase and lipase in the range of 92–99% (Harper & Cheslyn-Curtis 2011). Non-pancreatic hyperlipasaemia has been observed in a range of pathologies including peptic ulcer disease, mesenteric ischaemia, acute renal failure, bone fractures, crush injury and fat embolism. Serum lipase is not available locally.

Reference: Harper SJF and Cheslyn-Curtis S. Acute Pancreatitis (Review article). Ann Clin Biochem 2011; 48:23-37

Faecal immuno testing (FIT)

The Faecal Immuno testing (FIT) test allows the quantitative determination of human haemoglobin (Hb) in faeces. It can be used for screening many lower gastrointestinal tract conditions associated with bleeding such as colorectal carcinoma, colon polyps, Crohn's disease, and ulcerative colitis. The method is specific for human haemoglobin and no restricted diet (meat-free or peroxidase-free diet) is required.

Nice guidance on 2 WW referral published in July 2017 can be found at:

https://www.nice.org.uk/guidance/dg30

https://www.nice.org.uk/guidance/ng12

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Faecal calprotectin

Calprotectin is released into the gut in response to inflammation and therefore its measurement in faeces can be used to screen for inflammatory bowel diseases such as Crohn's disease or ulcerative colitis. Whilst these conditions can cause similar symptoms to irritable bowel syndrome measurement of faecal calprotectin can help distinguish between them and therefore minimise the need for extensive and expensive invasive investigations.

In October 2013 NICE published guidance (DG 11) endorsed and advocated the use of faecal calprotectin to help diagnose these conditions and reduce the need for colonoscopy.

10.4.7 Gonadal function including reproductive hormones, hirsutism, menopausal status.

Reproductive hormones and infertility

NOTE: The infertile couple should always be co-investigated, with the following scenarios / test selections being commonly used / encountered:

Female

Please indicate on request forms the presence or absence of menstruation, the date of the first day of the last menstrual period (LMP date), cycle length if not 28 days and the date of the sample. Please write on the form details of any hormone therapy being received or received in the previous two months. Details of body hair distribution, galactorrhoea, under or overweight are also helpful.

Infertility An LH and FSH are most useful in the follicular phase of the cycle. Progesterone is most useful about day 21 of the cycle, or 7 days before expected menstruation.

Oligo- or Amenorrhoea Pregnancy should be ruled out before requesting any further hormonal investigations. Prolactin is important especially if there is any suggestion of galactorrhoea. It is frequently justified to repeat the sample because the secretion is pulsatile. Details of all drugs should be written on the request form. Oestradiol is most useful if accompanied by FSH and LH analyses. TFTs, testosterone and sex hormone binding globulin can also be helpful. Other tests should be undertaken after liaison with consultant staff.

Hirsutism First line tests in female patients presenting with hirsutism should include FSH, LH, testosterone and SHBG. Further tests may logically be added when the first line test results become available. These may include DHEA sulphate, androstenedione, and 17-hydroxy progesterone. A useful link giving more information about the hirsute female can be found at:-http://www.endotext.com/female/female6/femaleframe6.htm

Male

Useful tests include FSH / LH, prolactin, HCG, oestradiol, thyroid function tests, and testosterone. The following notes may be helpful: -

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Impotence Exclude Diabetes Mellitus, include prolactin & testosterone in the request and ensure that you take the blood sample between 010.00-010.00h as circadian variation significantly reduces testosterone in many men thereafter.

Gynaecomastia Gynaecomastia is the enlargement of glandular tissue of the breast and should be distinguished from an increase in adipose tissue. It is usually bilateral, but may be strikingly asymmetrical, or unilateral. It results from an increase in the effective oestrogen: androgen ratio within the mammary tissue. Physiological gynaecomastia can occur at the extremes of life, but is most common during puberty, when it usually lasts for only a few months, although may persist into adulthood.

The index of suspicion and therefore the need for endocrine investigation of gynaecomastia will depend on the age of the subject and findings on detailed history and physical examination. Pathological gynaecomastia is likely when sudden enlargement occurs unrelated to puberty particularly in young boys or middle-aged men. The most common identifiable cause is drug therapy, but there is no obvious aetiology in up to 50% of cases.

Initial steps should include a detailed drug history. A positive drug history cannot exclude a breast cancer which should always be considered, especially in the elderly. Endocrine investigations should be considered if gynaecomastia is persistent or progressive and baseline investigations should include liver function tests, fasting glucose, reproductive hormones, oestradiol, TFT and b-HCG.

Seminal Fluid Clinicians should refer to local laboratory instructions and the Path Links Andrology Handbook section (page 160) for seminal analysis in infertile couples.

See also Anterior Pituitary, 10.5.3.

Menopause

Which patients to assess?

In practice, it is rarely useful to perform blood tests as hormone levels fluctuate widely over a very short time span and can be confusing. Blood tests (FSH/LH) are usually only indicated when a premature menopause is suspected in a younger woman, or to rule conditions that may cause similar symptoms such as anaemia or thyroid disease. The best way to diagnose the menopause is by taking a thorough history of symptoms and menstrual irregularities. The menopause can only be diagnosed with absolute certainty retrospectively, as ovulation could still occur after many months without menstrual periods. A woman is deemed to be menopausal if she has appropriate symptoms and her LMP was >1 yr. if she is >50yrs, or if she hasn't had a period for more than 2 years if <50.

Much useful advice can be found on the Faculty of Sexual Reproduction and Healthcare web page of the Royal College of Obstetricians and Gynaecologists under the 2010 guideline "Guidance on Contraception in women >40" at http://www.fsrh.org/

Investigating women with normal menstrual cycles

About 98% of women with regular menstrual cycles will ovulate normally. FSH/LH should be measured in the follicular phase, days 1-7 of the cycle (Note that FSH usually rises before LH during the climacteric).

An FSH of approx. >15 *may* indicate the start of the climacteric. LH may be of use to assess the possibility of a sample taken close to mid-cycle, when LH >> FSH.

Investigating women with abnormal menstrual cycles or post-hysterectomy

Samples cannot readily be related to stage of cycle in this group, although if still menstruating, then sampling at the time of menses can be helpful. An FSH of >30 U/L is suggestive of the menopause but does not necessarily rule out further ovulatory cycles. When FSH is unequivocally in the menopausal range, oestradiol assays are not needed, but if FSH is 20-30 U/L, it may be useful.

In those with irregular / absent cycles but who are otherwise of child-bearing age other causes should be considered e.g., pregnancy, drug therapy, hyperprolactinaemia, extremes in weight, stress and so on. Hypothyroidism, causing secondary hyperprolactinaemia, is a rare cause of oligomenorrhoea.

Post-hysterectomy, biochemical assessment is the only way to assess hormonal status as women reach menopause earlier and are more at risk of developing post-menopausal complications. It is probably advisable to check **FSH/LH** and oestradiol annually in those women of < 45 years of age especially as 20-30% may enter the menopause with no overt clinical symptoms.

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Monitoring hormone replacement therapy (HRT)

Biochemical measurements are of little value in monitoring oral or patch HRT therapy. Alleviation of symptoms is the best marker (measurement of oestradiol may be useful to check compliance or efficacy of skin patch absorption). NB: some HRT preparations are **equine** e.g., premarin and prempak-C. **These will show variable or no cross-reactivity with our oestradiol assay.** Accordingly, it is not recommended that oestradiol be measured in women on **oral** HRT. In those women being given human oestradiol by **implants** or **transdermally**, measurement should be done prior to re-implantation.

Other problem areas

Oestrogen-containing contraceptives will mask symptoms of ovarian failure as the progestogen component ensures regular monthly withdrawal bleeding irrespective of menopausal status. Reproductive hormones should not be measured in any patient on **combined oral contraceptives.** The progestogen-only pill suppresses gonadotrophins much less and will not relieve menopausal symptoms or amenorrhoea. Other reproductive hormones should not be measured in any patient on **ethinyl oestradiol.** The date of the menopause will be masked in women who start HRT before the menopause. As standard HRT is not contraceptive, use of a non-hormonal contraceptive is required.

10.4.8 Kidney function, Estimated Glomerular Filtration Rate (eGFR) & Acute Kidney Injury (AKI)

Chronic Kidney Disease (CKD)

NICE Clinical Guideline 182 recommends the use of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation to estimate GFR to monitor/diagnose CKD. Consider requesting eGFR as an alternative to creatinine clearance. Note that eGFR is not validated for use in children <18 years old, acute renal failure, pregnancy, oedematous states, muscle wasting disease states, amputees, or malnourished patients.

NICE guidance NG203 www.nice.org.uk/guidance/ng203 Published: 25 August 2021) states:

1.5.1 Give adults with CKD and their family members or carers (as appropriate) information about their 5-year risk of needing renal replacement therapy (measured using the 4-variable Kidney Failure Risk Equation).

The Kidney Failure Risk Equation (KFRE) is a well-validated risk prediction tool for a kidney replacement therapy (KRT) in the next two or five years in individuals with chronic kidney disease (CKD) stages 3a-5. To estimate the risk of KRT, the four-variable KFRE uses:

- • Age (>18 years)
- • Sex
- • Estimated glomerular filtration rate EPI calculation (EPI-eGFR)
- • Urine albumin:creatinine ratio (ACR) mg/mmol

The KFRE should be calculated in adults when an individual with CKD Stage 3a-5 (EPI-GFR <60) has an eGFR and ACR measured. This should be at least on an annual basis, but more frequently with more advanced disease. It is recommended that both eGFR and ACR should be within six months of each other, and ideally within a month.

The **KFRE** will give a risk of KRT over the next two or five years. Five-year predictions may be used in primary care to counsel patients regarding referral to secondary care.

Estimated GFR (eGFR) reference ranges (mL/min/1.73m^2)

>90 Indicates normal GFR, unless there is a structural abnormality or a functional abnormality such as persistent proteinuria or microscopic haematuria.

60-89 Does not indicate chronic kidney disease unless there is other existing laboratory/clinical evidence of disease.

30-59 Indicates moderate renal impairment.

15-29 Indicates established renal impairment.

<15 Indicates established renal failure.

Further information can be obtained from: NICE Clinical Guideline 182: chronic kidney disease in Adults: Assessment and Management (2014). Available at: www.nice.org.uk/guidance/cg182

Also, the UK Renal Association at:
http://www.renal.org/whatwedo/InformationResources/CKDeGUIDE.aspx

Cystatin C-based Estimate of GFR

NICE CG182 recommends considering the use of eGFRCystatinC at initial diagnosis to confirm or rule out CKD in people with an eGFR creatinine of 45-59 mL/min/1.732m^2, sustained for at least 90 days and no proteinuria (albumin:creatinine ratio [ACR] less than 3 mg/mmol) or other markers of kidney disease.

Note: eGFRCystatinC should be interpreted with caution in people with uncontrolled thyroid disease because eGFRCystatinC values may be falsely elevated in people with hypothyroidism and reduced in people with hyperthyroidism.

Acute Kidney Injury (AKI)

The Renal Association published a summary of clinical practice guidelines for AKI in March 2011 (see http://www.renal.org/clinical/guidelinessection/AcuteKidneyInjury.aspx) as a result of which hospital users can see a calculated index of AKI displayed on all in-patients in the Web V software application. This is based on serum creatinine increments compared with historical data accrued in the previous 12 months, as per these guidelines and is an automatic calculation in all patients where a serum creatinine has been measured during the current admission.

In 2013, NICE published clinical guidance 169: "Acute Kidney Injury: prevention, detection & management" (available at: www.nice.org.uk/guidance/cg169). This guideline details emphasises early intervention and stresses the importance of risk assessment and prevention, early recognition, and treatment of AKI. It offers best practice advice on the care of adults, children, and young people with or at risk of acute kidney injury.
In ULHT, further guidance has been produced "Guidelines for the Management of Acute Kidney Injury (AKI) including Acute Kidney Injury BOMB care bundle) and is available on the ULHT intranet site.

Serum creatinine measurement

The gold standard enzymatic method for serum creatinine measurement has been in use across Lincolnshire since January 2012, as recommended by the Renal Association. It offers improved specificity and more accurate quantitation of creatinine and GFR at lower creatinine levels. This method is used on our main Roche COBAS analysers and the calibrators are isotope-dilution mass spectrometry (IDMS)-traceable. The CKD-EPI creatinine equation is then used to calculate eGFR. Following implementation of this revised calibration, use of the CKD-EPI creatinine equation will reduce misclassification of CKD as it performs better and with less bias than the MDRD study equation, especially in patients with higher GFR. This equation is as follows:

$$GFR\ (mL/min/1.73\ m^2) = 141 \times \min(Scr/\kappa,1)^{\alpha} \times \max(Scr/\kappa,1)^{-1.209} \times 0.993^{Age} \times 1.018\ [\text{if female}] \times 1.159\ [\text{if black}]$$

κ = 0.7 if female; 0.9 if male
α = -0.29 if female; -0.411 if male
Min = the minimum of Scr/κ or 1
Max = the maximum of Scr/κ or 1

Samples for creatinine measurement

Note that significant meat intake will significantly elevate serum creatinine, an effect especially noticeable in those whose creatinine is usually <100 umol/L. If assessing creatinine / eGFR, it is therefore **essential** that the patient has fasted overnight and is known not to have had significant meat intake in the 12 hr prior to the test. Results are also affected in the case of extremes of muscle mass.

10.4.9 Lipid Management and guidelines

Adults

Reference should be made to the NICE clinical guidance 181 published in July 2014 "Cardiovascular disease: Risk Assessment & Reduction, including lipid modification" (available at: www.nice.org.uk/guidance/cg181). This guideline recommends a change in practice to use non-fasting samples to measure the lipid profile in most patients (and safer if they have diabetes). The full lipid profile includes measurement of total cholesterol, HDL-cholesterol, non-HDL cholesterol and triglyceride concentrations; with non-HDL cholesterol being a better cardiovascular disease (CVD) risk indicator than LDL-cholesterol.

Fasting is still required if LDL is needed (familial hypercholesterolaemia diagnosis) and for confirmation of severe hypertriglyceridaemia.

Note that dyslipidaemic patients requiring management advice from hospital consultant should be referred to one of the local endocrinologists who will review and advise on treating these patients.

Children

The Royal College of Paediatrics and Child Health published the OSCA consensus statement on the assessment of obese children & adolescents for paediatricians (2009) which contains advice on desirable lipid thresholds in children. These are

automatically reproduced on all lipid profiles in children up to the age of 16 years. The document can be viewed on the RCPCH website at: http://www.rcpch.ac.uk/system/files/protected/page/OSCA%20Investigation%20protocol.pdf
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Laboratory lipid profile and Management

1. The full laboratory lipid profile includes direct measurement of total cholesterol, HDL-cholesterol, and triglyceride concentrations with derivation of non-HDL cholesterol.

2. Measure **non-fasting** full lipid profile. Note that the depression of serum cholesterol following MI generally lasts no longer than 6 weeks but can be longer if there is a complicated recovery.

3. **If abnormal repeat** fasting (14hr) full lipid profile. Management decisions should not be made on a single lipid measurement.

4. If hyperlipidaemia is confirmed, exclude secondary causes (hypothyroidism, diabetes mellitus, renal failure, liver disease, ethanol, drugs, and obesity).
Contact the local laboratory for further advice when indicated and if further specialist analyses may be indicated. Secondary hyperlipidaemia (i.e., because of hypothyroidism, ethanol excess, diabetes, or nephrosis) should always be excluded before starting drug therapy.

10.4.10 Metabolic Screening

Path Links offers a metabolic screening service for the investigation of certain inherited disorders. The laboratory must be contacted prior to sending samples to ensure the tests are appropriate and any special requirements for collection of samples can be made.
Investigations for possible disorders of Amino Acid and Organic Acid metabolism and other rare inborn errors of metabolism are referred to Sheffield Children's hospital.

10.4.11 Porphyria screening

The investigations of the porphyria's can be classified into two broad groups:

i. Abdominal Pain and Neurological Symptoms
ii. Dermatological Manifestations

The initial investigations and samples required for first line investigations:

	Samples required	Investigation
Abdominal pain and neurological Symptoms	Fresh urine	Porphyrins & porphobilinogen
Dermatological Manifestations	Whole blood/plasma	Porphyrins
	Fresh urine	Porphyrins

Further samples may be requested depending on the outcome of the initial investigations. Please note that samples should be carefully labelled and protected from light. Our service utilises the laboratory and advisory service provided by Cardiff University Hospitals whose website can be viewed at http://www.cardiff-porphyria.org/. Another useful website can be found at http://www.porphyria-europe.com.

10.4.12 Sweat Testing

Sweat testing provides laboratory confirmation of a clinical diagnosis of cystic fibrosis (CF). Path Links laboratories use the Wescor iontophoresis system and follow the "Guidelines for the performance of the sweat test for investigation of Cystic Fibrosis in the UK 2nd version – An evidence based guideline" (March 2014) (available at: www.rcpch.ac.uk/system/files/protected/page/Sweat%20Guideline%20v3%20reformat_2.pdf). The Wescor system uses a pilocarpine-containing gel and has a microprocessor controlled current delivery which greatly reduces the possibility of skin burns.

In **children >6 months old** the results are interpreted as follows:

Sweat Chloride >60 mmol/l supports diagnosis of CF.
Sweat Chloride of 40 to 60 mmol/l is suggestive, but not diagnostic of CF.
Sweat Chloride <40 mmol/l is normal and indicates a low probability of CF.

In **children <6 months old**, results are interpreted as follows:

Sweat Chloride >60 mmol/L supports a diagnosis of CF.
Sweat Chloride of 30 to 60 mmol/L is suggestive, but not diagnostic of CF.
Sweat Chloride of <30 mmol/L is normal and indicates a low probability of CF.

10.4.13 Thyroid Function

The laboratory assessment of thyroid status and interpretation broadly follows the recommendations in the 2006 document UK Guidelines for the Use of Thyroid Function Tests which can be found on-line at http://www.british-thyroid-association.org/Guidelines/

Thyroid Stimulating Hormone (TSH)

The measurement of TSH in a basal blood sample by a sensitive immunometric assay provides the single most sensitive, specific, and reliable test of thyroid status in both overt and subclinical primary thyroid disorders. In primary hypothyroidism TSH is increased whilst in primary hyperthyroidism TSH is usually <0.01 mU/L. There are exceptions to this generalisation and abnormal TSH concentrations may be found in some euthyroid patients. TSH alone is not a reliable test for detecting thyroid dysfunction arising from hypothalamic-pituitary dysfunction and in other specific instances, for which there should be a high index of suspicion and close liaison with the laboratory.
All samples are assayed for TSH first. Please indicate on the request form if the patient is receiving thyroxine and / or anti-thyroid drugs, or if he or she has received iodine, oral contraceptives, or other drugs which may be relevant, e.g., Lithium or Amiodarone. Further tests are added at the discretion of senior laboratory staff dependent upon the TSH result and clinical information.

Free Thyroxine (FT_4) and free tri-iodothyronine (FT_3)

Free (unbound) thyroid hormones are regarded by many as the biologically active fraction of the total circulating thyroid hormone pool and are unaffected by changes in the concentration and affinity of thyroid-hormone binding proteins. Free hormones thus theoretically provide a more reliable means of diagnosing thyroid dysfunction than measurement of total hormone concentrations. Free hormones are normal in patients with mild (subclinical) thyroid disorders.

FT_4 is assayed if the TSH falls outside the reference range, or if the patient is taking amiodarone, carbimazole or is post I^{131} therapy. If secondary hypothyroidism (i.e., due to pituitary disease) is suspected then FT_4 should also be requested.

FT_3 is useful in the following situations: -

1. Borderline high T_4 and low TSH (on no treatment) to confirm thyrotoxicosis.
2. Normal T_4 and low TSH (on no treatment) to distinguish thyrotoxicosis caused solely by elevated levels of FT_3.
3. Patients with previously elevated FT_3 only, on treatment for hyperthyroidism.
4. Patients receiving liothyronine (T_3) replacement.
5. Amiodarone therapy. This anti-arrhythmic drug is an iodine-containing drug that has complex effects on thyroid metabolism. These include inhibition of T_4 to T_3 conversion, inhibition of thyroidal iodine uptake and inhibition of T_4 entry into cells. The drug may also induce a destructive thyroiditis. Patients may have an altered thyroid hormone profile without thyroid dysfunction but 14% -18% of patients taking amiodarone may develop clinically significant hypothyroidism or amiodarone induced thyrotoxicosis. Because of the long half-life of amiodarone, clinical problems may occur up to a year after stopping the drug.

Amiodarone-associated hyperthyroidism should be diagnosed only if high circulating FT_4 is associated with high or high/normal FT_3 and undetectable TSH since even in euthyroid subject's amiodarone therapy often causes modest elevation in serum FT_4 (and reduction in FT_3) because of its effect on peripheral deiodination of T_4 to T_3. A diagnosis of amiodarone-associated hyperthyroidism should prompt specialist referral since management may be complex and involve further investigations.

Measurement of FT_3 is **not** indicated in routine monitoring of patients on thyroxine replacement.
Thyroid peroxidase (TPO) antibodies are helpful if the patient has a goitre especially if recent or painful. They are also indicated if the FT_4 and TSH do not fit clinical features or if the clinical or/and biochemical pattern is changing without any

therapy, or if TSH is persistently raised and FT_4 is normal. They may also be requested before I^{131} iodine therapy because a high titre may lead to more rapid onset of hypothyroidism.
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These are present in the serum of patients with a wide range of immunologically mediated thyroid disorders (e.g., Hashimoto's thyroiditis, Graves' disease). They may also be found in a small proportion of apparently healthy individuals but the appearance of TPO Ab usually precedes the development of thyroid disorders.
The measurement of TPO Ab is of clinical use in diagnosis of autoimmune thyroid disorders.
as a risk factor for autoimmune thyroid disorders; as a risk factor for hypothyroidism during treatment with interferon alpha, interleukin-2 or lithium; as a risk factor for thyroid dysfunction during lithium or amiodarone therapy.

TSH-receptor antibodies (TSH-RAb), often referred to as Thyroid Binding Inhibiting Immunoglobulin's (TBII) or TSH Receptor Antibodies (TRAb) can be useful in several circumstances (NB: These assays do not distinguish between stimulatory or blocking properties of these antibodies).
In most patients the measurement of TSH-RAb is not an essential investigation for diagnostic purposes but their measurement of TSH-RAb ***is*** particularly useful in **pregnancy**. It can also be helpful in the following situations:

- To investigate hyperthyroidism of uncertain aetiology
- To investigate patients with suspected "euthyroid Graves' ophthalmopathy to identify neonates with transient hypothyroidism due to TSH-RAb

Occasionally other tests can be done by prior arrangement (e.g., TRH test)

Acutely ill Patients

It is recommended that thyroid function testing is delayed until convalescence in the acutely ill patient.

Radiographic Procedures

If renal function is moderate or good, intravenous urography or arteriograms have no effect on thyroid function. However, note that cholescystography and use of inorganic iodine-containing radiocontrast.
media will interfere with all thyroid function tests for at least 3 months after use. Most reliance should be placed on detailed clinical assessment of the patient. They may precipitate hypothyroidism in autoimmune disease as well as iodine-induced thyrotoxicosis (IIT). Their composition may be between 30 - 50% of iodine and many grams are used for roentgenologic visualization of organs. Those patients who have multinodular goitre, or live in areas where iodine intake is low, are especially at risk. Clinicians should be aware that IIT often develops several weeks after administration of X-ray contrast agents. Follow-up of such patients after X-ray procedures is therefore advisable although considering the wide use of X-ray contrast agents, the probability of inducing IIT by these substances must be low.

10.4.14 Xanthochromia Screening (CSF)

The following sampling protocol summary is taken from Cruickshank A *et al* (2008). Revised national guidelines for analysis of cerebrospinal fluid for bilirubin in suspected subarachnoid haemorrhage. Ann Clin Biochem 45: 238-244. The whole document is available on-line at: http://www.bilirubin.co.uk/Home/Revised%20National%20Guidelines.pdf

Principle

This test is performed to try to identify those patients who have had a SAH but in whom the CT scan is negative. The spectrophotometric scan detects bilirubin in CSF and this finding is consistent with a bleed into the CSF. The formation of bilirubin after haemorrhage is a time-dependent process and bilirubin may not be detectable soon after the event (e.g., onset of severe headache). CSF should not be sampled until **at least 12 h after a suspected event**. Opening pressure should always be recorded when performing a LP. LP is contraindicated in papilloedema, focal neurological deficit or reduced consciousness.

Sample Requirements

•Only applicable in **CT NEGATIVE** patients •Specimens
MUST be collected sequentially
•Sample for spectrophotometry/Xanthochromia **MUST** be sample number 4 and a sufficient volume must be sent - 1mL minimum
•Specimens **MUST** be protected from light and pneumatic tube transport **MUST NOT** be used •Specimens
SHOULD be received in the laboratory within 1 hour of sampling
•Simultaneous blood sample for bilirubin and total protein **MUST** be provided •Record timing
of sampling in relation to possible SAH, **which MUST** be >12 hrs

Procedure

CSF may also be required for microbiological examination and for protein and glucose estimation.
Sufficient CSF will therefore be needed for all of these investigations.

1. Label one fluoride-oxalate (**grey**-topped) bottle and three 28 mL sterile universal containers and with the patient's name, NHS number, ward, date of birth, **time** that the CSF was obtained, and the sequence order of sampling.

2. The first specimen should be a **minimum of 0.5mL** of CSF placed in the **GREY** top fluoride oxalate tube for glucose and protein estimations. This should be sent to Clinical Chemistry.

3. Microbiology requires **at least 5 mL** of CSF divided into 2 sequentially numbered sterile 28mL universal containers labelled "**second**" and "third". These should be sent to Microbiology.

4. A further **minimum** of **1mL** CSF should be placed in the final ("**fourth**") sterile 28 mL universal container for the spectrophotometric scan. (**1 mL** is about 20 drops from the Luer connector on a needle).
Protect this sample from the light by placing it in a **protective black bag (obtainable from the laboratory),** outside the usual plastic specimen bag or wrapping in a paper towel, place inside the usual sample bag and send to Biochemistry.

5. A **blood specimen** (appropriately labelled) should be taken at the same time for serum bilirubin, total protein and glucose estimation that are needed to aid interpretation.

Request form

Please indicate on the request form:
- Clinical indication for request.
- Result of CT scan.
- Time of onset of symptoms/event.
- Time of LP.
- If the differential diagnosis includes meningitis

Sample transport

Boston site
All samples must be delivered to the Pathology Department as soon as possible. Do NOT use the air tube delivery system. If this procedure is not followed analysis is likely to be compromised.

Grantham site
Users must liaise with the laboratory and arrange for CSF samples to be delivered by Taxi to the Boston site where they are delivered to the A&E Department for collection by laboratory staff.

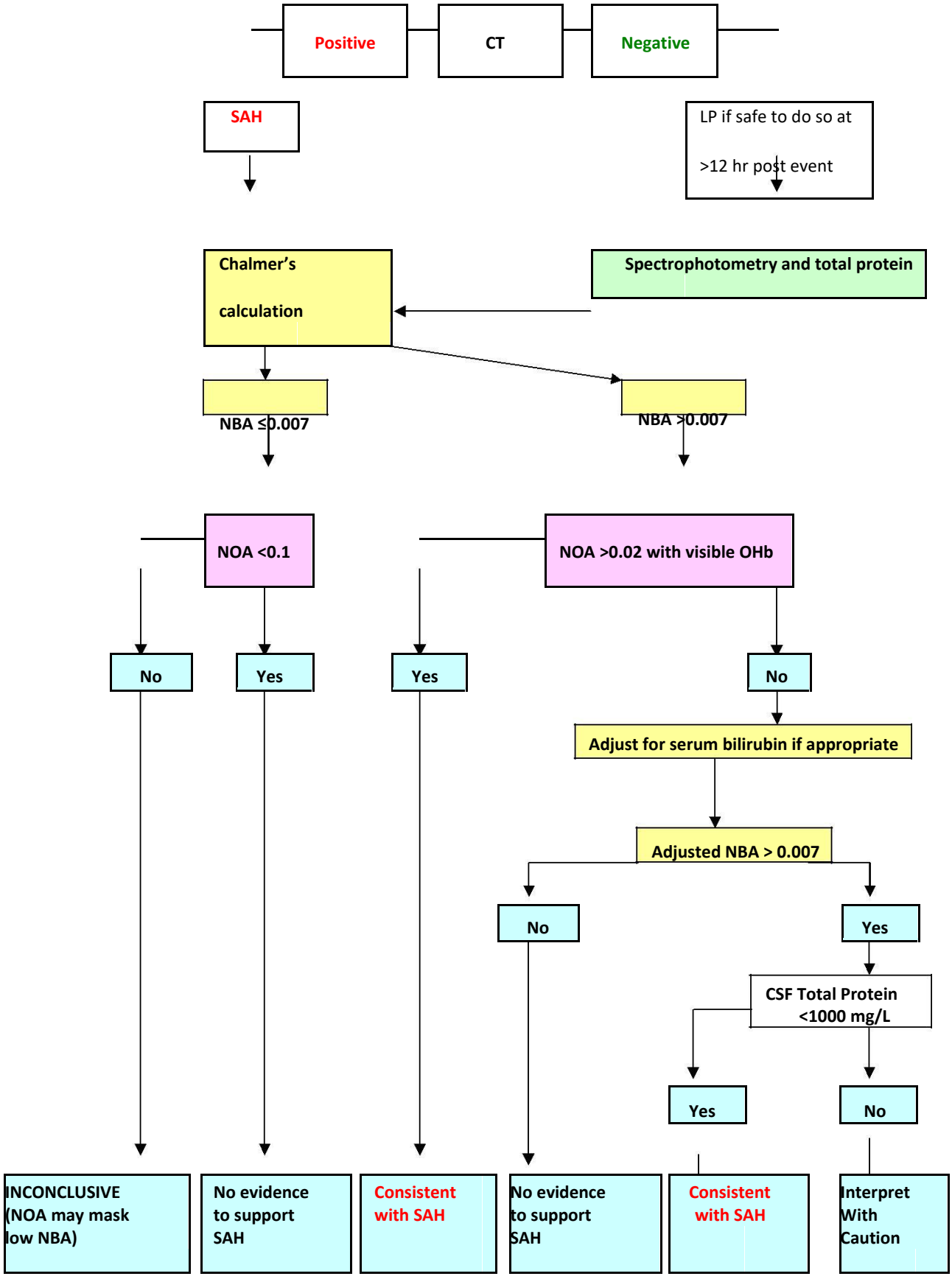
Grimsby site
Users must liaise with the laboratory and arrange for CSF samples to be delivered by Taxi to the Scunthorpe site where they are delivered to the A&E Department for collection by laboratory staff.

Lincoln site
All samples must be delivered to the Pathology Department as soon as possible. Do NOT use the air tube delivery system. If this procedure is not followed analysis is likely to be compromised.

Scunthorpe site
All samples must be delivered to the Pathology Department as soon as possible. Do NOT use the air tube delivery system. If this procedure is not followed analysis is likely to be compromised.

Interpretation

Interpretation of results is based on the Revised National Guidelines (2008) and a comment is appended to all reports. Interpretation is not always straightforward, especially when blood from whichever source is present in the CSF. The algorithm below is adapted from the revised National Guidelines (2008):



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10.4.15 Cardiac/homocysteine

High sensitive Troponin T

Troponin assays have been developed such that High Sensitivity assays are now able to detect release of troponin very soon after the onset of an acute coronary syndrome (ACS) and we are presently following NICE guideline DG15 (Oct 2014); Myocardial infarction (acute): Early rule out using high-sensitivity troponin tests (Elecsys Troponin T high-sensitive, ARCHITECT STAT High Sensitive Troponin-I and AccuTnI+3 assays).
We have implemented rapid assessment algorithms in conjunction with our acute care physicians and A/E consultants which assess dynamic changes in Troponin at admission (time zero) and after 3 hours which enable us to rule in and rule out ACS.

NT-proBNP

NT-proBNP is a peptide which is released into the blood stream in heart failure and in some cases its measurement can be used as a screening tool to rule our heart failure; thereby minimising the number of patient referrals for echocardiography.
In response to the recent NICE guideline; Acute heart failure: diagnosis and management (Act 2014) we now provide NT-proBNP testing on all Path Links sites and an interpretational guideline is printed on all patient reports.

Homocysteine

Homocysteine is an α-amino acid and a homologue of cysteine, differing by an additional methylene bridge (-CH_2-). It is biosynthesised from methionine by the removal of its terminal methyl group. Homocysteine can be recycled into methionine or converted into cysteine with the aid of certain B-vitamins e.g., B12/folate, pyridoxine. Therefore, blood levels can increase if these are deficient. Homocysteine levels can also increase with age, smoking and also secondary to drugs such as carbamazepine, methotrexate, and phenytoin.

Hyperhomocysteinaemia increases the risk of endothelial cell injury, which leads to inflammation in the blood vessels, which in turn may lead to atherogenesis, which can ultimately result in ischaemic injury. Hyperhomocysteinaemia is therefore a possible risk factor for coronary artery disease. However, although hyperhomocysteinaemia has been associated the occurrence of blood clots, heart attacks and strokes, it is unclear whether hyperhomocysteinemia is an independent risk factor for these conditions or whether correction improves patient outcomes. Hyperhomoscyteinaemia has also been associated with early pregnancy loss and with neural tube defects.

10.5 Dynamic Function Test Protocols

10.5.1 Creatinine Clearance Test

Creatinine is endogenously produced, released into body fluids at a constant rate and excreted via the kidneys. As plasma levels are maintained within relatively narrow limits, its clearance can be measured as an indication of glomerular filtration rate. This test has largely been supplanted by the automatic calculation of eGFR (NICE Clinical Guidance 182) but is still used in some circumstances, e.g., chemotherapy patients.

Patient preparation

- Patients should avoid diets high in animal protein and must have fasted overnight to avoid the post-prandial increases which occur in serum creatinine after a protein meal.
- Hydrate the patient with at least 600ml of water.

Procedure

1) Indicate all drugs on request form.
2) Have patient void and discard the urine. Note the time and from then on, collect all urine passed for 24 hours (or less if specially required e.g., 12 hours). Keep patient well hydrated during the collection period to ensure a urine flow rate of 1-2 ml/minute or greater. The urine must be collected into a plain container. The sample should be kept cool during the collection period and transported to the laboratory when complete.
3) A blood sample (ochre top) for creatinine should be taken some time during the 24-hour collection period and sent to the laboratory immediately after collection. If this is not practical, it is possible to do blood samples - one at the beginning of the 24-hour period and one at the end. The request form must be labelled clearly as "BLOOD FOR CREATININE CLEARANCE TEST, URINE SAMPLE TO FOLLOW". The patient details and times and dates must be on the samples of blood and urine.

Result Calculation

Creatinine clearance is calculated as follows:

$$\frac{\text{Ucrea} \times V}{\text{Pcrea}} \times \frac{1.73}{A}$$

Where, Ucrea = concentration of creatinine in urine
Pcrea = concentration of creatinine in plasma/serum (converted to the sample units as for urine)
V = volume of urine flow in mL/min (e.g., 24hr urine volume/24x60).
A = body surface area in square metres

(The factor (1.73 ÷ A) normalises clearance for average body surface, correcting as it does, for variation in creatinine excretion relative to lean body (muscle) mass. Whilst not usually required for adults, this procedure is essential in children, in which case the height and weight of the child must be provided. This is not done routinely, but laboratory can provide this correction if the requesting clinician liaises with the laboratory)

Sources of Error

- Error in recording timing of collection period, loss of a portion of the urine during collection, including the first void in the timed collection and urinary retention are the most common sources of error.
- Vigorous exercise during the collection period.
- Proper hydration of the patient to ensure urine flow rate of 2ml/minute or greater improves the accuracy of the measurement of filtration rate and tends to eliminate retention of urine in the bladder as a source of negative error.
- Patients should avoid significant meat intake for 12h prior to having a blood sample - in practice an overnight fast is the most practical approach. A significant meat protein intake can factitiously raise serum creatinine, especially in those with a creatinine which is usually <100 umol/L

Interpretation

Reference range: Males = 91 - 140 mL/min Females = 72 - 110 mL/min
Intra-individual variation is approximately +/- 15%.

References

- Nitrogen Metabolites and Renal Function, pages 1553 - 1537: Tietz Textbook of Clinical Chemistry, 3rd ed. (1999).
- Renal Function Testing, pages 50 *et seq*. Oxford Textbook of Clinical Nephrology, 2nd ed. (1998).

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10.5.2 Oral Glucose Tolerance Test for Diabetes Mellitus

Patient preparation

- The patient maintains an adequate carbohydrate intake for at least 3 days prior to the test (250g carbohydrate per day). For most, this will simply mean eating their normal mixed diet. Patients on calorie-restricted diets for e.g., losing weight should resume a "normal" diet.
- Discontinue, where possible, medications known to affect glucose tolerance. It is **NOT** appropriate to perform the test if patients are either on insulin or oral hypoglycaemic agents.
- On the day before the test the patient should fast overnight after the early evening meal, therefore fasting period must be between 10 and 16 hours. **This means NO food intake after rising on the morning of the test or until after the test is complete.**
- Only water is allowed at any time before or during the test. **ALL** other beverages should be avoided, including orange juice & milk of any description.
- The patient **must not smoke or eat** during the test and should remain seated at rest. NB: bed rest impairs glucose tolerance.

Ward/Phlebotomist requirements

- 250 ml bottle of ready to use GlucosePro obtainable from local Pathology department.
- Polycal (in liquid form) can be used as an alternative. Polycal can be obtained via hospital pathology departments at short notice. 113 mL contains an equivalent of 75g of glucose. To prepare the Polycal measure 113 mL into a beaker and add water to make the volume up to 200ml (mix well). This is drunk over a 5 min period followed by a further 100ml of water. Note that rapid consumption may cause transient abdominal discomfort. Otherwise, the test is performed exactly as you would use GlucosePro.
- Note that in children, an equivalent dose of 1.75 g glucose/kg body weight up to a maximum of 75g is given (up to a maximum of 250 ml of GlucosePro) or 2.6 mL/kg Polycal (up to a maximum of 113 mL) should be given. The reference table below should be used to ascertain the recommended GlucosePro dosage for oral GTT in children.

GlucosePro Dosage for Children																
Weight of patient (kg)	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Amount of GlucosePro (mL)	29	35	41	47	53	58	64	70	76	82	88	93	99	105	111	117

Procedure

PLEASE ENSURE ALL SAMPLES ARE CLEARLY LABELLED WITH PATIENT DETAILS AND THE COLLECTION TIMES

1) Ensure the patient has fasted. **If the patient has eaten on the morning of the test discontinue the test.**
2) Use the glucose meter (see relevant standard operating procedure) to measure the patient's fasting glucose in a fingerprick prick sample. Alternatively, a venous lithium heparin sample may be collected, and this used for glucose testing by meter. Note that a fluoride (grey cap) sample must not be used for testing in glucose meters. (If this procedure is undertaken in primary care, please record the fingerprick glucose result in the relevant result book, along with appropriate QC results).
3) Take the first FASTING fluoride **blood** sample and **label** appropriately. Ensure that time of collection is written on both sample and request form.
4) *If the fasting glucose result on the glucose meter is >10.0 mmol/L:* discontinue the test and allow the patient to leave. Send the fasting blood sample to the laboratory. Inform the requesting doctor as a courtesy. The test is discontinued if the fasting sample glucose is ≥ 10.0 mmol/L for two reasons. Firstly, because this would normally be considered diagnostic of Diabetes Mellitus and secondly, a high carbohydrate load may not be tolerated very well by a diabetic patient.
   If the fasting glucose result on the glucose meter is <10.0mmol/L: give the patient 250 mL GlucosePro (adults only).
   Note the time at which the patient starts to drink the GlucosePro/Polycal. The 2-hour sample should be taken exactly 2 hours from the point at which the drink is started.
5) Allow them to drink the GlucosePro/Polycal slowly over about 5 minutes.
6) Collect a further sample 2 hours **(or within 5 mins of this time)** after starting the ingestion of glucose. Ensure that time of collection is written on both sample and request form.

Note: If the patient vomits after drinking the GlucosePro and before the test is complete, stop the test but send the samples obtained up to that point to the laboratory.

Interpretation

Interpretation is based on the WHO criteria for Diagnosis of Diabetes Mellitus (2006):

	FASTING (mmol/L)	TWO HOURS after 75 g glucose (mmol/L)
Normoglycaemia	≤6.0	<7.8
Impaired Fasting Glycaemia	6.1 - 6.9	<7.8
Impaired Glucose Tolerance	<7.0	7.8 - 11.0
Diabetes Mellitus	≥7.0	≥11.1

According to NICE NG3 (Diabetes in pregnancy: management from preconception to the postnatal period), gestational diabetes is diagnosed if a woman has either:

- Fasting plasma glucose ≥5.6 mmol/L
- 2-hour plasma glucose ≥7.8 mmol/L

References

- WHO Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia (2006)
- NICE NG3 Diabetes in pregnancy: management from preconception to the postnatal period (published 2015, last updated 2020)
- Wiener K (1990). What is 75g of glucose? Ann Clin Bioc 27: 283-284

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10.5.3 Laboratory Investigation of Unexplained Hypoglycaemia in Neonates and Children

Specific information in respect of when to conduct investigations and correcting glucose levels should be sought from the *relevant local clinical protocol*. In all cases seek advice from an experienced paediatrician.

Relevant scenarios include:

- Newborn with persistent (x3) glucose tests <2.0mmol/L within the first 48hr
- Severe hypoglycaemia – glucose <1.0 mmol/L at any time
- Infants with unexplained glucose of less than 2.6 mmol/L

Sample collection:

- Unless stated, samples must be collected prior to correction of glucose but avoid prolonging hypoglycaemic episode.
- Inform the blood sciences laboratory at the time of sample collection so provision can be made for analysis or preserving the samples.

Initial sample requirements (prior to correction of hypoglycaemia)

Test	Sample Volume	Sample Bottle	Other Instructions
Blood gas	0.2 mL	Capillary	Check glucose & lactate
Glucose Lactate Intermediary metabolites	1.5 mL	1x grey Fl EDTA TUBE	To lab within 30 min
Insulin & C-peptide	1.3 mL	1x green top Li Hep (Or yellow gel)	To lab within 30 min
UE, LFT, Cortisol & GH	1.3 mL	1x green top Li Hep (Or yellow gel)	
Plasma Amino Acids	1.3 mL	1x green top Li Hep	To lab within 30 min
Acyl carnitines	1.3 mL	1x green top Li Hep	

Samples to follow (if not collected with above)

Ammonia	1.3 mL	1x purple EDTA	To lab within 15 min.
Urine Organic Acids & Urine Amino Acids	10-20 mL	Fresh urine – next passed white top universal	Record dipstix (? ketones)
Blood Culture	0.5 mL	Culture Bottle	As routine

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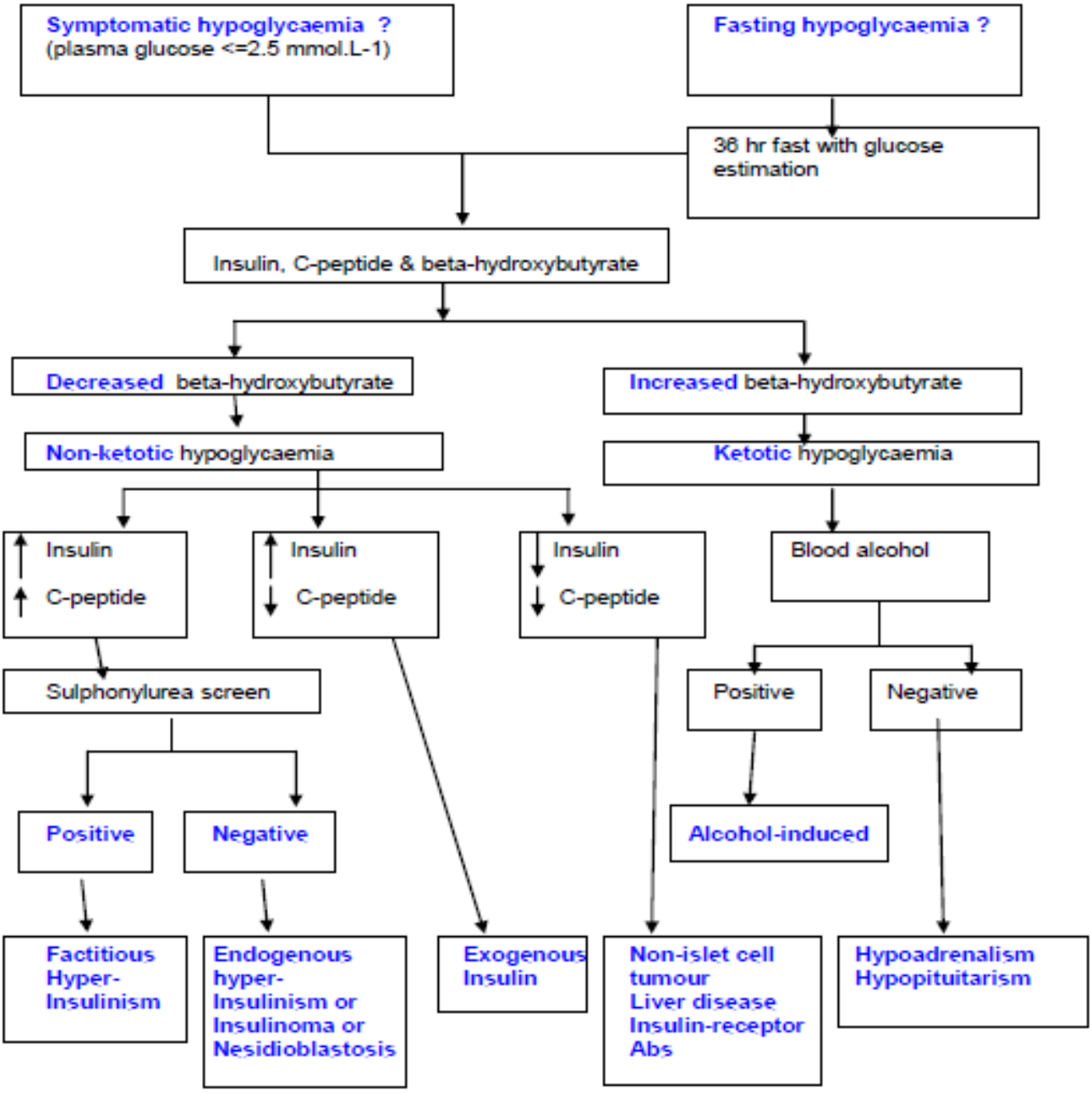
10.5.4 Laboratory Investigation of Unexplained Hypoglycaemia in Adults

- Acute hypoglycaemia may present with adrenergic symptoms, such as sweating, palpitation and tremor, or neuroglycopaenic symptoms such as cognitive impairment and ataxia.
- Symptoms occur with plasma glucose <2.5 mmol/L and resolve with glucose administration. The aim in these patients is to take appropriate samples for insulin/pro-insulin and other analytes in the presence of hypoglycaemia.
- After excluding iatrogenic hypoglycaemia in patients with diabetes, the commonest cause of acute hypoglycaemia is alcohol intoxication.
- More frequently patients present sub acutely with episodes of cognitive impairment or pre-syncopal symptoms, which resolve with carbohydrate ingestion. In these patients a 36 hr fast is probably as effective at eliciting evidence of hypoglycaemia as the traditional 72 hr fast, using a blood glucose < 3mmol/L as diagnostic evidence and adding ketone body estimation to the diagnostic evaluation.
- See the attached algorithm for assessing hypoglycaemia, which analytes to measure and when, and their interpretation.



10.5.5 Prolonged 36hr Fast for the Investigation of Hypoglycaemia

Precautions/Contraindications

Patients with insulinomas or other causes of hypoglycaemia may die from hypoglycaemia. The patient should therefore have an indwelling venous cannula throughout the test and be under continuous observation. 25 ml 25% dextrose should be drawn up so it may be administered as required.

Patient preparation

- Initial investigations prior to doing the extended fast should include U&E, LFT, 9am cortisol and TFT.
- The patient must have had an adequate carbohydrate intake for at least 3 days prior to the test. For most, this will simply mean eating their normal mixed diet. Patients on calorie-restricted diets for e.g., losing weight should resume a "normal" diet.
- On the day before the test the patient should fast overnight after the early evening meal. **This means NO food intake after rising on the morning of the test or until after the test is complete.** The patient is then admitted for observation under strict medical supervision and be prepared to fast for up to 36 hrs.
- Only water is allowed at any time during the test (**ALL** other beverages should be avoided, including orange juice & milk of any description), they **must not smoke or eat** during the test and should remain at rest.
- Note those patients with insulinoma or non-islet cell tumours will usually have their hypoglycaemia unmasked/provoked within this time scale.

Ward/Phlebotomist requirements

Grey-top (fluoride) blood bottles (for glucose and beta-hydroxybutyrate)
Ochre-top (plain) blood bottles (for insulin and C-peptide)

Procedure

1) Start the fast after an evening meal at 10.00hr and insert an IV cannula.
2) Take 1 grey-top (fluoride) blood tube for **glucose and beta-hydroxybutyrate**, and 1 ochre-top (plain) blood tube for **insulin and C-peptide**.
   Hand-label all samples appropriately, ensure time of collection is noted. Samples for insulin and C-peptide must be taken to the laboratory for immediate separation (contact the on-call personnel if required).
3) Continue to take samples for glucose (grey-top fluoride) and insulin and C-peptide (ochre-top plain) every 2 hours during the **day**, and whenever they are symptomatic. Note blood glucose can be monitored throughout the test using a glucose meter but simultaneous samples must also be sent to the lab. Continue for up to 36 hours.
4) The test should be terminated if the plasma glucose is persistently below 2.5 mmol/L on samples analysed in the laboratory, or if the patient's consciousness level deteriorates.
5) The test may be terminated by giving 10% dextrose IV and a substantial meal.

Procedure Variation

A small number of patients with insulinoma show only **post-prandial** hypoglycaemia. In this case the procedure is:

1) Patient should be admitted for an overnight fast.
2) Before breakfast, insert an IV cannula and take 1 grey-top (fluoride) blood tube for **glucose and beta-hydroxybutyrate**, and 1 ochre-top (plain) blood tube for **insulin and C-peptide**.
3) Thereafter, take samples hourly for the next 6 hours for glucose, insulin, C-peptide and beta-hydroxybutyrate. **Water only** is allowed until the test is completed or there is laboratory evidence of hypoglycaemia.

Interpretation

- *Plasma glucose:* In men this should not fall to <2.5 mmol/L. In normal women the plasma glucose has been observed to fall below 2.0 mmol/L during a prolonged fast.
- *Insulin and C-peptide:* Fasting Insulin and C-peptide levels are very assay-dependent and will be interpreted by the SAS Regional centre providing them. Note that they *may* be considerably increased in obesity and states of insulin resistance.
- Most patients having tumour-related hypoglycaemia will become symptomatic during the fasting period and longer fasting periods are usually unnecessary.

References

- Barth J H, Butler G E and P Hammond (2011). Biochemical Investigations in Laboratory Medicine. ACB Venture Publications, and at http://www.pathology.leedsth.nhs.uk/dnn_bilm/
- Besser M, Ed (1992): Clinical Endocrinology. Blackwell Scientific Publications Teale, DJ (SAS Laboratory, Guildford). Personal communication, 210801.
- V Marks (1992). Insulinomas and hypoglycaemia. In Clinical Endocrinology, Grossman A (Ed). Blackwell Scientific Publications.

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10.5.6 Short Synacthen Test

Intended as an initial screen for suspected hypoadrenalism in the non-critically ill patient.

Precautions/Contraindications

- Very severe hypersensitivity reactions to Synacthen have been reported, particularly in children with a history of allergic disorders. Patients having been given Synacthen should be kept under medical observation for at least one hour.
- The short Synacthen test gives unreliable results in the two weeks following pituitary surgery.
- It should be noted that prednisolone cross-reacts with cortisol assays.

Patient preparation

- The test does not require hospital admission.
- The test should be carried out starting at 10.00h *whenever possible*, as the response may vary by as much as 100 nmol/L at 30 min post Synacthen between morning and late afternoon.
- The patient does not need to be fasted.

Ward/Phlebotomist requirements

Adults: 250 micrograms of Synacthen (tetracosactrin - synthetic ACTH)
Children: 36 microgram/kg body weight up to a maximum of 250 microgram
Ochre-top (plain) blood bottles

Protocol

1) Take an initial blood sample for basal serum cortisol.
2) Inject 250 microgram of Synacthen intra-muscularly.
3) Take a further blood sample at **30 minutes post-synacthen** for cortisol.

Interpretation

Interpretation is based upon a method-specific threshold for the Roche Cobas Cortisol (Gen II) method, which correlates closely with mass spectrometry.

- Peak values usually >450 nmol/L at 30 minutes **post-Synacthen but the threshold is increased to 580nmol/L in women taking oral contraception. HRT will also increase the target threshold**.
- In ACTH deficiency the response may be normal or reduced.
- The response to Synacthen is not affected by obesity.
- There is no difference between IV and IM administration.

Sensitivity and specificity

- There are reports of patients with incipient adrenal failure having normal responses to Synacthen. The use of physiological doses e.g., 5 microgram may prove more useful at determining those subjects with poor responses to conventional (250 microgram) pharmacological doses.
- Beware acutely ill, stressed patients in whom basal cortisol may be raised in the high hundreds or sometimes higher and who's post-Synacthen response is blunted with only a small rise. Such cases are extremely difficult to interpret, and the test should ideally be repeated when the patient is convalescing.
- Patients who have been on long term steroids are likely to have a blunted response to Synacthen. It can take several months (up to a year) for adrenals to fully recover after long term steroids.

References

- Barth J H, Butler G E and P Hammond (2011). Biochemical Investigations in Laboratory Medicine. ACB Venture Publications, and at http://www.pathology.leedsth.nhs.uk/dnn_bilm/
- Besser M, Ed (1992): Clinical Endocrinology. Blackwell Scientific Publications.
- Clayton RN (1997). Assessment of the hypothalamo-pituitary-adrenal axis - is the insulin stress test obsolete? Proc ACB Nat Mtg May 1997.
- El-Farhan N, Pickett A, Ducroq D, et al. Determination of method-specific normal cortisol response to the short Synacthen test. Ann Clin Biochem 2011; 48(S1): 73.
- Patel RB, Selby C, Jeffcoate W (1991). The short synacthen test in acute hospital admissions. Clin Endo 35: 259-61.

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10.5.7 Long Synacthen Test (Depot)

For the investigation & diagnosis of suspected hypoadrenalism in those showing an inadequate response to a short Synacthen test. The availability of ACTH assays may now make this test protocol redundant.

Patient preparation

- The test should be carried out starting at 10.00h *whenever possible*, as the response may vary by as much as 100 nmol/L at 30 min post Synacthen between morning and late afternoon.
- The patient does not need to be fasted.

Precautions/Contraindications

- Very severe hypersensitivity reactions to Synacthen have been reported, particularly in children with a history of allergic disorders. Patients having been given Synacthen should be kept under medical observation for at least one hour.
- The short Synacthen test gives unreliable results in the two weeks following pituitary surgery.
- It should be noted that prednisolone cross-reacts with cortisol assays.
- Patients already taking corticosteroids should have been taking them for less than 2 weeks and should be switched to dexamethasone 24h before the test.
- Patients who have been on long term steroids are likely to have a blunted response to Synacthen. It can take several months (up to a year) for adrenal to fully recover after long term steroids.

Ward/Phlebotomist requirements

Synacthen depot (1 mg)
Ochre-top (plain) blood bottles

Protocol

1) Take an initial blood sample for basal serum cortisol.
2) Immediately inject 1 mg Depot Synacthen IM.
3) Take further samples at **1, 2, 4, 8 and 24h** post-Synacthen for cortisol.

Interpretation

The only published target data for a satisfactory response is rather old and not referable to our method. Given our lower cut-off for a short Synacthen test is about 25% lower than the widely used figure of 600, indicative thresholds derived from the commonly published data suggest the following approximations:

1 hr cortisol	>450 nmol/L
2 hr cortisol	>562 nmol/L
4 hr cortisol	>720 nmol/L
8 hr cortisol	>769 nmol/L
24 hr cortisol	>458 nmol/L

References

- Barth J H, Butler G E and P Hammond (2011). Biochemical Investigations in Laboratory Medicine. ACB Venture Publications, and at http://www.pathology.leedsth.nhs.uk/dnn_bilm/
- Besser M, Ed (1992): Clinical Endocrinology. Blackwell Scientific Publications. Ismail AAA (1981): Biochemical investigations in Endocrinology. Academic Press

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10.5.8 Overnight Dexamethasone Suppression Test (1mg)

To be used as a first line screening test in patients found to have endogenous hypercortisolism or suspected on clinical grounds of having Cushing's syndrome. This is one of the most difficult problems in clinical endocrinology. It is frequently suspected but rarely diagnosed. There are two phases: the diagnosis of hypercortisolism and secondly the localisation of the source. For the former, the suggested first-line screens are an overnight dexamethasone suppression test with/without 24-hour urinary cortisol. This has a relatively high false positive rate but very low false negative.

If the diagnosis is strongly suspected and the screening tests are negative, the diagnosis should not be discounted as there are well recorded cases of cyclical Cushing's disease with episodes of clinical and biochemical normality between episodes of typical clinical and biochemical disease.

Diagnostic tests for Cushing's disease:

	Sensitivity	Specificity
Urinary Free Cortisol (UFC)	95-100%	98%
1 mg Dexamethasone Suppression Test	98-100%	80%

Precautions/Contraindications

- Patients on enzyme inducing drugs e.g., anticonvulsants and rifampicin, may rapidly metabolise dexamethasone and give a false positive result, i.e., no suppression.
- Women on oestrogen therapy may fail to suppress adequately due to increased cortisol binding globulin. In these instances, a higher dose of dexamethasone should be used (2mg) or the COCP/HRT should be stopped for 6 weeks beforehand.

Patient preparation

No preparation of the patient is required.

Ward/Phlebotomist requirements

1 mg dexamethasone tablet (the dose for children is 15 micrograms/kg body weight)
Ochre-top (plain) blood bottles

Protocol

1) Give 1.0 mg dexamethasone orally between 22.00 & 23.00 hrs
2) Take a blood sample (ochre) between 08.00 & 09.00 hrs the next morning for serum cortisol**. Please ensure request form is clearly marked as "Overnight dexamethasone suppression test".**

Interpretation

Serum cortisol normally suppresses to <50 nmol/L. Normal subjects rarely (<2%) fail to suppress with overnight dexamethasone.

	False Positives	**False Negatives**
Overnight dexamethasone suppression test	1. Depression 2. Severe systemic illness 3. Renal failure on dialysis 4. Chronic alcohol abuse 5. Old age 6. Anorexia nervosa 7. Hepatic enzyme inducing drugs e.g., rifampicin, phenytoin 10. Drugs which increase CBG e.g., HRT, OCP, tamoxifen	Very rarely in patients with Cushing's disease – if clinical suspicion is high continue investigating!
24h urine free cortisol	1. Physical stress e.g., trauma, exercise & malnutrition 2. Mental stress e.g., depression, alcohol, or drug abuse/withdrawal 3. Metabolic e.g., raised CBG, glucocorticoid resistance, complicated diabetes	Renal failure

References

- Barth J H, Butler G E and P Hammond (2011). Biochemical Investigations in Laboratory Medicine. ACB Venture Publications, and at http://www.pathology.leedsth.nhs.uk/dnn_bilm/
- Honour JW (1994): The investigation of adrenocortical disorders. JIFCC 6, 154.
- Marshall WJ and Bangert SK, Eds (1995): Clinical Biochemistry, metabolic and clinical aspects, Churchill Livingstone.

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10.5.9 Low Dose Dexamethasone Suppression Test (0.5 mg, 3 day)

Used to establish the diagnosis of Cushing's syndrome. If performed as an inpatient it should not be started until 48 hr after admission, avoid all forms of stress and ensure that the patient has had adequate and normal sleep. Alternatively, it can be performed as an outpatient with careful patient selection and instruction. There appears to be no advantage in discrimination between 1mg and 1.5mg or 2mg. Although higher doses have been tried, the increased suppression in some patients with Cushing's syndrome significantly decreases the sensitivity of the test. Overall, both the original 2-day test and the overnight protocol appear to have comparable sensitivities (98-100%); however, with an appropriate cut-off the specificity is greater for the 2-day test (97-100%) compared to the overnight test (88%). A post-dexamethasone serum cortisol of <50 nmol/L has been proposed as providing the greatest sensitivity in the era of modern cortisol assays. In the series published by Morris & Ashman (see references) using the 2-day protocol in 150 patients with proven Cushing's syndrome, a cut-off serum cortisol of 50nmol/l resulted in a sensitivity of 98%.

Precautions/Contraindications

- Patients on enzyme inducing drugs e.g., anticonvulsants and rifampicin, may rapidly metabolise dexamethasone and give a false positive result, i.e., no suppression.
- Women on oestrogen therapy may fail to suppress adequately due to increased cortisol binding globulin. In these instances, a higher dose of dexamethasone should be used (2mg) or the COCP/HRT should be stopped for 6 weeks beforehand.
- Exercise caution in patients with diabetes mellitus and those who with heart failure, peptic ulcer, hypertension, hypomania, and depression.

Patient preparation

No preparation of the patient is required.

Ward/Phlebotomist requirements

Ochre-top (plain) blood bottles (for cortisol)
Purple EDTA blood bottles (for ACTH)
8 x 0.5 mg dexamethasone tablets (the dose for children is 15 microgram/kg body weight)

Protocol

DAY 1 08.30–09.00hrs: take samples for cortisol and ACTH. Give 0.5 mg dexamethasone orally.
Give 0.5 mg dexamethasone orally at **15.00h** and **21.00h**
DAY 2 Give 0.5 mg dexamethasone orally at **03.00h**, **09.00h**, **15.00h** and **21.00h**
DAY 3 Give 0.5 mg dexamethasone orally at **03.00h**
08.30–09.00hrs: take samples for cortisol and ACTH.

Interpretation

- Cortisol should suppress to <50 nmol/L after 48 hrs of dexamethasone. Note that testosterone and other androgens will also fall post-dexamethasone.
- Factors such as variable absorption and increased metabolism can influence dexamethasone test results. A history of symptoms of malabsorption and a careful drug history should be taken prior to using the test in a patient.
- Measurement of serum dexamethasone should be reserved for cases of suspected malabsorption as the assay is not widely available. Another solution is to use one of the published intravenous dexamethasone suppression tests.
- Hepatic enzyme inducers such as carbamazepine, phenytoin, phenobarbitone and rifampicin will reduce plasma dexamethasone concentrations, and will usually render the test uninterpretable.

References

- Roche Diagnostics COBAS 8000 Modular analysers: Cortisol Gen II data sheet (May 2015), Ref 06687733/ 190
- Barth J H, Butler G E and P Hammond (2011). Biochemical Investigations in Laboratory Medicine. ACB Venture Publications, and at http://www.pathology.leedsth.nhs.uk/dnn_bilm/
- Besser M, ed (1992): Clinical Endocrinology. Blackwell Sci. Pubns.
- Marshall WJ and Bangert SK, eds (1995): Clinical Biochemistry, metabolic and clinical aspects, Churchill Livingstone
- Morris DB and AB Grossman (2002).
- Cushing's syndrome *in* Endotext.org: Your Endocrine Source at http://www.endotext.com/neuroendo/neuroendo7 /neuroendoframe7.htm
- Evidence for The Low Dose Dexamethasone Suppression Test to Screen for Cushing's Syndrome - Recommendations for a Protocol for Biochemistry Laboratories. Annal Clin Biochem 1997; 34: 222 - 2210.

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10.5.10 High Dose Dexamethasone Suppression Test (2 mg)

To investigate further the differential diagnosis of patients with confirmed Cushing's syndrome. However, inadequate suppression can be seen in the ectopic ACTH syndrome, & since the advent of sensitive ACTH assays & adrenal imaging its main role has been the differentiation between the causes of ACTH-dependent Cushing's syndrome. The sensitivity and specificity of the test for the diagnosis of Cushing's disease versus the ectopic ACTH syndrome is 80-88% and 88-100% respectively.

Precautions/Contraindications

- Patients on enzyme inducing drugs e.g., anticonvulsants and rifampicin, may rapidly metabolise dexamethasone and give a false positive result, i.e., no suppression.
- Women on oestrogens may fail to suppress adequately due to increased cortisol binding globulin. In these instances, a higher dose of dexamethasone should be used (2mg) or the COCP/HRT should be stopped for 6 weeks beforehand.
- In diabetics, note that high dose dexamethasone may adversely affect glucose control during the test. Exercise caution in patients with diabetes mellitus and those who with heart failure, peptic ulcer, hypertension, hypomania, and depression

Patient preparation

No preparation of the patient is required but should only be performed as an in-patient.

Ward/Phlebotomist requirements

Ochre-top (plain) blood bottles (for cortisol)
Purple EDTA blood bottles (for ACTH)
16 x 1 mg dexamethasone tablets (the dose for children is 15 microgram/kg body weight)

Protocol

DAY 1 08.30–09.00 hrs: take samples for cortisol and ACTH, then give 2.0 mg dexamethasone orally.
Give 2.0 mg dexamethasone orally at each of **15.00h** & **21.00h**
DAY 2 Give 2.0 mg dexamethasone orally at each of **03.00h**, **09.00h**, **15.00h** and **21.00h**
DAY 3 Give 2.0 mg dexamethasone orally at **03.00h**
08.30–09.00 hrs: take samples for cortisol and ACTH.

Interpretation

Cortisol should classically suppress to 50% or less of the basal value in Cushing's disease (i.e., pituitary-dependent Cushing's), but not in ectopic ACTH secretion or adrenal carcinoma. However, there are significant exceptions and about 20% of those with Cushing's disease fail to suppress.

References

- Barth J H, Butler G E and P Hammond (2011). Biochemical Investigations in Laboratory Medicine. ACB Venture Publications, and at http://www.pathology.leedsth.nhs.uk/dnn_bilm/
- Honour JW (1994): The investigation of adrenocortical disorders. JIFCC 6, 154.
- Morris DB and AB Grossman (2002). Cushing's syndrome *in* Endotext.org :Your Endocrine Source at http://www.endotext.com/neuroendo/neuroendo7 /neuroendoframe7.htm
- Marshall WJ and Bangert SK, Eds (1995): Clinical Biochemistry, metabolic and clinical aspects, Churchill Livingstone.

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10.5.11 Cortisol Day Curve

Traditional hydrocortisone replacement utilised twice daily dosing, but many patients report fatigue or headache in the afternoon on this regimen. There is evidence that many patients 'feel better' on thrice daily regimes. The average daily requirement is approximately 20mg of hydrocortisone. This should be given as 10 mg on waking, 5 mg at lunchtime and 5 mg in the early evening. Enzyme-inducing drugs (especially, phenytoin, carbamazepine, and rifampicin) will increase metabolism of corticosteroids and should prompt an increment in replacement doses. Doses are therefore fine-tuned according to patient well-being and effectiveness of replacement as assessed by multiple serum cortisol levels (day curve, CDC) taken during the day. The test has not been fully evaluated but the following is a protocol that has proved useful.

Precautions

The results cannot be interpreted in patients on oestrogens.

Patient preparation

The patient should delay taking their morning dose of hydrocortisone until they arrive.

Ward/Phlebotomist requirements

Ochre-top (plain) blood bottles (for cortisol)

Procedure

The test will normally begin at approximately 10.00 h.

1) Take a basal blood sample for cortisol and **label** appropriately **including the time of day**.
2) Give the morning dose of hydrocortisone (include the time and dose information on the request form).
3) **Other hydrocortisone doses should be taken at the usual times.** Clearly indicate each time and dose on the request form.
4) Take further samples for cortisol at 11.00, 13.00, 15.00 & 17.00 h.

Interpretation

- The aim is to have adequate circulating levels of cortisol throughout the day (except for the basal sample, which is usually <50 nmol/L), whilst avoiding excessive peaks after each dose. The peak level after a dose should not exceed 900 nmol/L and the trough level before the next dose should not be less than 100 nmol/L. Frequently a mid-day dose is required to avoid excess levels after the morning dose but sufficient levels to provide adequate cover until the evening.
- Note: Literature-derived target ranges may be inaccurate due to the variation in cortisol assays. Discuss with the local Consultant Biochemist if in doubt.

References

- Chung T-T and JP Monson (2006) *in* Endotext.org: Your Endocrine Source at: http: //www.endotext.com/neuroendo/ neuroendo12/neuroendo12.htm
- Besser M, Ed (1992): Clinical Endocrinology. Blackwell Scientific Publications

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10.5.12 Growth Hormone Stimulation Test (Arginine Protocol)

Under NICE Guidelines, growth hormone (GH) deficiency must be demonstrated on two dynamic stimulation tests. Arginine stimulation tests are quick to carry out and can be administered directly after a glucagon stimulation test has finished.

Precautions/Contraindications

- Hypothyroidism impairs GH response (check thyroid function before arginine stimulation)
- Arginine can cause nausea and vomiting has been described in few patients.
- Irritation can occur at the infusion site.

Patient preparation

- The child should be fasted for 8 hours before starting the test, with only water to drink.
- If a glucagon stimulation test is being performed as well, the arginine infusion should be started **once the last sample from the glucagon test has been taken**.
- If combined pituitary function tests are being performed, Synacthen can be administered once the arginine infusion has finished.

Ward/Phlebotomist requirements

Arginine for intravenous infusion (supplied as a 10% solution of arginine monochloride in 0.9% sodium chloride), 0.5g/kg body weight up to a maximum of 30g.
Ochre-top (plain) blood bottles (for GH)
Grey-top (fluoride) blood bottles (for glucose)

Protocol

1) Take a basal blood for GH (t = 0)
2) Give arginine (0.5g/kg) via IV infusion over 30 minutes.
3) Take samples for GH at end of the infusion (i.e., t = 30 min) and then at t = 45 min and t = 60 min. Ensure that all samples are CLEARLY labelled with time & patient details
4) In children with suspected hypopituitarism, blood glucose samples should also be taken at each timepoint as in the glucagon stimulation test.
5) Children should be allowed to eat and drink as normal once the test is complete, ensure that the blood glucose is normal, and that the child has eaten before discharge.

Interpretation

Interpretation is based upon cut-offs stated in the Imperial Centre for Endocrinology Endocrine bible:

- A normal GH response of >5.7 micrograms/L excludes GH deficiency.
- A GH response of 2.7 – 5.7 micrograms/L may indicate partial GH deficiency and should be investigated by a second forma stimulation test.
- A GH response of <2.7 micrograms/L should also generally be confirmed by a second test.
- A child with pubertal growth delay may show a subnormal GH response if the test is performed without sex hormone priming, however there should be a normal response after priming.

Sensitivity and Specificity

The % of children who are not GH deficient and who show a normal response varies from 45-93%. Generally, 20% of normal children fail to respond to a formal test and this is the reason for doing 2 tests before proceeding to GH therapy. For example, 71% of normal children will respond to both insulin tolerance and arginine stimulation tests. However, the others will respond to one of the tests (13% to insulin only, 16% to arginine only).

References

- Brook's Clinical Paediatric Endocrinology, 5th Edition 2005
- ICH Endocrinology Handbook http://www.imperialendo.com/for-doctors/endocrine-bible

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10.5.13 Growth Hormone Stimulation Test (Clonidine Protocol)

For the investigation of growth hormone (GH) secretion in children of short stature. Note that, as in other provocative tests of GH secretion, up to 25% of these tests may be negative in normal children.

Patient preparation

- Children who are pre-/peri-pubertal (aged 10 yrs or younger for girls, 11 yrs or younger for boys) will not respond to provocative tests of GH release unless primed beforehand with the appropriate sex steroid:
- Priming with sex steroids is recommended in pre-pubertal children who are over 10 years of age (either chronological or bone age). Prescribe stilboestrol 1mg 12 hourly for 48 hours prior to test.
- This test must be performed fasting and, in the morning, (at least 4 hours after any food and drink - water is permitted).

Ward/Phlebotomist requirements

- It is recommended that the patient be fitted with a butterfly for blood sampling.
- The weight (kg) & height (m) of the child should be known to calculate their surface area (square metres).
- Clonidine, to be given orally at a dose of 0.15 mg/square metre. **Note that Clonidine may cause hypotension in some patients.**
- Ochre-top (plain) blood bottles (for GH)

Protocol

1) Start the test at 0830-0900 hrs & take a basal blood for growth hormone (GH) into a plain tube.
2) Give oral clonidine at dose calculated (above).
3) Take further samples for GH at **30, 60, 90-, 120-, 150- & 180-min** post-clonidine.

Interpretation

- Peak GH response is usually seen at 90 min post-clonidine, but interpretation is fraught with problems and as many as 30% of normal children may not respond “normally”.
- Normal, “positive” responders usually show a peak of >6.7 micrograms/L (ug/L) post-clonidine.

References

- Barth J H, Butler G E and P Hammond (2011). Biochemical Investigations in Laboratory Medicine. ACB Venture Publications, and at http://www.pathology.leedsth.nhs.uk/dnn_bilm/
- Besser M, ed (1992) Clinical Endocrinology. Blackwell Scientific Publications
- Burtis CA and Ashwood ER, Eds (1994): Tietz textbook of Clinical Chemistry. Saunders.
- (Dr P Wood, SAS Endocrine Laboratory, Southampton gave the recommendations for the pre-stimulation steroid doses)
- Evans C, Gregory JW (2004). All Wales Clin Biochem audit group. J Clin Pathol 2004; 57:126-130

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10.5.14 Oral Glucose Tolerance Test (GH Suppression Test) for Acromegaly

Initial investigations: Acromegaly may be initially investigated as an outpatient by a sample taken about 2 hours after food. Request growth hormone (GH) and insulin-like growth factor (IGF-1) (ochre cap bottle). Feeding should suppress growth hormone therefore a result of <1 microgram (ug/L) on a sample taken 2 hours after eating virtually excludes acromegaly. Alternatively, IGF-1 can be used as a screening test for acromegaly and can be requested at any time during the day. However, if there is a strong index of clinical suspicion, a full suppression test should be undertaken.

Patient preparation

- The patient should have been on adequate carbohydrate intake for at least 3 days before the test. If the patient has been calorie-restricted for any reason, a "normal" mixed diet should have been resumed for at least 3 days beforehand.
- The patient should fast overnight & remain at rest before and during the test; refrain from smoking & eating; refrain from drinking anything except water during the test.

Ward/Phlebotomist requirements

250-ml carton of GlucosePro is obtainable from local Pathology department. (Do **not** use Lucozade).
Polycal (in liquid form) can be used as an alternative. Polycal can be obtained via hospital pharmacies at short notice. One hundred and thirteen (113mL) mL contain an equivalent of 75g of glucose - measure 113 mL into a beaker and add water to make the volume up to 200ml (mix well). This is drunk over a 5 min period followed by a further 100mL of water. Note that rapid consumption may cause transient abdominal discomfort. Otherwise, the test is done exactly as you would use GlucosePro.
Ochre-top (plain) blood bottles (for GH and IGF-1)
Grey top fluoride bottles (for glucose)

Protocol

1) Collect baseline (time = 0 min) samples for glucose, GH, and IGF-1.
2) Allow them to drink the GlucosePro/Polycal slowly over about 5 minutes.
3) Collect samples for glucose and GH at 30, 60, 90 and 120 mins.

Interpretation

Interpretation is based upon cut-offs stated in the Imperial Centre for Endocrinology Endocrine bible:

- Growth hormone should be suppressed by glucose administration to <1 microgram/L (ug/L) and Growth Hormone concentrations remaining above 1 microgram/L (ug/L) during the test are highly suggestive of acromegaly.
- Patients with acromegaly might fail to show complete suppression and may show a paradoxical rise.
- Failure to suppress in the absence of acromegaly may be seen in normal adolescence, liver disease, poorly controlled diabetes, renal failure, malnutrition, and Laron-type dwarfism.

References

- IGF-1 measurement in diagnosis and management of Acromegaly. Ann Clin Biochem 2001; 38:297-303. Clinical Biochemistry, metabolic and clinical aspects. Marshall and Bangert 1995.
- Barth J H, Butler G E and P Hammond (2011). Biochemical Investigations in Laboratory Medicine. ACB Venture Publications, and at http://www.pathology.leedsth.nhs.uk/dnn_bilm/
- Barth JH & PEC Sibley (2008). Standardisation of the IMMULITE systems Growth Hormone assay with the recombinant IS 98/574. Annal Clin Biochem *in litt*.
- Besser M, ed (1992): Clinical Endocrinology. Blackwell Scientific Publications
- Marshall WJ and Bangert SK, Eds (1995): Clinical Biochemistry, metabolic and clinical aspects, Churchill Livingstone.
- Colao A and G Lombardi (2008). Should We Still Use Glucose-Suppressed Growth Hormone Levels for the Evaluation of Acromegaly? JCEM 93(4): 1181-2.
- Giustina A, Barkan A *et al* (2000). Criteria for Cure of Acromegaly: A Consensus Statement. JCEM 85: 526-5210.

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10.5.15 Growth Hormone Day Curve

In patients with acromegaly, it can be used as an indicator of degree of cure after pituitary surgery, and as early as day 4 post-op. Note that IGF-1 may also be used, but is not reliable until 6 weeks post op.

Patient preparation
The patient should be off somatostatin analogues.

Ward/Phlebotomist requirements
Ochre-top (plain) blood bottles (for GH and IGF-1)

Procedure
1) The test will normally begin at approximately 09.30 h.
2) Insert an indwelling catheter and wait 15 minutes before taking the first sample at 10.00 h.
3) Take a basal blood sample for GH and IGF-1, and **label with date and time of day**.
4) Take further samples for GH at 11.00, 13.00, 15.00 & 17.00 h.

Interpretation
- A curative procedure is mean GH of <1.7 ug/L (from the 5 samples taken)
- Mean GH <1.7 ug/L and nadir <0.6 ug/L on OGTT but IGF-1 >ULN – consider medical treatment.
- Mean GH >1.7 ug/L and/or nadir >0.6 ug/L on OGTT with IGF-1 in upper half of reference range – consider medical treatment.
- Mean GH >1.7ug/L and nadir >0.6 ug/L on OGTT and IGF-1 >ULN – further treatment required, consider surgical referral.
- If GH is undetectable on day curve and IGF-1 within normal range at 6 weeks post op, then annual random GH and IGF-1 should be sufficient for follow-up.

References
ICH Endocrinology Handbook http://www.imperialendo.co.uk/Bible2010v1.htm

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10.5.16 TRH Stimulation Test

The development of TSH assays capable of accurate measurements <0.01 mU/L has obviated the need for the TRH test in most cases of thyroid practice except perhaps in the differential diagnosis of TSHoma and thyroid hormone resistance (high TSH and high thyroxine).

Precautions/Contraindications

- In view of its postulated action on smooth muscle, the test should be used with caution in those with bronchial asthma, other types of obstructive airway disease & myocardial ischaemia.
- The TRH test should **not** be used in pregnant women.
- The test is usually well tolerated in other patients, but side effects may include nausea, facial flushing, an urge to urinate or defaecate, slight dizziness & altered taste sensations. It is advisable therefore to always have medical supervision.

Patient preparation

None, although patients should remain at rest throughout & refrain from smoking.

Ward/Phlebotomist requirements

TRH for intra-venous injection (200 **micro**gram bolus) In children, give 7 microgram/kg body weight up to 200 micrograms.
Ochre-top (plain) blood bottles.

Protocol

1) Take a basal blood for TSH. Immediately give the TRH by IV injection as a single bolus.
2) Take further ochre top (plain) bloods for TSH at 20 & 60 minutes after the injection. Ensure that all samples are clearlylabelled with time & full patient details.

Interpretation

Interpretation is based upon cut-offs stated in Biochemical Investigations in Laboratory Medicine (2011).

- Normal basal TSH values should be 0.4–4.5 mU/L (this is the adult reference range for TSH).
- The normal increment in TSH at 20 min should be 5–30 mU/L with a slight fall from the peak at 60 min.
- An exaggerated TSH response is seen in primary hypothyroidism.
- A peak response less than 5 mU/L is seen in primary hyperthyroidism, but also in some apparently euthyroid patients with ophthalmic Graves' disease or multi-nodular goitre.
- Some patients with hypothalamic dysfunction show a delayed response and a peak at 60 min, but this is not reliable.
- The TSH response is **reduced** by glucocorticoids, dopamine agonists (e.g., 1-DOPA, bromocriptine and fluoxetine) and enhanced by dopamine antagonists (e.g., metoclopramide, oestrogens, theophylline, and sertraline)
- In neonates, peak TSH responses <35 mU/L are not associated with subsequent hypothyroidism, whereas responses >35 mU/L are associated with a rate of subsequent hypothyroidism of 35%.
- The TSH response is flat in most cases of TSHoma whereas in thyroid hormone resistance the TSH response is brisk.

References

- Barth J H, Butler G E and P Hammond (2011). Biochemical Investigations in Laboratory Medicine. ACB Venture Publications, and at http://www.pathology.leedsth.nhs.uk/dnn_bilm/
- Marshall WJ and Bangert SK, Eds (1995): Clinical Biochemistry, metabolic and clinical aspects, Churchill Livingstone.

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10.5.17 GnRH Stimulation Test

GnRH stimulates gonadotrophin release by the pituitary, with the magnitude of LH release being greater than that of FSH. The test is intended to diagnose hypothalamic-pituitary disease in precocious and delayed puberty in both sexes in children with low basal gonadotrophin concentrations. It does not reliably differentiate between pituitary & hypophyseal causes of gonadotrophin deficiency. As such the test should be restricted to very specific cases where other tests have been inconclusive.

Note: This test may be combined with TRH and glucagon stimulation test or insulin stress test as part of a triple pituitary function test if required.

Patient preparation

Prepare the patient on the morning of the test with an in-dwelling venous needle.
The test lasts for approximately 3 hours.

Ward/Phlebotomist requirements

GnRH @ 2.5 micrograms/kg body weight to a maximum of 100 micrograms for I.V. injection
Ochre-top (plain) blood bottles (for FSH & LH)
Weighing scales

Protocol

1) After inserting the I.V. cannula take a blood sample for FSH and LH. Label with time and date.
2) Immediately afterwards, inject the GnRH over 30 seconds.
3) Take further samples for FSH and LH at **20 and 60 minutes after** GnRH.

Interpretation

Interpretation is based upon cut-offs stated in Biochemical Investigations in Laboratory Medicine (2011).

- Normal basal reference values in pre-pubertal children for both FSH and LH are <2.0 IU/L.
- After GnRH, LH may peak at either 20 or 60 min and show at least a doubling of values compared with basal. FSH response is very variable, with 15% showing no rise.
- Note that the response will vary throughout the menstrual cycle: early (day 4) < late follicular (day 11) = "luteal" (day 21), maximum response occurs at the mid-cycle (day 14).
- An exaggerated response is seen in primary and secondary gonadal failure.
- A flat response (<5 IU/L) occurs in pre-pubertal children and with pituitary and/or hypothalamic disease.
- Note that a normal response does NOT exclude pituitary and/or hypothalamic disease since the response will be affected by the exact anatomy of the disorder.
- The magnitude of the LH response is proportional to the mean nocturnal LH values and therefore the evolution of puberty.

References

- Barth J H, Butler G E and P Hammond (2011). Biochemical Investigations in Laboratory Medicine. ACB Venture Publications, and at http://www.pathology.leedsth.nhs.uk/dnn_bilm/
- Marshall WJ and Bangert SK, eds (1995): Clinical Biochemistry, metabolic and clinical aspects, Churchill Livingstone.

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10.5.18 HCG Stimulation Test

Intended for the assessment of the functional integrity of the Leydig cells of the testes and to assess their reserve capacity to synthesise & secrete testosterone. Indicated in infants with ambiguous genitalia and palpable gonads; males with delayed puberty and/or undescended testes; to confirm the presence of testes.

Patient preparation
No patient preparation is required & hospital admission is unnecessary.

Ward/Phlebotomist requirements
One ampoule of 1500 U HCG for infants or 5000 U for children over 2 years for intra-muscular injection.
Ochre-top (plain) blood bottles

Protocol

Day 0 Before breakfast take 1 ochre-cap blood tube for testosterone, androstenedione, and dihydrotestosterone. Clearly label with the time & date and send to the laboratory.
Inject 1500 U (infants) or 5000 U (> 2 years) of HCG intra-muscularly.

Day 4 Before breakfast take ONE ochre-cap blood tube for testosterone, androstenedione, and dihydrotestosterone. Clearly label with the time & date and send to the laboratory.

Interpretation

- In **normal adult males**, the basal testosterone is within the reference range and should double after 48 or 72 hours; the increment is usually >10 nmol/L.
- In **normal boys**, the response is even greater with testosterone concentrations usually increasing from 2-9 times and may reach adult male concentrations by the finish.
- In the **absence of functioning testicular tissue**, testosterone (which may be sub-normal initially) fails to increase after HCG.
- A rise in testosterone indicates that a testis is present which may be intra-abdominal if the scrotum is empty.
- In unilateral / bilateral cryptorchidism, the testosterone rise is impaired, and stimulation may need to be extended over 4 weeks.

Hypogonadotrophic states: In gonadotrophin deficiency without testicular abnormality, there is a rise in testosterone, and the low basal value should triple post-HCG. If the initial rise is small the test may need to be extended over 4 weeks to elicit a definite increase.

Testosterone/DH testosterone ratio: Useful in the assessment of suspected 5 alpha-reductase deficiency. Interpretation is based upon cut-offs stated in Biochemical Investigations in Laboratory Medicine (2011).

	Testosterone (nmol/L)	DHT (nmol/L)	T/DHT ratio after HCG
Normal adult male	**8-27**	**<2.9**	**<17**
Normal male children (6 months – puberty)	**<0.9**	**<0.1**	**<27**
5 alpha-reductase deficiency	**<0.5**		**>27**

References

- Barth J H, Butler G E and P Hammond (2011). Biochemical Investigations in Laboratory Medicine. ACB Venture Publications, and at http://www.pathology.leedsth.nhs.uk/dnn_bilm/
- Ismail AAA (1981): Biochemical investigations in Endocrinology. Academic Press Besser M, ed (1992): Clinical Endocrinology. Blackwell Scientific Publications.

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10.5.19 Combined Anterior Pituitary Stimulation Test (Paediatrics)

Patient preparation

Note that GnRH tests are not indicated in children aged 18 months to 10 years, except those demonstrating premature sexual maturation. Priming with sex steroids ***is*** recommended in pre-pubertal children who are over 10 years of age (either chronological or bone age). Prescribe stilboestrol 1mg 12 hourly for 48 hours prior to the test.

If a child has not had thyroid function tests before then it will be necessary to do basal thyroid functions and a TRH stimulation test; if basal TFTs done on a previous occasion are normal, then a TRH test is probably not necessary. However, blood for baseline TSH and Free T4 must be taken in all cases. Fasting is not necessary for the test, although they may only drink plain water during the test.

Ward/Phlebotomist requirements

Obtain TRH, clonidine or glucagon and GnRH from Pharmacy, prior to patient admission.
Ochre-top (plain) blood bottles
Grey cap (fluoride) blood bottles

Procedure

At time 0 mins:	Site IV	Take basal bloods.
	TRH	7 microgram (ug) per kg IV, to a maximum of 200 ug
	GnRH	2.5 microgram (ug) per kg IV, to a maximum of 100 ug
	Clonidine	150 microgram (ug) per m^2 (or Glucagon IM), 100 ug per kg to maximum 1 mg

Continue to collect samples at 20, 60, 90, 120, 150 and 180 minutes.

ENSURE ALL SAMPLES ARE CLEARLY LABELLED WITH PATIENT DETAILS AND THE TIMES

TIME (mins)	GH	CORTISOL*	GLUCOSE $	TSH	LH/FSH	IGF-1	IGFBP-3
0	X	X	X	X	X	X	X
20	X	X	X	X	X		
60	X	X	X	X	X		
90	X	X	X				
120	X	X	X				
150	X	X	X				
180	X	X	X				

* Cortisol at baseline only if clonidine used, as indicated if glucagon used
$ If glucagon used, additional GREY top samples for glucose may be sent at each time point.

Sample Notes

1) In the event of insufficient blood on any of the samples - discuss with the Consultant Biochemist.
2) Only perform assays that have been requested, this may vary depending on each specific case - discuss with the Consultant Biochemist.

Interpretation

Refer to TRH, GnRH and Clonidine protocols.

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10.5.20 Glucagon Stimulation Test (Adults)

The Glucagon Stimulation Test is a much safer alternative pituitary test for ACTH and growth hormone reserve than the Insulin Stress Test and is therefore the test of choice for investigation of suspected hypopituitarism.

Do basal pituitary/end-organ hormones initially (FSH, LH, prolactin, TSH, Free T4, Free T3, testosterone (male patients), oestradiol (female pre-menopausal patients), collected into ochre cap blood bottles) and wait for results prior to deciding to perform this procedure.

Precautions/Contraindications

Patients who have not eaten for 48 hrs, glycogen storage disease, phaeochromocytoma, insulinoma, severe cortisol deficiency (i.e., situations where glycogen stores are low).

Patient preparation

The patient should have been fasted from midnight and patients taking hydrocortisone should **omit** their morning and evening doses prior to the day of the test.

Ward/Phlebotomist requirements

An indwelling venous needle.
Ochre-top (plain) blood bottles (for cortisol and GH)
Glucagon dose **Adult** 1 mg intra-muscularly or 1.5 mg if >90 kg.
Child 15 microgram/ kg (up to a max of 1 mg) intra-muscularly.
Weighing scales

Protocol

1) Fast patient overnight, except for water. Start the test at or near 10.00 and insert a venous cannula.
2) After 10 mins rest, collect a basal blood sample for cortisol and GH.
3) Give an intra-muscular injection of glucagon (as per the protocol above). For **adult** patients weighing less than 40 kg use 0.5 mg and patients weighing more than 90 kg use 1.5 mg.
4) Collect blood samples for cortisol and GH at 90, 120, 150-, 180-, 210- and 240-minutes post glucagon injection. Mark all bottles clearly with the patient details **and** sample times.

Important notes

- Nausea (and sometimes vomiting), with sweating and headache occur in approximately 20 % of patients (usually between 120 and 150 mins) and rare fainting occurrences (0.8% of patients) have been reported.
- The patient must remain fasting throughout the duration of the test but is allowed small sips of water.
- Please keep all the blood samples on the ward until the test is complete and then send them all to the laboratory.

Interpretation

Interpretation is based upon cut-offs stated in Biochemical Investigations in Laboratory Medicine (2011).

- A normal cortisol response is defined as a peak cortisol of >450 nmol/L (usually occurring between 120 or 180 mins)
- A normal growth hormone response is defined as >6.7 micrograms per litre (ug/L). Note that the GH cut-off is not as well established as cortisol. A UK consensus for GH response in stimulation tests is currently being sought (August 2008).

References

- Barth J H, Butler G E and P Hammond (2011). Biochemical Investigations in Laboratory Medicine. ACB Venture Publications, and at http://www.pathology.leedsth.nhs.uk/dnn_bilm/
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- Leong et al. An audit of 500 subcutaneous glucagon stimulation tests to assess growth hormones and ACTH secretion in patients with hypothalamic-pituitary disease. Clin End 2001: 54;463-4610.

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10.5.21 Insulin Stress Test (Adults)

This test is potentially dangerous and must be undertaken with great care. A doctor or nurse must be in attendance at all times. A safer alternative is the glucagon stimulation test. This protocol must not be used without prior consultation with the Consultant Biochemist.

Intended for the assessment of ACTH/cortisol and GH reserve in **adult** patients in whom other stimulation tests have proven negative equivocal. **Must not be used in children.**

Precautions/Contraindications

- 100 mg hydrocortisone and 10% glucose for I.V. infusion should be kept on hand in the event of a severe hypoglycaemic reaction.
- Age > 60 years
- Seizure disorders
- Ischaemic heart disease / cardiovascular insufficiency
- Severe panhypopituitarism, hypoadrenalism (10.00 cortisol <100 nmol/L)
- Hypothyroidism impairs the GH and cortisol response. Patients should have corticosteroid replacement commenced prior to thyroxine as the latter has been reported to precipitate an Addisonian crisis in patients with dual deficiency. If adrenal insufficiency is confirmed, the need for a repeat ITT may need to be reconsidered after 3 months of thyroxine.

Patient preparation

The test is done after an overnight fast and with the patient at bed rest.

Ward/Phlebotomist requirements

An indwelling venous needle
Grey cap (fluoride) blood bottles (for glucose)
Ochre-top (plain) blood bottles (for GH and cortisol)
Actrapid Insulin dose

Adults with normal pituitary function dose	0.15	units/kg
Hypopituitary subjects	0.10	units/kg
Acromegaly, diabetes, or Cushing's syndrome	0.2-0.3	units/kg

Weighing scales
POCT glucose meter

Protocol

1) Notify the laboratory before starting the test so that arrangements can be made to analyse the glucose samples urgently.
2) After an overnight fast weigh, the patient and insert an indwelling venous cannula at 10.00 hrs. The patient is allowed to relax for 30 minutes.
3) Take a FASTING SAMPLES, one ochre-cap (for GH and cortisol) and one grey/white fluoride (for glucose).
4) Give the patient insulin by intravenous injection. Dose determined as above.
5) Take further blood samples for glucose, cortisol, and GH at 30, 45-, 60-, 90- and 120-min post-insulin.

Important: The patient should develop adequate symptomatic hypoglycaemic stress after insulin (sweating, tremor). Send blood samples to the laboratory immediately to monitor the development of adequate hypoglycaemia (< 2.5 mmol/L). If this has not occurred by 45 minutes post-insulin, further IV insulin may be given, in which case sampling should be extended by a further 30 minutes.

Hypoglycaemia should be reversed if there are severe symptoms i.e., loss of consciousness, cardiac symptoms, extreme anxiety, or fits. If necessary, I.V. 10% glucose should be administered, and blood sampling continued

Interpretation

Interpretation is based upon cut-offs stated in Biochemical Investigations in Laboratory Medicine (2011).

- Adequate hypoglycaemia after insulin must be achieved to interpret the test (glucose <2.2 mmol/L).
- **Hypopituitarism** – an adequate cortisol response is defined as a rise in plasma cortisol to >450 nmol/L. Patients with impaired cortisol responses, i.e., <450 but >400 nmol/L, may only need cortisol cover for major illnesses or stresses. An increase in GH >6.7 micrograms/L (ug/L) indicates normal pituitary reserve.
- **Cushing's syndrome** – there is a rise of <170 nmol/L above the basal cortisol.

Sensitivity and specificity – 5-15% of normal patients may show a suboptimal response as defined by the maximal cortisol concentration. 20% of those with Cushing's will show a rise >170 nmol/L. A rise of less than this is rare in depression or alcoholic pseudo-Cushing's syndromes which are the principal differential diagnoses.

References

Barth J H, Butler G E and P Hammond (2011). Biochemical Investigations in Laboratory Medicine. ACB Venture Publications, and at http://www.pathology.leedsth.nhs.uk/dnn_bilm/

Burtis C A and E R Ashwood, Eds. (1994). Tietz Textbook of Clinical Chemistry, 2nd edition. W B Saunders Company.

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10.5.22 Primary Hyperaldosteronism – First Line Aldosterone/Renin Screen

Conventional practice suggests that the diagnosis of hyperaldosteronism should be considered in hypertensive patients with spontaneous or diuretic-induced hypokalaemia. However, data from clinics which routinely screen hypertensive indicates that several patients would be undiagnosed on this basis since approximately 50% of patients with aldosterone secreting adenoma are normokalaemic.

The renin-aldosterone axis is primarily regulated by renal blood flow. Subjects under investigation should, therefore, not be taking any drugs that interfere with fluid balance or potassium. Neither Doxazosin nor Prazosin interferes and those subjects requiring hypotensive therapy should ideally be transferred to one of these agents. Secondly, it is essential that subject should be normally hydrated and has an adequate oral intake of sodium. Hypokalaemia must be avoided since it suppresses aldosterone secretion. It is important to note that the effect of increasing oral sodium will be to considerably increase urinary potassium excretion.

Patient preparation

Correct pre-existing hypokalaemia: Hypokalaemia suppresses aldosterone & confuses interpretation. It must be corrected beforehand as best as is practical by oral KCl (Slow K) as a slow-release preparation (to avoid intestinal ulceration) and in whatever dose is required to raise plasma potassium into, or as close to as possible, the reference range (3.5-5.5 mmol/L). Replacement should be stopped on the day of the test.

Assess drug therapy: The test is surprisingly robust in the face of drugs but false positives & negatives can occur so the following steps should be taken:

- Spironolactone must be stopped for 6 weeks to be certain that any elevation in plasma renin activity is not due its inhibition of aldosterone.
- Ideally all interfering drugs should be stopped, but if this is impractical, a best pragmatic approach is to stop ACE inhibitors, beta-blockers for 2 weeks and to avoid Ca-channel blockers on the day of the test; although Valloton (1996) states that the aldosterone/renin ratio is robust and antihypertensive therapy does not need to be stopped when the ratio is used as a first line test.
- The optimal approach is to use either Doxazosin or Prazosin as neither appears to affect the renin-aldosterone axis.

Do not stop medications because of unacceptable risks but maintain control.

Drug	Physiological Effect	Time to Remove Interference
ACE inhibitors	Increase PRA & reduce aldosterone	2 weeks
Beta-blockers	Reduce PRA more than aldosterone	2 weeks
Calcium channel blockers	Reduce aldosterone & stimulate renin production	2 weeks
Diuretics	Increase PRA & aldosterone	2 weeks
Hypokalaemia	Inhibits aldosterone secretion	
NSAIDs	Retain sodium & reduce PRA? effect on aldosterone	2 weeks
Oestradiol	Increase renin substrate	6 weeks
Spironolactone	Increase PRA, variable effect on aldosterone	6 weeks

Patient activity: The patient should not have walked or exercised vigorously in the previous 60 minutes & prior to blood sampling should have been seated for 10 minutes.

Ward/Phlebotomist requirements

4 mL heparin bottles

Procedure

1) The patient should remain seated for 10 min prior to venepuncture.
2) The blood sample should optimally be taken at 8 am when aldosterone is physiologically high - take a random blood sample into a 4 mL lithium heparin tube for plasma renin activity (PRA), aldosterone (PA) and renal profile.

Interpretation

In primary hyperaldosteronism there may be a gradation in aldosterone/PRA levels. It may be diagnosed biochemically when aldosterone is virtually normal, but PRA is reduced, producing a raised A/PRA ratio. More

advanced cases will have a high aldosterone and a profoundly reduced PRA and thus a markedly elevated A/PRA ratio. Thus:

- In a patient not receiving anti-hypertensive therapy and with an A/PRA ratio of >2000, primary hyperaldosteronism is almost a certainty.
- If the A/PRA ratio is <800, then primary hyperaldosteronism is very unlikely.

Patients left on calcium-channel blockers with normal ratios but a high level of clinical suspicion, and also those with a ratio of >1000, require further investigation. See “Renin/Aldosterone Screen Follow-up Testing” below.

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10.5.23 Primary Hyperaldosteronism – Aldosterone/Renin Postural Studies

Patient preparation

- As in the screening test, patients should be taken off spironolactone - spironolactone must be stopped for 6 weeks to be certain that any elevation in plasma renin activity (PRA) is not due to antagonism of aldosterone action.
- Other drugs such as calcium-channel blockers, beta-blockers and ACE inhibitors should be discontinued for 2 weeks.
- When necessary, hypertension can be controlled with an alpha-blocker such as Doxazosin or Prazosin, neither of which appear to affect the renin-aldosterone axis.
- Subjects should be placed on a diet containing an adequate quantity of sodium (100-150 mmol/day) and potassium (75-125 mmol/day). This should be assessed by at least one 24h urine collection prior to the investigation.

Ward/Phlebotomist requirements

Green-top (lith hep) blood bottles
Ochre-top (plain) blood bottles

Procedure

1) The subject should not rise from bed after 22.00 h on the night prior to investigation, not even to go to the toilet. This is essential to ensure that basal levels of hormones are measured in the first sample.
2) At 10.30h **immediately after** overnight recumbency & **before** change in posture or breakfast take 4 mL of blood into a heparinised bottle. Take to the laboratory immediately for separation. Also take a serum for renal profile and cortisol.
3) After the patient has been out of bed for **30 minutes** and before breakfast repeat the blood sample as outlined above (heparin sample only) & take to the laboratory immediately for separation.
4) After the patient has been out of bed for **4 hours** repeat the blood samples as outlined above (heparinised for renin and aldosterone and ochre-cap vacuette for cortisol) & take to the laboratory immediately for separation.

Guidance and potential pitfalls

- The above procedures should have been carried out in full to the satisfaction of the investigating Physician. **Patients should NOT be referred for possible surgery based on a single abnormal A/R ratio**.
- If an initial A/R ratio is not raised in someone with unprovoked hypokalaemia, it should be repeated after potassium replacement.
- The baseline sample is taken to confirm the presence of hyperaldosteronism and to evaluate the aldosterone-PRA ratio under the controlled conditions on which the reference data has been collected.
- The second sample is taken to establish that the effect of posture is to cause a physiological rise in PRA. No change in PRA suggests that aldosterone secretion is autonomous.
- The 4h sample may give additional information & help classify the nature of the adenoma. Aldosterone-producing adenomas are either angiotensin- dependent or ACTH-dependent. The former is suggested by a midday concentration of aldosterone which rises to twice the 08.30h value. In ACTH-dependent adenoma, a fall in aldosterone to half the morning concentration usually occurs. The latter requires confirmation by assessment of the physiological fall in ACTH at midday by a fall in plasma cortisol from 0830 to 1200 noon.
- The aldosterone secretion in bilateral adrenal hyperplasia is generally angiotensin- dependent and will rise in the midday sample.
- Patients with Familial hyperaldosteronism type 1 (glucocorticoid suppressible) also show a fall in aldosterone in the midday sample.
- Patients with renal insufficiency need especially careful assessment. These patients may have a high A/R ratio due to hyperkalaemic stimulation of aldosterone production as well as suppression of PRA because of fewer functioning juxtaglomerular cells.
- CT or MRI scanning of the adrenals may be misleading - bilateral adrenal hyperplasia may be mistaken for an adenoma; the presence of an adenoma on a scan does not necessarily mean it is a functioning one.
- If there is doubt over the diagnosis or surgical intervention is contra-indicated the patient should be maintained on spironolactone. In any case, in the event of any difficulties with the diagnosis after the above procedures please discuss the case with the Laboratory Consultant.

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10.5.24 Primary Hyperaldosteronism – Furosemide Stimulation Test

Even after the long in-patient procedure above, has been performed, some patients may still give equivocal results. A suggested dynamic function test as a follow-up is the **Furosemide stimulation test** as follows:

Patient preparation

- As in the screening test, patients should be taken off spironolactone and oestrogens for 6 weeks - spironolactone must be stopped for 6 weeks to be certain that any elevation in plasma renin activity (PRA) is not due to antagonism of aldosterone action.
- Other drugs such as calcium-channel blockers, beta-blockers and ACE inhibitors should be discontinued for 2 weeks.
- When necessary, hypertension can be controlled with an alpha-blocker such as Doxazosin or Prazosin, neither of which appear to affect the renin-aldosterone axis.

Ward/Phlebotomist requirements

Two Furosemide 40mg tablets
Green-top (lith hep) blood bottles

Procedure

DAY 1	0900-1100h	Patient should remain upright & ambulant at 1100h take blood for plasma renin activity & aldosterone
DAY 2	1800h	Give Furosemide 40 mg tablet.
Day 3	0900h	Give Furosemide 40 mg tablet.
	0900-1100h	Patient should remain upright & ambulant at 1100h take blood for plasma renin activity & aldosterone

Interpretation

Interpretation is based upon cut-offs stated in Biochemical Investigations in Laboratory Medicine (2011).

- Failure of PRA to rise above 1.5 nmol/L/h indicates primary hyperaldosteronism.

Sensitivity and specificity

This test separates all forms of mineralocorticoid excess from other causes of hypertension but does not indicate the cause i.e. bilateral adrenal hyperplasia, adrenal adenoma, idiopathic hyperaldosteronism, apparent mineralocorticoid excess, deoxycorticosterone-producing adenoma and so on.

References

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10.5.25 Primary Hyperaldosteronism – Saline Suppression Test

May be used in further investigation as an alternative to the frusemide stimulation test.

Patient preparation

- As in the screening test, patients should be taken off spironolactone and oestrogens for 6 weeks, other drugs such as calcium channel blockers, beta-blockers and ACE inhibitors should be discontinued for 2 weeks. Where necessary, hypertension can be controlled with alpha blockers such as doxazosin, prazosin, or verapamil.
- Patient must be normokalaemic (ideally >/= 4.0 mmol/L)
- Examine patient for signs of cardiac failure. This test **should not be performed** in patients with severe uncontrolled hypertension, renal insufficiency, cardiac insufficiency, cardiac arrhythmia, or severe hypokalemia.

Procedure

1) Cannulate and take blood for plasma aldosterone, plasma renin activity, U & Es.
2) Patient should be in the seated position for at least 30 mins before infusion begins.
3) Infuse 2 litres of 0.9% saline over 4 hours, starting at 10.00 a.m.
4) Blood pressure, oxygen saturation and heart rate are monitored throughout the test.
5) After 4 hours (i.e., 13:00pm), take further blood sample for aldosterone, U & Es (i.v. saline infusion can promote hypokalaemia).

Interpretation

Principle of test is that the lack of suppression of aldosterone excretion with intravascular expansion indicates primary hyperaldosteronism.

Interpretation is based upon cut-offs stated in the Imperial Centre for Endocrinology Endocrine bible:

- Post-infusion plasma aldosterone:
    - <120 pmol/L make the diagnosis of primary hyperadosteronism unlikely.
    - >240 pmol/L very probable sign of primary hyperaldosteronism
    - Values between 120 – 240 pmol/L are indeterminate.

References

- Imperial College Centre for Endocrinology Handbook http://imperialendo.co.uk/Bible2018.pdf accessed 12/12/18.
- Rossi et al (2007) Journal of Hypertension 25:1433
- Funder et al (2009) J Clin Endocrinol Metab 93:3266

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10.5.26 Water Deprivation Test

Preliminary (Outpatient Procedure)

1) A random, first morning urine osmolality is mandatory, before proceeding to a full water deprivation test, repeating if borderline results are obtained.
2) Collect a 24-hour urine to measure the volume and verify that true polyuria is occurring. The standard bottle holds 5000 mL which is adequate in most cases; occasionally two will be required to diagnose polyuria in adults. Polyuria is defined as a 24-hour urine volume of >3.0 L.
3) Collect fasting blood a.m. for glucose, cortisol, free T4, TSH, sodium, potassium, calcium, urea, and creatinine. Please quote drug therapy.

Precautions/Contraindications - READ CAREFULLY!

The water/fluid deprivation test should be done only during the day & under constant medical supervision. It is potentially dangerous in some circumstances e.g., in the presence of renal disease and/or diabetes mellitus. It may also produce severe dehydration in diabetes insipidus. For these reasons the patient's weight MUST be recorded accurately at the beginning of the test and HOURLY thereafter. If the patient's weight falls by *3% or more* the test should be terminated.

- Patients suspected of primary polydipsia should be supervised VERY carefully as they may attempt to gain access to ANY source of water on the ward.
- Note that if excessive water drinking is permitted after vasopressin injection, water intoxication may result.
- The test starts early morning. The patient is allowed a light **breakfast but NO tea, coffee, or other fluids**. Fluids or wet foods are not allowed until 1630 h or until after the test has finished. Smoking is not permitted.
- ***DO NOT PROCEED IF ALREADY DEHYDRATED***

Ward/Phlebotomist requirements

- Accurate weighing scales (preferably digital.)
- Desmopressin (ADH analogue), available as Pitressin, from Pharmacy in ampoule form for I.M. injection, 4 microgram/ml. **Note that 2 microgram (= 0.5 mL) is required for this test**.
- White topped MSU bottles or large mouth containers (supplied by lab)
- Measuring cylinder (supplied by the laboratory) if laboratory staff supervising test.

Procedure

Osmolality measurements: The test should usually be booked in advance with the Consultant Biochemist. If laboratory staff are unavailable to volume samples on the ward, please ensure that samples are sent to the laboratory PROMPTLY. Note that samples should be analysed as the test proceeds. The osmolalities of the first urine samples and/or subsequent response to water deprivation may allow the test to be terminated by mid-day in some (normal) patients.

The test starts early morning. The patient is allowed a light breakfast but NO tea, coffee, or other fluids. Fluids or wet foods are not allowed until 1630 hrs or until after the test has finished. Smoking is not permitted.

0830 h	The patient empties their bladder & this urine is discarded. Record the patient's weight.
0900 h	Take ochre-top (plain) blood sample. Label "**serum 1**" & send to the laboratory for U&E and osmolality.
0930 h	Patient empties bladder, collecting all the sample. Record volume, label "**urine 1**". Record the patient's weight
1030h	Patient empties bladder, collecting all the sample. Record volume, label "**urine 2**". Record the patient's weight
1130h	Patient empties bladder, collecting all the sample. Record volume, label "**urine 3**". Record the patient's weight
1200 h	Take ochre-top (plain) blood sample, label "**serum 2**" & send to the laboratory
1230 h	Patient empties bladder, collecting all the sample. Record volume, label "**urine 4**". Record the patient's weight
1330 h	Patient empties bladder, collecting all the sample.

Record volume, label "**urine 5**". Record the patient's weight

1430 h Patient empties bladder, collecting all the sample.
Record volume, label "**urine 6**". Record the patient's weight

1500 h Take ochre-top (plain) blood sample, label "**serum 3**" & send to the laboratory.

1530 h Patient empties bladder, collecting all the sample.
Record volume, label "**urine 7**". Record the patient's weight

1600 h Take ochre-top (plain) blood sample, label "**serum 4**" & send to the laboratory.

1630 h Patient empties bladder, collecting all the sample.
Record volume, label "**urine 8**". Record the patient's weight

Immediately after the last urine collection give **2 microgram** desmopressin intra-muscularly.

Collect ALL urines passed in entirety during the next 16 hours & record volumes. SAVE AN ALIQUOT FOR OSMOLALITY MEASUREMENT from all samples passed. If staff cannot collect urine saves separately, pool into a 24h urine container all urines passed before sleep. If possible, also request an aliquot from the first morning urine void the day after.

The patient may eat & drink limited amounts of fluid during this period. Smoking is not permitted. Send all samples to the laboratory for osmolality determinations.

Interpretation

- Normal AVP secretion is characterised by a urine concentration of greater than 600 mosm/kg and serum osmolality remaining within reference limits.
- Cranial Diabetes Insipidus is diagnosed by a rise in serum osmolality above 300 mmol/kg, while the urine remains dilute only increasing after desmopressin (to >600 mmol/kg). Partial Cranial DI is characterised by some evidence of urine concentration (urine osmolality>serum osmolality) pre-desmopressin and a normal concentration (to > 600 mmol/kg) after its administration.
- Nephrogenic DI (NDI) is diagnosed by an increased serum osmolality and diluted urine without any notable concentration of urine in the presence of desmopressin. Partial NDI is characterised by evidence of partial concentration of urine (urine osmolality> 300 mmol/kg) but little or no response to exogenous desmopressin.

NOTES:
Interpretation can be difficult in psychogenic polydipsia where renal concentrating ability is compromised because of “medullary washout”. Such patients might also surreptitiously drink water during the test.

Anomalous results can also occur if the patient does not completely empty the bladder at each urine collection.

References

- Clinical Biochemistry- Metabolic Aspects. Ed Marshall and Bangert, 1995, p312.
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10.5.27 Urinary Acidification Test for Renal Tubular Acidosis

Note: Must be discussed with the laboratory prior to arranging the test.

Acid excretion is one of the main roles of the kidney and congenital and acquired disorders of the nephron results in acidosis. Whilst chronic renal failure remains the commonest form of acidosis, isolated tubular defects represent a more challenging diagnostic dilemma. The inherited forms are characterised by a hyperchloraemic acidosis with near normal GFR and plasma inorganic anions e.g., phosphate. Prior to formal tests of urinary acidification, the first section here details simple tests which can be done at the outset before applying these (often unpleasant) tests:

Summary of urine and blood tests to be completed *prior* to formal urinary acidification tests.

	Classic distal RTA (Type I)	Incomplete distal RTA (Type I)	Voltage dependent distal RTA (Type I)	proximal RTA (Type II)	Type IV RTA
Urine pH*	>5.5	>5.5	>5.5	<5.5	<5.5
Urine anion gap*	Positive	Negative	Positive	Negative	Positive
Fractional bicarbonate excretion**	<5-10%	<5-10%	<5-10%	>15%	5-15%
Furosemide test	Abnormal	Abnormal	Abnormal (If reversible)	Normal	Normal
Urine calcium*	High	Normal/high	High	Normal	Normal
Urine citrate*	Low	Low/normal	Low	Normal	Normal
Renal stones	Common	Common	Common	Rare	Rare
Metabolic bone disease	Rare	Rare	Rare	Common	Rare
Other tubular defects	Rare	Rare	Rare	Common	Rare
Plasma potassium*	Normal/low	Normal	High	Normal/low	High

* Determined when plasma bicarbonate < 20 mmol/L
** determined when plasma bicarbonate > 26 mmol/L

Urine pH: The ideal sample for measuring pH is a fresh early morning urine taken before breakfast. It is important to be certain that the urine is sterile as urea splitting organisms release ammonia and increase the pH.

Urine anion gap (UAG): This is an indirect method for measuring urine [ammonia] and can be measured on a random urine sample. It is only valid when the urine pH < 6.5 as at greater pH, urine bicarbonate is a significant anion.
UAG = [urine Na] + [urine K] - [urine Cl]
The use of the UAG as an estimate of urine ammonium ion is disputed by some investigators (Kirschbaum *et al*)

Fractional bicarbonate excretion (F_E (HCO_3^-)): F_E can be assessed on a random urine sample. Take care to ensure that the sample container is full and there is a minimum air space available for loss of bicarbonate through dissociation.
F_E (HCO_3^-) = (plasma HCO_3^- * urine creatinine) / (plasma creatinine * urine HCO_3^-)
NB ensure that creatinine and bicarbonate are in the same units.

Hypokalaemia: Hypokalaemia or chronic acidosis may prevent normal urinary acidification and urine pH will be > 5.5, due to increased tubular ammoniagenesis. Hyponatraemia may also prevent acidification due to reduced cation available for exchange in the distal tubules.

The urinary acidification test is intended to confirm the diagnosis of distal renal tubular acidosis (RTA). This condition is characterised by a hyperchloraemic acidosis with normal anion gap. Note that the diagnosis is confirmed if the urine is not maximally acidified (pH of <5.5 in a first morning urine) despite a metabolic acidosis.

The test is not necessary if the pH of a urinary specimen collected after an overnight fast, is less than 5.5. The ammonium ion NH_4^+ is potentially acid because it can dissociate to ammonia and H^+. After ingestion of ammonium chloride, the kidneys usually secrete the H^+ and the urinary pH falls.

However, the use of ammonium chloride is contra-indicated in the presence of liver problems, but calcium chloride can be used as an alternative (see below). The rationale for this test is based on the fact that $CaCl_2$ will react in the gut to produce calcium carbonate, Cl, carbon dioxide and water; acidosis is induced by HCO_3^- consumption.

Precautions/Contraindications

- This test is potentially dangerous and must be undertaken with great care. It should NOT be performed if the urine is alkaline in the presence of a metabolic acidosis, in patients with liver disease or in patients taking alkali replacement.
- Bacterial urine infection may give a falsely high pH due to urea hydrolysis.
- This test should not be performed in hypokalaemia or hypercalcaemia as these conditions interfere with tubular function and may mimic RTA.

Patient preparation

The patient must fast overnight.
Determine their body weight on the morning of the test.

Ward/Phlebotomist requirements

- Ammonium chloride (NH_4Cl): administer 100mg/kg bodyweight. Use iced water & lemon or other flavouring to dissolve the NH_4Cl.It is a strong emetic, so patients need to be covered with an anti-emetic (e.g., metoclopramide 10 mg orally). It can also be obtained as an enteric-coated preparation if preferred. **Note: this is unpleasant to drink & flavouring is essential to ensure complete consumption**
- Urine collection containers

Procedure

1) Ensure the laboratory has been notified **before** starting the test.
2) 0700 - 0800 hrs: Collect all urine passed during the first hour after waking. Weigh the patient and take a heparinised venous blood sample for **pH/bicarbonate** assay.
3) Next, calculate the NH_4Cl required & prepare as above, (2).
4) 0800 hr: Give the NH_4Cl drink in a tumbler with ice cubes. Stir frequently to ensure complete dissolution of all the crystals. **Important:** Allow the patient up to **TWO HOURS** to consume the drink to avoid gastric irritation & subsequent vomiting. Additional sips of water/beverage between drinks help to alleviate the bitterness & ensure adequate urine output. The patient may experience nausea, mild diarrhoea, and abdominal discomfort. Some patients will vomit but allowing an ingestion period of two hours can reduce this.
5) 0900 - 1700 hr: Collect urine samples on an HOURLY basis and take to the laboratory immediately for urine pH measurement. LABEL THE CONTAINER with the time of collection. Continue collecting hourly urine samples until 1700 hrs.
6) 1000 hr: Take a second venous heparinised BLOOD sample for pH / bicarbonate & send to the laboratory.

Interpretation

- In normal subjects the urine pH falls to 5.5 or below at between 2 and 8 hours after the dose. In generalised tubular disease the response of the functioning nephrons may give a normal result. In distal renal tubular acidosis this degree of acidification does not occur. Urinary acidification is normal in proximal tubular acidosis.
- Plasma renin and aldosterone measurement is helpful if Type IV RTA is being considered. Hyporeninaemic hypoaldosteronism is a relatively common feature of long-standing DM.

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10.5.28 Calcium Chloride Loading Test for Renal Tubular Acidosis

This test is used to investigate Renal Tubular Acidosis and is used as an alternative to the ammonium chloride loading test in patients who may have compromised liver function.

Patient preparation

Patient consumes a normal diet prior to the test and is allowed to eat their usual dinner on the evening of the test but is not allowed any further food until the test is complete. However, 50 mL of water per hour is allowed during the test.

Procedure

1) The patients are encouraged to remain recumbent, except to void urine, throughout the duration of the test.
2) At 7 am, the patient empties the bladder and discards the urine.
3) At 8 am the patient voids urine and collects it all into a plain container (provided by the lab) for urine pH, volume, creatinine, chloride, and calcium - this must be transported to the lab immediately (LABEL WITH TIME AND PATIENT DETAILS). An arterialised capillary blood sample is also taken for blood gases. Venous blood (yellow cap vacuette) is also taken for urea/electrolytes, bicarbonate, calcium, and chloride.
4) Calcium chloride (equivalent to 1mmol/kg, (0.147g/kg of dihydrate), body weight) is then given to the patient – this is dissolved in 190 ml of water and 10 ml of sodium free artificial flavoured syrup (quite large amounts may be required because of the poor palatability of $CaCl_2$). The dissolved calcium chloride can be drunk slowly over a period of 15-20 minutes.
5) Hourly urine samples (for pH, volume, creatinine, chloride, and calcium and transported to the lab immediately), arterialised blood * (for blood gases) and venous blood (for urea/electrolytes, bicarbonate, calcium, and chloride) are then taken for a further 6 hours.
6) Hand needs to be kept at about 45 C for 10-15 mins before capillary samples are taken.
7) The test is curtailed when the last samples are taken at 2 p.m.
8) Tabulate all results in result chart.

Calcium Chloride Loading Test Results							
Patient Name:							
Patient DOB:							
Patient No:							
Date of Test:							
Blood Sample Times							
Test	**8am**	**9am**	**10am**	**11am**	**12am**	**1pm**	**2pm**
Blood pH							
PCO2							
PO2							
Act Bic							
Base Xs							
Serum Na							
Serum K							
Serum CL							
Serum Urea							
Serum Creat							
Serum adj Calcium							
Urine times							
Urine pH							
Urine Volume							
Urine Creat							
Urine Chloride							
Urine Calcium							
Urine Calcium/ Creat ratio							

Interpretation

Acid ingestion produces significant decreases in blood pH and bicarbonate concentrations. There should also be increases in serum chloride, serum calcium and urine calcium concentrations. The measurement of these parameters demonstrates adequate $CaCl_2$ absorption.

"Normal" patients should acidify urine to a pH of below 5.0 when the base excess has reached – 6 mmol/L (4) but Oster (3) suggests 5.3 to be the "normal threshold".

For further information see the references.

References

- Backman et al. A short duration renal acidification test. Scan J Urol Neprol 1976; 10 suppl 35: 33-410. Barth J H, Butler G E and P Hammond (2011). Biochemical Investigations in Laboratory Medicine. ACB Venture Publications, and at http://1210.11.156.204/Bilm/
- Kirschbaum B, Sica D, Anderson P (1999).Urine electrolytes and the urine anion and osmolar gaps. J Lab Clin Med 133:597-604.
- Marshall WJ and Bangert SK, Eds (1995): Clinical Biochemistry, metabolic and clinical aspects, Churchill Livingstone.
- Oster JR et al. A short duration renal acidification test using calcium chloride. Nephron 1975;14: 281-292 Silva J, Pennell P and P D Main. Clinical Chemistry, Diagnosis & Treatment. Page 105. 5th ed.
- Penny MD and Oleeskey DA. Renal tubular acidosis. Ann Clin Biochem 1999;36: 406-407.

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10.5.29 Lactose Tolerance Test

Intended for the further investigation of patients suspected of having lactase deficiency in which watery diarrhoea, cramps or bloating follow ingestion of milk, or starch. (Lactose hydrogen breath testing can be considered as an alternative; currently it is unavailable locally although Path Links are looking into possible repatriation).

Patient preparation

- The test should be performed when the patient has no diarrhoea and when the patient has not been ingesting the offending disaccharide for a few days.
- The patient should fast overnight prior to the test
- Diabetes mellitus should have been ruled out beforehand (see Interpretation)

Ward/Phlebotomist requirements

Lactose (obtainable from pharmacy), dose as follows:

ADULTS 50g in 200 mL of water

CHILDREN 2g / kg bodyweight to a maximum of 50g in 200 mL of flavoured water. The test can be unpleasant for young children - suggest discussing with the paediatricians in advance if in doubt.

Fluoride (grey/white-topped) blood bottles.

Procedure

1) After an overnight fast, take a blood sample into a fluoride bottle for glucose.
2) Give the appropriate lactose dose as a drink (see 1, above.).
3) Take further blood samples for glucose @ 15, 30, 60, 90 and 120 minutes after the lactose load.

ENSURE ALL SAMPLES ARE CLEARLY LABELLED WITH PATIENT DETAILS AND THE TIMES

Interpretation

Adults: Normally there should be a rise in plasma glucose of > 1.1 mmol/L. Those who do not show this rise should have the test repeated using equimolar amounts of glucose and galactose. Intestinal lactase deficiency may be presumed if there is a normal rise in plasma glucose following the glucose/ galactose mixture, but not after lactose. Abdominal symptoms following the latter but not the former support the diagnosis. Patients who have diabetes mellitus and lactase deficiency will show a normal rise in glucose but accompanied by abdominal symptoms post-lactose.

Paediatric Cases: An increase in blood glucose of more than 1.7mmol/l above the fasting level is recognised as normal. A rise of 1.1 - 1.7mmol/l is doubtful and a rise of less than 1.1mmol/l is abnormal. Rather more important than the rise in blood sugar levels in children is observation of the stools after the oral load and in particular the development of diarrhoea. Following an oral lactose load the demonstration of a flat lactose tolerance test without diarrhoea and accompanied by excess stool reducing substances is not clinically significant. It should not on its own be regarded as an indication that the child should be treated for lactose malabsorption. It could be due to delayed gastric emptying or indicate some disorder of sugar handling which is not severe enough to produce symptoms.

References

Clinical Biochemistry and the Sick Child. 2nd Ed. Page 386 - 387. Editors: Barbara Clayton and Joan Round
Marshall WJ and Bangert SK, Eds (1995): Clinical Biochemistry, metabolic and clinical aspects, Churchill Livingstone.

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10.5.30 Ischaemic Lactate Test

For the investigation of patients with muscle weakness, fatigue, and cramps possibly due to an enzymatic defect in converting muscle glycogen or glucose to lactate (McArdle's disease (muscle phosphorylase deficiency, GSD type V) and phosphofructokinase deficiency, GSD type VII.)

Sensitivity and specificity: Failure of lactate to rise after this test confirms a diagnosis of a disorder of glycolysis but this test cannot be used to exclude partial expression of these diseases.

Precautions/Contraindications
There are reports of rhabdomyolysis developing in patients with underlying acquired (e.g., alcoholic, or hypothyroid) or inherited myopathies when they are strenuously exercised. Ensure the laboratory is notified before commencing the test.

Patient preparation
The patient should have rested for 30 minutes prior to the test.

Ward/Phlebotomist requirements
EDTA (lavender cap) tube for ammonia
Fluoride tube (grey/white cap) for lactate
Ice
Sphygmomanometer

Procedure
1) Place a sphygmomanometer cuff on the patient's upper arm and insert an intravenous cannula in the antecubital vein that is kept patent with Hepsal.
2) Take blood samples for lactate (grey/white cap tube) and ammonia (lavender cap tube) (sample 1), label clearly with time of sampling and **place on ice**.
3) Inflate the cuff until above systolic pressure.
4) The patient should repeatedly squeeze the sphygmomanometer bulb until limited by pain (affected individuals are usually unable to perform the work for more than 40 - 50 seconds.)
5) After one minute of ischaemia the cuff is released. After a further 5 seconds a blood sample is taken for lactate and ammonia (sample 2). Label sample clearly with time and **place on ice**.
6) Further blood samples for lactate and ammonia are taken at 2, 3, 5 and 7 minutes (samples 3, 4, 5 and 6 – label clearly with time and **place on ice**).

NB Samples for ammonia and lactate should be **sent immediately to the laboratory, on ice**, to arrive **within 15 min of commencing venepuncture.**

Interpretation
- Normal individuals obtain relatively instantaneous relief of pain and can move their fingers immediately on release of the cuff.
- Patients with metabolic defects cannot exercise for 2 minutes or more, develop forearm contracture, and are unable to extend their fingers.
- A normal response is shown by maximum rises in both plasma lactate (to > 2.2 mmol/L) and in plasma ammonia (to > 70 micromol/L). A normal increment in one analyte does not exclude the disease. A failure of both lactate and ammonia to rise suggests that the subject did not exercise adequately, and the test should be repeated.
- Affected individuals characteristically show no rise in blood lactate in response to ischaemic exercise - the absence of a venous lactate response to ischaemic exercise is characteristic of all diseases in which there is impairment in the conversion of glycogen to glucose or lactate in muscle.
- An absent rise in ammonia with a normal rise in lactate is characteristic of myoadenylate deaminase deficiency.
- Where abnormal results are obtained it is essential to measure the various muscle enzymes involved (phosphorylase, phosphofructokinase-1 & others) on a muscle biopsy to establish a definitive diagnosis. Note that during attacks, myoglobinuria may be noted with CK and LD elevated in serum.

References

- Barth J H, Butler G E and P Hammond (2011). Biochemical Investigations in Laboratory Medicine. ACB Venture Publications, and at http://www.pathology.leedsth.nhs.uk/dnn_bilm/
- Holton J B, Ed (1987). The Inherited Metabolic Diseases. Churchill Livingstone.

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9.5 Toxicology and therapeutic drug monitoring service

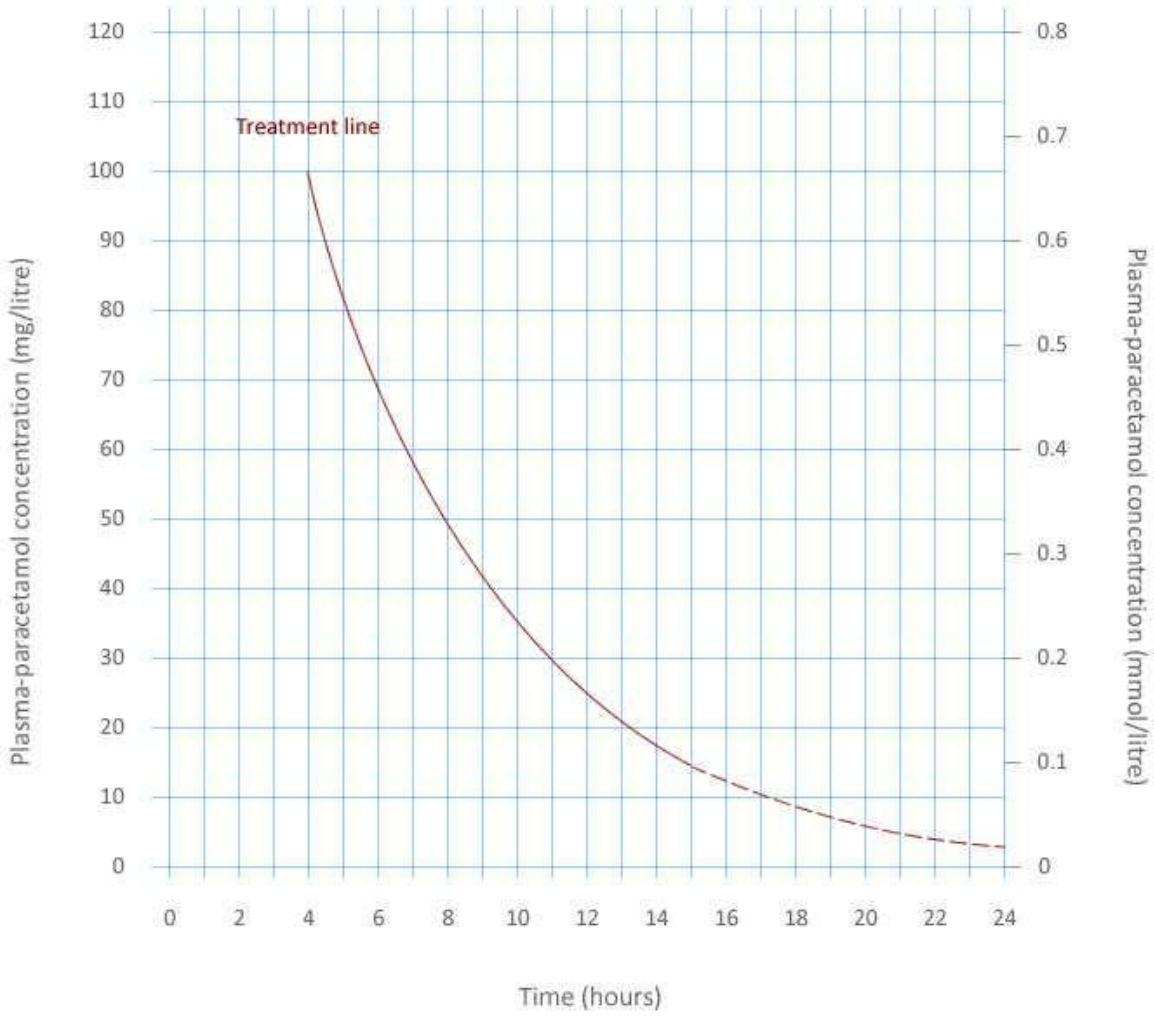
10.6.1 Paracetamol overdose

Please refer to the treatment graph below. Note the importance of time since ingestion. Paracetamol poisoning is potentially fatal - **please call your nearest NPIS centre for advice in severe cases** (0870 600 6266). Also see their website at http://www.toxbase.org/Chemicals/Management-Pages/Paracetamol-Index-UK/
The licensed indication for acetylcysteine is now:

– Paracetamol overdose irrespective of the plasma paracetamol level in circumstances where the overdose is staggered or there is doubt over the time of paracetamol ingestion; or

– Paracetamol overdose with a timed plasma paracetamol concentration on or above a single treatment line joining points of 100 mg/L at 4 hours and 15 mg/L at 15 hours nomogram regardless of risk factors of hepatotoxicity. **Note that the units used are mg/L**

Paracetamol treatment nomogram (courtesy of Toxbase web site) updated September 2012



10.6.2 Salicylate overdose

NB Salicylate poisoning is potentially fatal - please call your nearest NPIS centre for severe cases (0844 892 0111)

Common acid-base changes:

Adults and children over the age of 4 years:
A mixed respiratory alkalosis and metabolic acidosis is the rule with normal or reduced hydrogen ion concentration.

Children aged 4 years or less:
A dominant metabolic acidosis with high arterial hydrogen ion concentration is common.

Acidosis may increase salicylate transfer across the blood brain barrier.

Assessment of the severity of poisoning

The severity of poisoning cannot be assessed from plasma salicylate concentrations alone although most adult deaths occur in patients whose concentrations exceed 700 mg/L

10.6.3 Ethylene glycol ingestion / overdose

NB Ethylene glycol poisoning is potentially fatal - please call your nearest NPIS centre for severe cases (0844 892 0111)

There is no local laboratory service for detecting and monitoring ethylene glycol ingestion, this service is provided by a laboratory outside the county. It is therefore essential to liaise with the on-call Consultant Clinical Biochemist who will arrange sample transport when appropriate (fluoride-oxalate, grey-topped sample bottle). As with other dangerous toxins, please consult www.toxbase.org for full advice.

10.6.4 Drugs of Abuse - guide for users of the Drugs of Abuse Service.

Urine drugs of abuse (DOA) samples are routinely referred away for analysis using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). The drugs screen includes a range of classically abused drugs (opiates, opioids, cocaine, amphetamines, benzodiazepines, and some prescribed and synthetic drugs. Please give details if specific drugs need to be tested for e.g., cannabinoids, prescription drugs. Further information can be obtained from the local laboratory or Consultant Clinical Biochemist.
All samples are routinely tested for creatinine to check for sample dilution, and those with a creatinine concentration <1.8 mmol/L have the caveat added that the results should be treated with caution.

The results turnaround times for LC-MS/MS DOA screen is 1-2 days.

10.6.5 Organophosphorous (OP) compounds – screening for occupational exposure

Background

OP insecticides are usually supplied as powders or dissolved in organic solvents. All are absorbed through the bronchi and skin as well as through the gut. They inhibit cholinesterase activity and prolong / intensify the effects of acetylcholine. Toxicity varies between compounds and onset of symptoms may be considerably delayed after absorption through the skin.

Occupational monitoring (see also "Frequency of measurement", below)

Serial serum enzyme measurements (serum butyrylcholinesterase (BCHS) (formerly known as pseudocholinesterase) are used to monitor exposure. This is a liver enzyme with cholinesterase-like activity which is inhibited by OP compounds and falls before red cell acetylcholinesterase.

95% reference range:

Plasma butyrylcholinesterase: >5300IU/L

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Enzyme changes and symptoms

· A 30-40% reduction in serum activity occurs before symptoms are felt.
· A fall in enzyme activity can only be determined with certainty if a baseline activity is determined before exposure to OP begins (See "Frequency of measurement", below)
· In an accidental exposure a baseline needs to be determined after a 2-month exposure-free period.
· On average a drop of 80% is seen before serious neuromuscular effects become apparent.
· Near zero levels of enzyme activity require emergency treatment with agents such as atropine, to reverse the muscarinic effects, and pralidoxime mesylate an enzyme reactivator.
· NOTE that a small percentage of the population has genetically determined variants of plasma cholinesterase which manifest themselves as low levels of serum activity. It is not thought that these people are at extra risk from OP's.

Confounding factors affecting BCHS levels.

Decreased values are seen as follows: in pregnant patients, in patients with acute infections, pulmonary embolism, muscular dystrophy, post-MI, chronic renal disease as well as after surgical procedures.

Marginal increases are seen in thyrotoxicosis, haemochromatosis, obese diabetics and in some anxiety states.

Sample required.

5ml plain clotted blood

Frequency of measurement

1. Workers having occupational exposure to OP compounds should have plasma cholinesterase levels checked *before the season starts* (Note that there should be a minimum of **60 days** without exposure). *Monthly* monitoring is reasonable for most workers but for those in occupations where early symptoms such as blurred vision and impaired co-ordination could present a special hazard to the person affected, other employees or the general public (e.g. pilots involved in aerial spraying) a shorter interval should be considered.

2. Frequency of measurements will also depend on successive results. The verbatim HSE advice (MS 17 series leaflet) is as follows:

> "if, during routine monitoring, *plasma BCHS activity has fallen by >30% of pre-exposure levels*, the worker should be medically examined. The medical officer may then, at his own discretion, considering the nature of the work involved and the clinical symptoms, recommend that the worker be suspended from further exposure to OP compounds until considered fit to resume normal work. The rate of recovery of blood enzyme activity varies with the chemical structure of the OP compound to which the individual has been exposed. The return of enzyme activity to pre-exposure level may take up to 60 days. It is not necessary for pre-cholinesterase levels to be reached before resumption of normal work. The medical officer should base his decision on both clinical evidence and the results of biological monitoring."

3. The advice regarding red blood cell is less clear but its assay is said to offer little advantage with the qualifying statement ". in cases of over-exposure when the base line measurements are not available, measurements of both enzymes are desirable".

RBC CHS measurement can only be done by the Bristol Cholinesterase Investigation Unit, Southmead, Bristol Please seek advice from the Consultant Clinical Biochemist.

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OP poisoning

For advice in cases of suspected poisoning, any enquiries should be referred to www.toxbase.org or the Medical Toxicology enquiry lines at Guy's Hospital. For advice regarding potential antidotes, refer enquiries to www.bnf.org for the latest information. The HSE Guidance Note MS 17 is available for reference on the Pathology S-drive in folder Chem/Cholinesterase.

Note Pralidoxime is of no use against carbamate cholinesterase inhibitors (e.g., neostigmine or pesticides such as carbaryl)

Identification of a specific OP

The H & S Laboratory (Buxton) offers a urine dialkylphosphate analysis service using capillary GC which identifies a range of these OP metabolites. Sample requirement is for 25 mL of urine.

References

Burtis C A and E R Ashwood, eds. (1999). Tietz Textbook of Clinical Chemistry, 3rd edition. W B Saunders Company.
BMA and Royal Pharmaceutical Soc. of GB (1997). British National Formulary, number 37 (March 1999). Health and Safety Executive. Biological monitoring of workers exposed to organo-phosphorous pesticides. Guidance Note MS 17, revised October 1987.
Health and Safety laboratory. Guidance on laboratory Techniques in Occupational Medicine. 11th edition 2007. Crown copyright.
Toxbase Poison Information Service: www.toxbase.org

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10.6.6 Therapeutic Drug Monitoring

Most analyses are performed daily. Specific information can be obtained by contacting the laboratory. For a valid interpretation of the results obtained the following information **must** accompany each request:

Type of preparation, daily dose, time of last dose, time blood sample taken, other drugs being taken.

For meaningful interpretation, knowledge of time of the last dose is imperative. During long term therapy blood samples should be taken in the 'steady state', i.e., after treatment with a constant dose over at least 5 half lives and after the distribution phase is complete, which in the case of digoxin takes at least 6 hours. It is important, even in the case of drugs with long half lives, for sampling times to be consistent if you wish to make comparative measurements. Samples taken immediately before the next dose generally fulfil the above criteria and the quoted ranges refer to such a time. The re-sampling time quoted below is the time required to achieve new 'steady state' i.e., the earliest time after which samples should be taken following dose adjustments.

Therapeutic Ranges should only be used as a guide because other factors may alter the effect of a drug concentration at its site of action, e.g., protein binding, age, concurrent illness, pregnancy, renal failure, other medication, tolerance, and tissue receptors.

Caffeine

Since caffeine toxicity is much less troublesome than theophylline toxicity, and the simpler, more predictable dosage regimes, there is no routine service for caffeine monitoring. In the event of possible toxicity, or a lack of response to doses usually producing results in most patients, samples can be referred for analysis. Please contact the laboratory for more information.

Collection Time	Trough level
Other Names	NA

Therapeutic Range	15-30mg/L
Re-Sampling time	4 days
Half Life	40-230 hr

Carbamazepine

Collection Time	Trough level
Other Names	Tegretol
Therapeutic Range	4-10 mg/L
Time to steady state	2-6 days
Re-Sampling time	4 days
Half Life	25-45 hr single dose, 8-24 hr chronic dose

Digoxin

Collection Time	> 6hours post dose
Other Names	Lanoxin
Therapeutic Range	0.5-1.0 microgram/L
Re-Sampling time	14 days
Half Life	36-48 hours, (>100hours in anuria), 18-33 hrs (infants), 12-24 hrs(children)

The recommended target range for digoxin is 0.5-1.0 ug/L for patients being treated for heart failure. However, a range of 0.5-2.0 ug/L may be appropriate for some patients. There is an increased risk of digoxin toxicity, even if the level is within the therapeutic range, if there is hypokalaemia and note that renal impairment will increase blood digoxin concentrations secondary to decreased excretion.
Concentrations of more than 3.0 microgram/L are in the toxic range. Signs of toxicity may be found in some patients at levels between 2.0 and 3.0 microgram/L at 6 hours post-dose. Higher concentrations are tolerated by children.

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Lithium

Collection Time	Trough level, 12 hours post dose
Other Names	Priadel, Camcolit, Liskonum
Therapeutic Range	0.4-1.0 mmol/L*
Re-Sampling time	5 days
Half Life	4-45 hr increases with age and in renal disease.

Lithium monitoring

Dosing

Lithium should be administered **once daily**, preferably at night.

* Therapeutic range

Although the therapeutic range is from 0.4 - 1.0 mmol/L, most people should be run at values well below the upper limit, apart from during episodes of mania. A level of >1.5 mmol/L is considered toxic. Patients should have their therapeutic levels individualised and be maintained at the lowest effective serum concentration. There is good evidence to suggest that people can be adequately controlled at levels as low as 0.5 mmol/L.

Renal effects of lithium therapy:

1. Patients on long-term therapy appear to be susceptible to progressive impairment of concentrating ability.
2. Those on lithium having had previous treatment with neuroleptics experience worse concentrating ability. The functional lesion is not fully reversible on cessation of lithium therapy. Renal biopsy in these patients often confirms the presence of a focal interstitial nephropathy, but…
3. Similar lesions have been found in psychiatric patients never exposed to lithium.

4. Polyuria appears to be progressive for the first decade of lithium therapy.
5. Avoid thiazides for controlling polyuria, they cause volume contraction and subsequently increases sodium and lithium resorption. This may induce acute lithium intoxication (Amiloride has been used by some, although it has not gained widespread acceptance)
6. Lithium therapy is associated with an increased incidence of hypercalcaemia due to alteration in the renal handling of calcium and primary hyperparathyroidism, so calcium should be checked before starting therapy and 6-monthly thereafter.

Minimising the renal effects of lithium:

1. Assiduously avoid episodes of renal toxicity including lithium toxicity
2. Monitor lithium to achieve optimal efficacy at the lowest possible concentration.
3. Check serum creatinine 6-monthly and 24 hr urine volume (when polyuria is suspected)
4. If renal function deteriorates request full medical evaluation.

Monitoring thyroid function:
Hypothyroidism is quite commonly seen in lithium-treated patients and TSH should be checked 6-monthly.

Phenobarbitone

Collection Time	Trough level. Sampling time not important but consistency is needed.
Other Names	
Therapeutic Range	10-40mg/L*
Time to steady state	10-25 days (adult), 8-20 days (children)
Re-Sampling time	14 days
Half Life	100 hr, less in children.

* A therapeutic range of 10-20mg/L shows effective prophylaxis against febrile convulsions in children.

Interpret the therapeutic limits with flexibility as very low concentrations may have a significant anti-epileptic effect, particularly in those who have been on the drug for a long time. In those having **valproate** added in there may be a sharp increase in phenobarbitone, necessitating a dose reduction. Patients on Primidone should have phenobarbitone measured as the main active metabolite, and with the same reference range.

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Phenytoin

Collection Time	Trough level. Sampling time not important but consistency is needed.
Other Names	Dilantin, Epanutin
Therapeutic Range	< 13weeks: 6.0-14.0 mg/L, > 13weeks: 10.0-20.0 mg/L 4-24
Time to steady state	days
Re-Sampling time	Variable
Half Life	9-22 hours (single dose), 20-40 hours chronic therapy*
Max rate of elimination	100-1000 mg/day

Toxic symptoms appear with increasing severity at levels between 20 - 40 mg/L. At > 40 mg/L, there is severe toxicity with the possibility of permanent neurological damage.
* Half life may be up to 100 hours in chronic therapy. Half-life in **pre-term neonates** is about 75 hr, in **term neonates** about 21 hr. Half-life in **infants / children** is as short as 7.5 hr.

Some patients may need levels of > 20 mg/L to achieve seizure control. In the absence of unacceptable toxicity, dosages should not be altered as loss of seizure control may be precipitated.

A wide variety of drugs alter phenytoin metabolism, usually by induction or inhibition of the enzyme systems involved. Reduced absorption and displacement of phenytoin from binding proteins may also occur. Increase monitoring vigilance when such drugs are added or withdrawn (check BNF for individual details.)

Theophylline

Collection Time	Trough (oral dose), 30 mins after load (IV dose) or 4-6 hrs after continuous infusion.
Other Names	
Therapeutic Range	10-20 mg/L (5-10 mg/L in neonates)
Time to Steady State	2-3 days (adults); 1-2 days (children); 1-5 days (infants)
Re-sampling Time	
Half-life	3-12 hours (non-smoker), 4 hours (smoker), 2-10 hours (children)

Valproate

Collection Time	Trough
Other Names	Epilim
Therapeutic Range	50-100 mg/L
Time to Steady State	2-4 days
Re-sampling Time	
Half-life	6-17 hours (adults), 5-15 hours (infants and children), 15-60 hours (neonates <2 months) There is poor correlation between serum drug levels and clinical effectiveness. Monitoring has limited value in many patients unless querying compliance or toxicity

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10.7 POCT: Point of Care Testing

In line with the guidelines issued by the Joint Working Group on Quality Assurance and the MHRA, there are arrangements in place for managing for POCT with a POCT committee in each Trust. Each is currently chaired by a Consultant Clinical Scientist. The policies and terms of reference are aligned to ensure consistency across the whole Path Links area.

POCT management

Point of Care testing (POCT) of patient samples may be a valuable procedure used in patient care in NHS Hospital trusts. The rapid growth in POCT devices is largely a result of technological advances and such devices may increasingly form an important role in disease management.
There may be considerable benefits to patients by using the latest methodology to carry out tests in close proximity to patients. Non-pathology staff or the patients themselves may perform some of these tests. However, if not used appropriately there is a risk that patients may be put at risk from inappropriate testing or wrong results. Therefore, for the successful use of these devices it is essential that the purchase, implementation, continuing application of quality assurance and competency assessment be carried out appropriately. The appropriate use of these tests should be considered as a Clinical Governance issue and subject to examination of clinical effectiveness.

Which devices are categorised as "POCT Devices"?

Broadly speaking, these will include devices that are being used outside the laboratory to assay biological samples. Most usually the samples will be either blood or urine, but not samples of breath. The commonest generic types of POCT devices are as follows:
Blood Glucose Meters; Blood Gas Analysers; Bilirubin meters (SCBU); Urine Dipsticks of all kinds; Drugs of Abuse Urine Dipsticks; Urine Pregnancy Tests; Cardiac readers (Troponins, CK-MB, myoglobin); Haemoglobin meters; other analysers measuring a variety of analytes such as HbA_{1c}, cholesterol, sodium, potassium.

POCT committees in ULH & NLAG NHS Trusts

There is a POCT Committee within both NHS Trusts in Lincolnshire. These advise the Hospitals and Health Care Professionals within the Trust Hospitals on the appropriate use of POCT Devices in extra-laboratory areas. The POCT committee in the ULH Trust is directly responsible to the Trust's Clinical Effectiveness Committee and POCT committee in the NLAG Trust is responsible to the Governance committee. The Chairs of the POCT committee's report directly to these meetings. Staff representatives sitting on the POCT committees, together with their contact details are listed in the two Trusts' POCT Policy documents.

POCT policies and guidelines

In the ULH Trust there is POCT information available on the Intranet - http://ulhintranet/point-of-care-testing

Similarly, in NLAG Trust POCT information is also available via the Intranet –
http://nlgnet.nlg.nhs.uk/pathlinks/SitePages/Point%20of%20Care.aspx

Staff training and support

ULH

All policies and guidelines relating to POCT devices in ULH Trust are available on the Intranet web pages, these can be found by typing 'POCT' in the search bar of the ULHT main page. Use of POCT devices is outlined in the ULHT Point of Care Testing Policy on the Trust Intranet. Training and update sessions are arranged through contacting ulth.poct@nhs.net or in the case of the glucose meters through the sites Diabetes Specialist Nurse (DSN). Initial training is delivered by the POCT device manufactures training specialist. Further training is then delivered by an approved cascade trainer. Training records are kept on the data management systems for blood glucose meters and blood gas analysers. For any other POCT devices, training records and competencies are kept at ward and user level. Therefore, it is the ward and users' responsibility to keep these up to date, in line with the device relevant ULHT SOP/Policy. Representatives of all the Trust Directorates attend the Clinical Effectiveness Committee and Risk Management meetings and receive direct reports from the POCT committee meetings. The POCT committee meet bimonthly to discuss/review ULHT POCT, including the consideration of any new request for a POCT device within any ULHT site.

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NLAG

All policies and guidelines relating to point of care (POCT) devices in NL&G Trust are available in the Quick Links section under the Point of Care tab in the Trust Hub home page. Use of POCT devices is outlined in the NL&G Point of Care Testing Policy which is available on the POCT site.
Initial training is delivered by the POCT device manufacturer's training specialist. Further training is then delivered by an approved cascade trainer or can be organised by contacting the Point of Care team on nlg.tr-pointofcare@nhs.net .
Several blood gas analyser training events are held throughout the year and are advertised on the Hub. Training for glucose meters is arranged through each site's Diabetes Specialist Nursing Team (DSN) and training sessions are advertised on the Hub. Upcoming sessions can be found on the Diabetes and Endocrinology page.
Training records are stored on the data management systems for blood glucose meters and blood gas analysers. For other POCT devices, training records and competencies are stored at ward or user level. It is, therefore, the ward and users' responsibility to keep these records up to date.

At SGH the blood gas analysers are maintained by laboratory point of care staff, who also ensure the analysers are supplied with consumables. In DPOW analyser maintenance and troubleshooting is overseen by the Medical Engineering Department, with each area responsible for ordering their own consumables.
The Point of Care committee meets bi-monthly to discuss issues relating to POCT. Requests for any new POCT equipment must be made by submitting a POCT Device Request form to the Point of Care Committee as described in the POCT Testing Policy.

Internal quality control (IQC) and external quality assurance (EQA)

All users of POCT devices should use regular IQC solutions (see POCT Policy) and participate in an EQA scheme where available. There is a county wide glucose meter EQA programme run from the SGH/Goole laboratory, and recent implementation of connectivity-enabled meters has introduced mandatory internal quality control and user identification in hospital-based glucose meters.
Blood gas analysers in the Trusts are password protected and accessible only by competency assessed clinical staff. POCT equipment should only be used by competency assessed staff who receive annual training update days. Competence is recorded by means of a standard Competence proforma. This is completed by each user and signed off in the presence of the lead nurse or nurse manager. The signed proforma is kept by the individual staff member in their training portfolio. Training records are available on the POCT intranet sites. Capillary glucose results will be available on the ward-based results system, Web View, as will blood gas analyser results.

Glucose

Grey Capped Tube (gel serum sample is suitable only if < 2hours old on receipt in lab). This test is also available in the Radiometer ABL90 FLEX PLUS blood gas analysers - local data indicates no clinically significant difference between results. If more information is required, please contact your local Consultant Biochemist.

Potassium

Gel Tube. This test is also available in the Radiometer ABL90 FLEX PLUS blood gas analysers - local data indicates no clinically significant difference between results in non-haemolysed samples. If more information is required, please contact your local Consultant Biochemist.

Potassium is released from platelets during clotting. Literature[1] suggests that plasma and whole blood potassium concentrations are 0.1-0.7 mmol/L lower than those in serum.
[1] http://www.acb.org.uk/Nat%20Lab%20Med%20Hbk/Potassium.pdf
If more information is required then please contact your local Consultant Biochemist.

Sodium

Gel Tube. This test is also available in the Radiometer ABL90 FLEX PLUS blood gas analysers - local data indicates no clinically significant difference between results. If more information is required, please contact your local Consultant Biochemist.

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Lactate

Grey capped tube. Send immediately to the laboratory for centrifugation. This test is also available in the Radiometer ABL90 FLEX PLUS blood gas analysers - local data indicates no clinically significant difference between results. If more information is required, please contact your local Consultant Biochemist.

Haemoglobin

Lavender capped tube. This test is also available in the Radiometer ABL90 FLEX PLUS blood gas analysers - local data indicates no clinically significant difference between results. If more information is required, please contact your local Consultant Biochemist.

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SECTION 11	IMMUNOLOGY HANDBOOK

IMMUNOLOGY SAMPLE REQUIREMENTS

11.1 General serum samples

Most tests are performed on serum separated at room temperature; the exceptions are cryoglobulins. For serum, blood should be collected into a **white topped** 4ml Vacuette tube with no anticoagulant. **DO NOT** use EDTA tubes for serum tests as this invalidates some assays.

11.2 Cryoglobulin and cryofibrinogen

If cryoglobulins are suspected a red topped 4ml Vacuette tube (i.e., no additives, and no gel) and purple EDTA 4ml Vacuette tube *both pre-warmed* in water at 37°C should be used and the sample should be immediately delivered to the Laboratory during normal working hours in a thermos flask containing water at 37°C before 11.00 hr. Please contact the Immunology Department **in advance** if a Scunthorpe patient, or the Biochemistry Department if at another Path Links site. The laboratory keeps appropriate equipment to transport cryoglobulin samples if required. It is generally easier to transfer the patient to the phlebotomy department, where the samples can be taken and transported correctly. The assay is subject to external quality assessment.

11.3 Complement studies (not C3, C4)

Complement studies such as CH50/CH100, complement breakdown products, C3-nephritic factor and others require very special handling. **Do not request these tests without prior discussion** with the Immunology Consultant staff and arranging with the lab in advance. Samples will need to be collected and rapidly separated and frozen for transport.

11.4 Cellular assays

These samples deteriorate within hours, so do **NOT** send samples after 2PM on Friday afternoons or at weekends when the laboratory will not be able to process them. **Only** known HIV-positive patients can have routine CD4 counts, **all** other tests must be agreed with the laboratory in advance. CD4 counts need an EDTA tube without separating gel; other tubes may be required for other tests. **Cellular samples MUST NOT BE REFRIGERATED, store at room temperature at all times.**

11.5 Urgent ANCA/GBM tests

Requests for these **must** be telephoned to the laboratory in advance (01724 303716) for discussion with a senior member of staff who will assess the urgency of the test with you and will discuss sample transport arrangements. You will need to take the sample to your pathology department **by hand** and they will arrange for urgent transport to Immunology.

11.6 Special situations

If the test is not listed in this handbook, it is very likely that special sample conditions, and/or tubes, and/or transport conditions will be needed – so please discuss **in advance** with senior lab/clinical staff so that patients will not be bled unnecessarily.

Test repertoire

Note on assay names used in this list.

Autoantibodies are listed under the antigen part of their name, so nuclear antibodies, rather than anti-nuclear antibodies.
Specific IgE tests are listed under IgE, rather than by allergen.
Alternative names and standard abbreviations are also shown.

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A

Acetylcholine receptor antibodies (AChR)

Background

IgG Anti-AChR antibodies are found in 80-90% of subjects with myasthenia gravis, and 60% of those with ocular myasthenia. They are virtually never found in the normal population but are seen in 3-10% of unaffected first-degree relatives of myasthenic patients. 50% of patients with non-myasthenic thymoma are positive, and in 4% of patients on penicillamine with no clinical evidence of myasthenia. Most neonates born to seropositive myasthenic mothers are transiently anti-AChR positive due to passive transfer of maternal immunoglobulin, however only a small percentage develop clinical neonatal myasthenia.
Note: MuSK antibodies are tested alongside AChR antibodies as they may be useful in patients with a convincing history of myasthenia who are negative for AChR antibodies. Clinical assays for antibodies to LRP4 are not yet available.
Note: acetylcholinesterase is a different test, for 'scoline apnoea'/cholinesterase deficiency. Please see the Biochemistry handbook for details.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Sent to a reference laboratory (**1**); positive/negative screen result followed by quantitation if positive result seen, normal ranges will be printed on the result form.
Turnaround: ACR – 34 days, MuSK 26 days

Important factors affecting the result.

Immunosuppressive treatment and protein loss may reduce autoantibody levels; intravenous immunoglobulin preparations given therapeutically may themselves contain autoantibodies. The numerical imprecision of this assay is available on request.

Adrenal antibodies

Background

Autoantibodies in Addison's disease, autoimmune endocrinopathies, premature ovarian failure and gonadal failure have been described and there is a degree of cross reactivity between these antibodies which react with adrenal cortex, various ovary cells and the anterior pituitary. Note that adrenal insufficiency can be present without these autoantibodies.

The antigens are the cytochrome P450 enzymes in the steroid biosynthetic pathway.

Adrenal antibodies are seen in isolated Addison's disease (approx 60% of cases but are not required to make the diagnosis of Addison's), and with antibodies to ovary cells in primary ovarian failure. Multiple antibodies can be seen in autoimmune polyendocrinopathies.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Results are reported as positive or negative.
Turnaround: within two weeks

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunofluorescence assay, so are subject to inter-operator variability. Internal controls are always used, and the assay is subject to a sample share quality assessment.

Allergy tests
See IgE – specific, tryptase, and investigation section.

ANCA
See Neutrophil cytoplasm antibodies.

Autoantibodies
There are over 100 different autoantibodies known. Simply requesting an 'autoantibody screen' will result in us undertaking an antinuclear antibody test only. We will try to undertake additional relevant tests if we can discern these from the clinical details provided – but where possible please specify exactly which tests you require. We are always happy to discuss the most effective testing strategy with you in advance.

B

B cells
See lymphocyte subsets.

B27
See HLA-B27 test.

Bence Jones proteins (BJP)
See paraproteins (urine)

Beta-2-glycoprotein-1 antibodies

Background
Beta-2-gp-1 is a phospholipid binding protein and antibodies directed against this antigen are seen in patients suffering from the Anti-Phospholipid Syndrome (APS). Currently there is little advantage to measuring these antibodies in preference to anti-Cardiolipin antibodies. They are recommended in patients suspected of having APS in whom the Lupus Anticoagulant and anti-cardiolipin assays are negative. Further advice about these antibodies is available from the British Society for Haematology 2012 guidelines: http://www.bcshguidelines.com/documents/antiphospholipids_2012.pdf

Sample
Plain serum (4 ml white topped tube filled to line)

Results
Negative 0-20 CU (units)
Turnaround: within 5 working days

Important factors affecting the result.
Immunosuppressive treatment and protein loss may reduce autoantibody levels; intravenous immunoglobulin preparations given therapeutically may themselves contain autoantibodies. The numerical imprecision of this assay is available on request from the reference laboratory.

C

C1 inhibitor (C1 esterase inhibitor, C1INH)

Background
Deficiencies of C1inhibitor can be hereditary (HAE, hereditary angioedema) or acquired (AAE). Both cause episodes of angioedema, so this is an important investigation in patients presenting with pure angioedema (but not in those with urticaria and angioedema), and both can also present as recurrent abdominal pain. There are at least two forms of HAE, type I has low levels of C1inh, type II has normal or high levels of a dysfunctional protein. Hence both quantitative and functional assays are required. This laboratory uses the presence of normal complement C3 with low (<0.10 g/L) C4 as a screening test; samples with normal C4 levels are not usually tested for C1inh quantity or function, unless special arrangements have been made in advance. Acquired forms can be due to connective tissue diseases or lymphoproliferative disorders. Both HAE and AAE can present in adult life, but AAE is more likely in elderly patients. Abnormal results require confirmatory testing and discussion with immunology clinical staff.

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Sample

Plain serum (4 ml white topped tube filled to line)

Results

Sent to a reference laboratory (**1**)
Reported as quantity (g/L) and function (%)
Abnormal results will all receive clinical comments.
Samples with normal C4 values will not usually be tested.
Turnaround: within one month.

Important factors affecting the result.

Complement proteins degrade rapidly so sampling, transport and storage conditions can significantly affect the result. These assays are run by a reference laboratory – performance characteristics of the test are available on request.

C1q antibodies

Background

Antibodies against the first component of the classical pathway, C1q, are seen in some forms of systemic lupus erythematosus, and in hypocomplementaemic urticarial vasculitis. This assay is performed off-site by a reference laboratory. It is not generally useful in patients with normal C3, C4 levels.

Sample

Plain serum (4 ml white topped tube filled to line)
Requires discussion with lab in advance.

Results

Sent to a reference laboratory (**1**); normal ranges will be printed on the result form.
Turnaround: within 6 weeks.

Important factors affecting the result.

Immunosuppressive treatment and protein loss may reduce autoantibody levels; intravenous immunoglobulin preparations given therapeutically may themselves contain autoantibodies. The numerical imprecision of this assay is available on request from the reference laboratory.

Cardiac muscle antibodies

Background

Antibodies to cardiac muscle are seen in myocarditis, idiopathic dilated cardiomyopathy, rheumatic carditis and Dressler's syndrome.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Sent to a reference laboratory (**1**); normal ranges will be printed on the result form.
Turnaround: within two weeks

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunofluorescence assay, so are subject to inter-operator variability. Internal controls are always used, and the assay is subject to external quality assessment.

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Cardiolipin antibodies (anti-phospholipid antibodies)

Background

Anti-cardiolipin antibodies (ACA) are autoantibodies to positively charged phospholipids and are found in 90% of patients with the anti-phospholipid syndrome (Hughes Syndrome). They are closely related to, but not identical to the antibodies which cause a false positive VDRL and the lupus anticoagulant (LA) phenomenon. Because anticardiolipin antibodies, false positive VDRL and the lupus anticoagulant represent overlapping but not identical populations of antibodies, ACA and LA should be measured when the anti-phospholipid syndrome is suspected.

Clinical features of the anti-phospholipid syndrome include recurrent abortions, unexplained venous and arterial thromboses, thrombocytopaenia, livedo reticularis and neurological abnormalities (particularly chorea, stroke, psychosis, myelopathy, and convulsions). Anti-phospholipid antibodies have also been reported to be associated with various other conditions. The anti-phospholipid syndrome consists of any combination of these abnormalities in the presence of anti-cardiolipin antibodies, lupus anticoagulant or persistently false positive VDRL. It may occur, particularly in young females, in the absence of any connective tissue disease (primary anti-phospholipid syndrome) or more commonly when complicating SLE or any other connective tissue disease.

Raised anti-cardiolipin antibodies may also be seen in patients taking drugs such as chlorpromazine, patients with underlying malignancy, and transiently following infection or pulmonary emboli. *Persistently elevated titres of anti-cardiolipin antibodies, over at least 12 weeks, are needed to support a diagnosis of anti-phospholipid syndrome.* It has been noted that the level of anti-cardiolipin antibodies *may fall* during a thrombotic event or miscarriage. Thus, a normal result obtained at this time does not rule out the anti-phospholipid syndrome and repeat testing during convalescence (at least 6-8 weeks later, ideally 12 weeks) is needed.

All new positive results must be confirmed by a repeat test 12 weeks later. Single episodes of positive results *do not* make the diagnosis of an antiphospholipid antibody syndrome. Further advice about these antibodies is available from the British Society for Haematology 2012 guidelines: http://www.bcshguidelines.com/documents/antiphospholipids_2012.pdf

Sample

Plain serum (4 ml white topped tube filled to line) (also consider testing lupus anticoagulant, needs a citrated sample)

Results

Negative 0-20 CU (units)
Turnaround: within 5 working days

Important factors affecting the result.
Immunosuppressive treatment and protein loss may affect autoantibody levels. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

CCP antibodies

See cyclic citrullinated peptide antibodies.

CD4 counts.

See lymphocyte subsets.

Centromere antibodies

Background

Anti-centromere antibodies are found in about 70% of patients with CREST syndrome or limited cutaneous scleroderma. This pattern is also seen in 10% of patients with generalised scleroderma and is usually associated with a relatively good prognosis. Also seen in primary Raynaud's and primary biliary cirrhosis, as well as in first-degree relatives of patients with CREST syndrome. Patients usually remain positive for life, so repeated testing of known positive patients is not necessary. However, 40% of patients with scleroderma do not have either centromere or Scl-70 antibodies.

Sample

Plain serum (4 ml white topped tube filled to line)

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Results

Reported as negative, or positive with titre.
Turnaround: one week

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunofluorescence assay, so are subject to inter-operator variability, typically of ±1 titre dilution step (i.e., 1/40 could be 1/20 – 1/80). Internal controls are always used, and the assay is subject to external quality assessment.

Complement C3, C4

Background

C3 is common to both alternative and classical pathways, and C4 is part of the classical complement pathway. C3 and C4 are usually normal in complement deficiency – so are not used to test for complement deficiencies (see classical pathway tests). Total C3 and C4 are useful in the investigation and monitoring of SLE, immune-complex diseases (including cryoglobulins), some glomerulonephritides, angioedema and some vasculitis syndromes.
The C3 and C4 together are useful for diagnostic purposes:
Low C3 & C4 together occur in sepsis, SLE, rheumatoid vasculitis, or urticarial vasculitis.
Low C3 with normal C4 is seen in alternative pathway complement consumption. Consider Gram negative sepsis, post streptococcal glomerulonephritis, the presence of C3 nephritic factor or endocarditis.
Low C4 with normal C3 suggests cryoglobulins, SLE or hereditary angioedema.
High C3 or C4 indicates an acute phase response. Many complement components are synthesised in the liver. Hence in extreme cases of hepatic failure and following hepatic transplantation low complement values may be recorded.
Serial measurements of C3 and C4 are useful in monitoring established SLE; sudden reductions can indicate a lupus flare-up.

Sample

Plain serum (4 ml white topped tube filled to line)
Complement components readily degrade, so although C3 and C4 are relatively stable, samples should be sent to the laboratory promptly.

Results

Reported as quantity.
C3 0.90-1.80 g/L
C4 0.10-0.40 g/L
Paediatric normal ranges not defined – adult values are used.
Abnormal values receive clinical comments when clinical details are provided.
Turnaround: within 3 days

Important factors affecting the result.

Complement proteins degrade rapidly so sampling, transport and storage conditions can affect the result. The immunoassay used is subject to external quality assessment. Performance characteristics of the test are available on request.

Complement classical and alternative pathway tests (CH50, CH100, AP50, AP100)

Background

These are the tests for complement deficiencies. Classical pathway assays such as the CH100 measure the integrity of the entire pathway from activation to formation of the membrane attack complex. This test is now only used when a complement deficiency syndrome is suspected (e.g., in recurrent meningococcal meningitis). The alternative pathway tests should be used if properdin deficiency is suspected (e.g., recurrent meningitis in brothers).
Both tests require special samples, special transport conditions and must be discussed with senior immunology staff in advance.
These tests are no longer used to monitor SLE activity.

Sample

Sent to a reference laboratory (**1**); Tests must be discussed with senior lab staff in advance – serum samples are needed. Arrangements will be made for rapid plasma and serum separation and freezing.

Results

Quantitative values are reported.

Absent CH100 or AP100 suggests a complement deficiency – but must be repeated to confirm, and then further tests would be needed to identify which complement component is defective.
Turnaround: may take several weeks

Important factors affecting the result.

Complement proteins degrade rapidly so sampling, transport and storage conditions can significantly affect the result. These assays are run by a reference laboratory – performance characteristics of the test are available on request.

Cryoglobulins and cryofibrinogen

Background

Cryoproteins are serum proteins which reversibly precipitate at temperatures below 37C. There are two types of cryoproteins; cryoglobulins and cryofibrinogens. Cryoglobulins are immunoglobulins which precipitate in both serum and plasma whereas cryofibrinogens, which contain fibrinogen-fibrin complexes, only precipitate in plasma. There are three types of cryoglobulin: **Type I** is a monoclonal cryoglobulin (i.e., a paraprotein). **Type II** are monoclonal paraproteins in association with polyclonal immunoglobulins due to rheumatoid factor activity. **Type III** are polyclonal immune complexes in the absence of monoclonal immunoglobulin.
Cryoglobulins are found in a wide spectrum of disorders but are often transient during viral or bacterial infection. Cryoprotein studies are indicated in any patient showing clinical manifestations which include intolerance of cold with pain in exposed areas. Clinical manifestations affect the skin, peripheral nerves and most seriously the kidneys. Type I and II having a monoclonal component require investigation for B cell lymphoproliferative disorders. There is an association with hepatitis C in some cases. Cryofibrinogen presents clinically like type I cryoglobulin but precipitate is seen in plasma samples only. Cryofibrinogen can also be artefactually induced by heparin.

Sample

Sampling conditions are critical – if the sample cools at all between venepuncture and processing in the laboratory, the cryoglobulin will precipitate out and the result will be negative, therefore if a cryoglobulin is strongly suspected, particularly if complement testing shows normal C3 and low C4, the test should be repeated with scrupulous attention to detail.
Samples need to be collected into pre-warmed tubes and transported in a flask.

Results
Results are reported as negative, or positive with type.
Turnaround: within two weeks (one week refrigeration required)

Important factors affecting the result.

This assay is critically dependent upon good sampling technique and maintenance of sample temperature at 37°C. Unexpected negative results should be repeated. Positive results are confirmed by immunofixation so as to prevent misinterpretation of other cryo-proteins e.g., cryofibrinogenaemia.

Cyclic citrullinated peptide (CCP) antibodies

Background

Antibodies against cyclic citrullinated peptides (CCP) may be used in the diagnosis of rheumatoid arthritis (RA). CCP antibodies are now known to be the same as several other autoantibodies which were thought to be associated with RA including antiperinuclear factor, filaggrin antibodies and keratin antibodies. Manufacturer's data for this second-generation assay suggests that positive results (>10 U/ml) have approximately 80-88% sensitivity and >95% specificity for RA. It is important to appreciate that the test does **not** indicate disease activity, and is unlikely to change with time, so does not need repeating in the same individual. CCP antibodies are useful when there is diagnostic difficulty in making a firm diagnosis of RA but are not necessary to measure in clear-cut classical rheumatoid arthritis. CCP antibodies give different information to rheumatoid factor (see below). Negative CCP antibodies in RF-negative RA patients who have responded to treatment, may indicate that a trial of withdrawal of therapy is appropriate.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as concentration (U/ml)
Positive >17 U/ml
Turnaround: within one week.

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

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D

Dihydrorhodamine test (DHR)

Background

Dihydrhodamine (DHR) assay measure's neutrophil function and the functional activity of the enzyme NADPH oxidase. Stimulated neutrophils will oxidise DHR to rhodamine by respiratory burst mechanism. Rhodamine is a measurable fluorescent product. Reduced neutrophil function is associated with chronic granulomatous disease (CGD). CGD results in failure to thrive and has a poor prognosis due to failure in intracellular killing resulting in formation of granulomas, especially in the liver, lungs, and gut. There are X-linked and autosomal recessive forms of CGD with a mortality rate of 20% in 10 years for the X-linked form and 35% in 10 years for the autosomal recessive form. Prognosis improves with early diagnosis and treatment. CGD should be suspected in children with chronic abscesses, recurrent staphylococcal infections, colitis, aspergillous infections or Pseudomonas cepaciae infections. The NBT test **must** be discussed with immunology clinical staff beforehand as this test must be booked in advance with the laboratory.

Sample

EDTA sample (purple topped tube *without* gel) with separate control sample taken at the same time from non-family member. Sample must reach the lab during working hours Mon-Thurs as previously discussed and arranged with a senior BMS. They **MUST NOT** be sent after 2pm on Thursday afternoons, Fridays or at weekends. Please note that a sample from a healthy normal non-family member volunteer will also be required. Sent to a reference laboratory (**1**).

Results

Reported as % of stimulated and unstimulated neutrophils of patient and control samples.
The samples will be processed immediately due to the nature of the samples and results will be available within 2 weeks .

Important factors affecting the result.

If the patient is hyperglycaemic or septic this will invalidate the test. Patients with G6PD deficiency may also invalidate the test. If abnormal, this test is not diagnostic of CGD but will have to be repeated with alternative confirmatory tests.

Double-stranded DNA antibodies

Background

Antibodies to double-stranded DNA (dsDNA) are seen in systemic lupus erythematosus (SLE) and to a lesser extent, in autoimmune hepatitis type I. Not all patients with SLE have dsDNA autoantibodies. To some extent, the antibody level indicates disease activity, but this should be gauged by clinical assessment, in combination with other markers such as complement C3, C4 values. Antibody levels against dsDNA may rise prior to a 'flare' of SLE and usually fall suddenly during the flare. Increases of >20% suggest a flare is imminent, and closer patient monitoring is recommended. In some individuals with SLE, dsDNA antibodies are always high, and in this case do not reflect disease activity.
Samples positive for dsDNA are also tested using *Crithidia* as a substrate; samples which are Crithidia-positive contain high-affinity dsDNA antibodies and are more closely linked to SLE; Crithidia-negative samples contain low-affinity antibodies and the association with SLE is poor. The Crithidia test is performed on first time positive dsDNA-positive samples. Testing is not routinely performed on ANA-negative samples.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Negative 0-27 IU/ml
Crithidia results are reported as positive or negative.
Turnaround: within 13 working days

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

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E

Electrophoresis

See Paraproteins

Endomysial Antibodies

Background

Endomysial antibodies of the IgA type are associated with coeliac disease. In this laboratory, samples are initially tested for tissue transglutaminase antibodies, and the endomysial antibody test is used to confirm positive results; requests for 'endomysial antibodies' will be screened for tTG antibodies first. Please see tTG antibodies for guidance with this test. IgG endomysial antibodies are tested when the patient has low IgA.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Are reported as positive or negative.
Turnaround: within one week

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunofluorescence assay, so are subject to inter-operator variability. Internal controls are always used, and the assay is subject to external quality assessment.

Extractable nuclear antigen (ENA) antibodies

Background

Antibodies against extractable nuclear antigens are seen in a range of connective tissue diseases. Examples of ENA include Ro, La, Sm, U1RNP, Jo-1 and Scl-70. The type of ENA detected is useful for diagnosis but not for disease monitoring. All samples for ENA testing are screened with an antinuclear antibody test first, using a sensitive substrate called Hep2 cells. Only ANA-positive samples are tested for ENA antibodies as our method of detection is sensitive enough to detect all clinically relevant ENA antibodies. ENA testing uses a sensitive screening assay, with positive samples undergoing further testing to identify the type of ENA detected. Once ENA are detected, it is not usually necessary to repeat the test, as the antibodies usually remain positive and subsequent positivity is not usually related to disease activity. Please see individual ENA antibodies for more information. Rare and unusual ENA antibodies may be available from reference laboratories by prior discussion.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Only samples positive for antinuclear antibodies are tested.
Results are reported as negative, or individual positives are identified by name.
Turnaround: within 13 working days.

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

F

Free Light Chains – serum (sFLC)

Background

The assays should be used at diagnosis in all new cases of plasma cell dyscrasias and be used at follow up of non-secretory, oligosecretory or light chain myeloma. They are also useful is cases of myeloma relapse, solitary plasmacytoma, smouldering (asymptomatic) myeloma in monitoring monoclonal gammopathy of undetermined significance and in primary amyloidosis.

The NICE Guidelines on Myeloma: diagnosis and management. the diagnosis of myeloma is available here: https://www.nice.org.uk/guidance/ng35

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Sample
Plain serum (4 ml white topped tube filled to line)

Results
Reported as mg/L.
kappa sFLC: 3.3 – 110.4 mg/L
lambda sFLC: 5.7 – 26.3 mg/L
□/□ ratio: 0.26 – 1.65
Turnaround time is within 1 week.

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

G

Ganglioside antibodies

See glycolipid antibodies.

Gastric parietal cell antibodies (GPC)

Background

Antibodies to gastric parietal cells (GPC) are seen in autoimmune gastritis and pernicious anaemia (~90%). However, they can also be detected in other autoimmune diseases, and in the healthy elderly population. We use GPC antibodies as a screening test for pernicious anaemia – positive samples are automatically tested for intrinsic factor antibodies (which are a more specific, but less sensitive test for pernicious anaemia).

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as positive or negative.
Turnaround: within one week

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunofluorescence assay. The assay is subject to external quality assessment.

Glomerular basement membrane antibodies (GBM)

Background

Antibodies against type IV collagen are seen in Goodpasture's syndrome, a pulmonary-renal form of vasculitis associated with rapidly progressing renal failure and haemoptysis. Anti-GBM antibodies are available during routine working hours as an urgent assay, but please see the procedure to follow for urgent test requests. About 10% of patients with Wegener's granulomatosis may also have GBM antibodies. Performance characteristics of the test are available on request.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Negative 0-20 CU (units)

Turnaround: available urgently within 6 hours during the working day, or routinely within 5 working days.

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Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

Glutamic acid decarboxylase antibodies (GAD)

Background

Antibodies against glutamic acid decarboxylase (GAD) are seen in stiff-person syndrome (aka stiff man syndrome) and in the early phase of type 1 diabetes mellitus. The type of antibodies found in each disease are different: GAD antibodies in stiff person syndrome are only found in about 60% of patients and are against a 65kDa linear epitope; GAD antibodies in type 1 diabetes are against conformational epitopes of 65kDa GAD. GAD antibodies cannot be used to reliably distinguish type 1 from type 2 diabetes and are lost once diabetes has become established. Islet cell antibodies may be a suitable alternative autoantibody. **GAD antibodies are not available as a routine test in established diabetes** but are available following discussion and with appropriate clinical details: approximately 70-90% of adult patients with latent autoimmune diabetes in adults (LADA) have GAD antibodies, as do small percentages of patients with type 2 diabetes.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Sent to a reference laboratory (**1**); normal ranges are printed on the result form.
Turnaround: within one month

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Internal controls are always used, and the assay is subject to external quality assessment by the reference laboratory. Performance characteristics of the test are available on request.

Goodpasture antibodies

See glomerular basement membrane antibodies.

Glycolipid antibodies (ganglioside antibodies)

Background

Antibodies to glycolipids are associated with a wide range of neurological disorders including Guillian-Barré syndrome and the Miller-Fischer variant, chronic demyelinating polyneuropathies, and multifocal motor neuropathy. These antibodies are not diagnostic of individual conditions but can support a clinical diagnosis. The nomenclature of individual antibodies refers to the different types of glycolipids they bind. The concentration and class of antibody is important in interpreting the results, and a clinical comment will be appended to positive results.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Sent to a reference laboratory (**3**)
Reported as negative or positive with class of antibody and titre, and clinical comment.
Turnaround: 10 days

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

H

Haemophilus influenzae type b antibodies (Hib antibodies)

Background

IgG antibodies against *H influenzae* type b are used to investigate specific antibody defects in patients with recurrent respiratory tract infections but normal total immunoglobulin levels. Although a polysaccharide antigen, Hib is conjugated to a protein, so IgG anti-Hib responses are not a true indication of the response to T-independent antigens. These tests are usually only available following specialist advice in the work-up of patients with recurrent infections.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Sent to a reference laboratory (**1**)
Reported as concentration; paediatric normal ranges are not defined; clinical comments will be given where necessary
Turnaround: within one month

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect antibody levels. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

HLA-B27 test

Background

The major histocompatibility complex antigen HLA-B27 is expressed in approximately 8% of healthy Caucasians, approximately 90% of patients with ankylosing spondylitis, and is a non-specific association with many other diseases and syndromes. This means that HLA-B27 is not a diagnostic test for ankylosing spondylitis and plays no role in the routine work-up of back pain. We use a flow-cytometric assay for B27 testing which means that samples will degrade if not received and processed within 48 hours.

Sample

EDTA sample (purple topped tube without separating gel).
Must be processed within approximately 72 hours.

Results

Results are expressed as positive or negative.
Equivocal results from the flow cytometric assay are sent to a reference laboratory (**4**) for molecular typing.
Turnaround: within one week (longer if molecular typing required)

Important factors affecting the result.

Sample storage and transport conditions can significantly affect cell viability, in particular samples must not be refrigerated. This flow-cytometric assay is subject to external quality assessment. Performance characteristics of the test are available on request.

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Hu antibodies

See Purkinje antibodies.

I

Immunoglobulins (IgG, IgA, IgM)

Background

Serum immunoglobulin levels have numerous diagnostics uses, including testing for suspected antibody deficiency syndromes, myelomas and checking for chronic inflammatory responses. Serum IgG, IgA, and IgM levels are determined by turbidimetry, and all samples also undergo electrophoresis to check for paraproteins. A urine can also be sent for monoclonal free light chain analysis (Bence Jones proteins). Clinical details are particularly important for the laboratory to provide interpretation of immunoglobulin results. If a paraprotein (monoclonal immunoglobulin) is detected, the type will be determined, and it will be quantified. Note that the presence of a paraprotein is not in itself diagnostic of myeloma, and because 20% of myelomas only

secrete light chains in the urine, the absence of a serum paraprotein does not exclude myeloma. If IgA deficiency is detected, confirmatory tests will be done by a reference laboratory (4). The 2014 BCSH Guidelines for diagnosing myeloma are available here: https://b-s-h.org.uk/guidelines/?search=M+protein and https://www.nice.org.uk/guidance/ng35

Sample

Plain serum (4 ml white topped tube filled to line)
DO NOT SEND uncoagulated (Li-Heparin or EDTA) samples– they will produce a false positive band on electrophoresis.

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Results

Immunoglobulin concentrations are reported for IgG, IgA, and IgM in g/L.
The normal range is age-dependent and is printed on the report.
Features of the electrophoresis are reported qualitatively (e.g., normal, low, raised) and paraproteins are typed and quantified.
Turnaround: within one week (longer if complex typing required)

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect antibody levels. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

Iglon5 antibodies

Background

IgLON5 is a member of a family of adhesion molecules used in the brain for neuronal connectivity.

Antibodies against IgLON5 have been found in patients showing acute and chronic sleeping disorders, gait instability and dysphagia.

Sample

1ml serum (serum preferred but CSF acceptable)

Results

Referred to a reference laboratory (5); Reported as negative or positive.

Turnaround: 14 days

Immunoglobulin G subclasses (IgG subclasses)

Background

IgG subclasses are not available as a routine assay. Although it was initially thought that IgG subclass deficiencies explained infections in patients with normal total IgG levels, the link is now disputed. Testing for antibody levels against specific pathogens has now superseded IgG subclass testing except for a few rare indications such as diagnosing lymphocytic pancreatitis. All IgG subclass tests requests should be discussed with the laboratory in advance.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Sent to a reference laboratory (**1**)
Age-related normal ranges for each isotype are printed on the result form.
Turnaround: within one month

Important factors affecting the result.

Immunosuppressive treatment and protein loss may reduce IgG subclass levels; intravenous immunoglobulin preparations given therapeutically may themselves contain autoantibodies. The numerical imprecision of this assay is available on request from the reference laboratory. The assay is subject to external quality assurance. Performance characteristics of the test are available on request.

Immunoglobulin D (IgD)

Background

IgD is polyclonally raised in children with the rare hyper-IgD syndrome. This is not a routine assay and is only available following discussion with the laboratory.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Sent to a reference laboratory (**1**)
Age related normal ranges will be printed on the result form.
Turnaround: within one month

Important factors affecting the result.

Immunosuppressive treatment and protein loss may reduce IgD levels. Performance characteristics of the test are available on request.

Immunoglobulin E (Total IgE)

Background

Total IgE levels do not indicate whether a person has or has not got allergies but are useful in the interpretation of IgE levels against specific allergens, for example latex allergy (see below). Very high total IgE levels (e.g., >1000 kU/L) may cause false-positive specific IgE tests; conversely, low levels of IgE (e.g., <10 kU/L) may cause false negative specific IgE tests. Total IgE is useful in monitoring allergic bronchopulmonary aspergillosis and Churg Strauss syndrome.
IgE may be elevated in atopic individuals (asthma, eczema, rhinitis) as well as in parasite infections (hookworm, schistosomiasis, filariasis, larva migrans), lymphoma (especially Hodgkin's disease), liver disease, Churg Strauss syndrome, EBV infection, systemic sclerosis, bullous pemphigoid, Wiskott-Aldrich syndrome, Ommen's syndrome and Job's syndrome.
Total IgE is not routinely measured in screening for allergy to aeroallergens.

Sample

Plain serum sample (4 ml white topped tube filled to line)

Results

Reported in kU/L
An age-related normal range is printed on the result form.
Turnaround: within 5 working days.

Immunoglobulin E (Allergen-specific IgE, RAST)

Background

IgE against specific allergens ('specific IgE') tests used to be called 'RAST tests but a different type of assay is now used. A very wide range of allergens are available including most inhalant allergens and foodstuffs. The allergens to be tested must be written on the request form; general screening tests are not available, and if not quoted or indecipherable the sample will be stored for up to two weeks pending clarification.
Note in the presence of a convincing history, a negative specific IgE test does not necessarily exclude allergy.
Following anaphylaxis, specific IgE testing should be delayed for approximately 6 weeks.
Skin prick testing may be required – see Clinical Immunology/Allergy service for referral.
Specific IgE testing to drugs is only available for penicillin and lidocaine, but is very insensitive, so a positive result is helpful, but a negative result does not exclude allergy.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Results are given in kAU/L.
<0.35 kAU/L is negative.

Turnaround: within 5 working days unless an unusual allergen is required, when the sample may have to be sent to a referral laboratory

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect antibody levels. Recent anaphylaxis may depress allergen specific IgE levels. Internal controls are always used, and the assay is subject to external quality assessment. A high total IgE (typically >1000kAU/L) may cause false positive results, low total IgE (<2kAU/L) may cause false negative results. Performance characteristics of the test are available on request.

Intrinsic factor antibodies

Background

Intrinsic factor antibodies are seen in pernicious anaemia. The test is insensitive, with only approximately 50% of patients with pernicious anaemia having intrinsic factor antibodies, but the test is very specific for this disease. For this reason, gastric parietal cell antibodies are used to screen for pernicious anaemia, and intrinsic factor antibodies are used as a confirmatory test accepting that not all patients with pernicious anaemia will have intrinsic factor antibodies. Only GPC-positive samples will be tested for intrinsic factor antibodies unless there are specific reasons given on the request form.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as positive or negative.
Turnaround: within 8 working days
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Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

Islet cell antibodies (ICA) (pancreatic islet cell antibodies)

Background

Antibodies to pancreatic islet cells are transiently positive in patients with type 1 diabetes mellitus. In first degree relatives of patients with this condition, the antibodies are associated with a high risk of developing diabetes. (Relative risk 75). As the antibodies are lost within the first year of onset, they are not a reliable way to distinguish type 1 from type 2 diabetes. **This test is not available as a routine test but** is available following discussion and when appropriate clinical details are provided. Approximately 50-70% of adults with latent autoimmune diabetes of adults (LADA) have ICA, as well as small numbers of patients with type 2 diabetes.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as positive or negative.
Turnaround: within two weeks

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunofluorescence assay, so are subject to inter-operator variability. Internal controls are always used, and the assay is subject to external quality assessment.

Isoelectric focussing (oligoclonal bands)

See Oligoclonal Bands

J

Jo-1 antibodies

Background

Antibodies to Jo-1 are a type of ENA antibody and are detected using the ENA screen followed by a confirmatory test. Jo-1 antibodies are seen in some cases of myositis, particularly overlapping with Raynaud's and interstitial lung disease. This 'anti-synthetase' syndrome can be associated with other very rare autoantibodies, so if clinically suspected please mention this on the request form as additional tests are also available. Jo-1 antibodies should be specifically requested as the ANA screening test is insensitive for Jo-1 antibodies.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as positive, equivocal, or negative.
Turnaround: within one week

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunoblot assay, so are subject to inter-operator variability. Internal controls are always used, and the assay is subject to external quality assessment.

K

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L

Latex allergy test

Background

Latex allergy should be suspected in individuals who have features of an IgE-mediated immediate type hypersensitivity reaction (e.g., asthma, urticaria, angioedema, anaphylaxis) on contact with latex. Please see the Trust's latex allergy policy for more information. Screening for latex allergy is only undertaken in patients with a high probability of latex allergy, not on unselected patients. A positive result in a patient with an appropriate clinical history is suggestive of latex allergy. If the total IgE is <100 iu/mL the test for specific IgE against latex becomes insensitive, so in that case negative results do not exclude allergy, and skin prick testing may be required (available through the clinical immunology/allergy service).

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as level in iU/mL, <0.35 is negative.
Turnaround: within one week

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect antibody levels. Recent anaphylaxis may depress allergen specific IgE levels. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

Liver kidney microsomal antibodies (LKM)

Background

Antibodies to microsomes in liver and kidney tissues are seen in autoimmune hepatitis type 2. This autoantibody is seen as a characteristic pattern on indirect immunofluorescence, and positive results are confirmed using a specific immunoblot assay.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as positive, equivocal, or negative.
Turnaround: within 5 working days

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunofluorescence assay, so are subject to inter-operator variability, typically of ±1 titre dilution step (i.e., 1/40 could be 1/20 – 1/80). Internal controls are always used, and the assay is subject to external quality assessment.

La antibodies (SS-B antibodies, ENA antibodies)

Background

La antibodies are a type of ENA antibody seen in approximately 80% of cases of systemic lupus erythematosus (SLE) and approximately 80% of cases of primary Sjögren's syndrome. They are not required for the diagnosis of Sjögren's. La antibodies are usually accompanied by Ro antibodies. Only samples positive for antinuclear antibodies are tested for ENA, and positive ENA-screened samples undergo specific testing for La antibodies.

Sample

Plain serum (4 ml white topped tube filled to line)

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Results

Reported as positive, equivocal, or negative.
Turnaround: within one week

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunoblot assay, so are subject to inter-operator variability. Internal controls are always used, and the assay is subject to external quality assessment.

Lymphocyte subsets (CD4 count, B cells, T cells, NK cells)

Background

Lymphocyte subsets include T cells (both $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells), B cells and NK cells. The number and proportions of these can be used to diagnose and monitor a range of immunodeficiencies. Normal ranges are age dependent and lymphocyte levels are greatly influenced by concurrent illness; specimens degrade rapidly and are temperature sensitive – requiring transport and storage at room temperature only. CD4 T cell counts are used in HIV management for monitoring the efficacy of anti-retroviral therapies, and to decide at what point prophylactic antibiotics should be used. CD4 T cell counts must never be used as a surrogate marker of HIV infection, as HIV-positive patients may have normal counts, and low CD4 counts are possible in a large range of other diseases.
For this reason, lymphocyte subsets are only routinely available for patients with established HIV infection. All other requests must be discussed with the laboratory in advance. This laboratory does **not** process samples suspected of having haematological malignancies – please see Haematology handbook. For suspected primary immunodeficiencies, additional lymphocyte subsets may be available by prior arrangement. Monitoring frequency of CD4 counts in HIV depends on a variety of factors as outlined in the BHIVA guidelines; www.bhiva.org/monitoring-guidelines

Sample

EDTA sample (purple topped tube *without* gel)
Sample must reach the lab during working hours Mon-Fri, so should **NOT** be sent after 2PM on Friday afternoons or at weekends, no longer than 48 hours after collection.

Results

Reported as concentration of each subset, with age-related normal values, and as percentage of total lymphocytes. **NOTE ON UNITS**: absolute counts are expressed as multiples of 10^9 cells per litre (e.g., 0.456 $x10^9$/L) just as in a full blood count. To convert to numbers of cells per microliter, multiply the number before the $x10^9$ by 1000. So, 0.456 $x10^9$/L = 456/µl

Turnaround: within 5 working days

Important factors affecting the result.

Sample storage and transport conditions can significantly affect cell viability, in particular samples **must not be refrigerated**. This flow-cytometric assay is subject to external quality assessment. Performance characteristics of the test are available on request.

M

Mast cell tryptase

See tryptase.

Mitochondrial antibodies (AMA)

Background

Antibodies against mitochondria are typically associated with primary biliary cholangitis (PBC). Although mitochondrial antibodies can also be detected in primary Sjogren's syndrome and similar autoimmune conditions such as autoimmune thyroiditis, rheumatoid arthritis, and scleroderma. All these conditions can be associated with PBC so long-term. monitoring of liver function is recommended if AMA are detected. The major subtype responsible for PBC is the M2 subtype, and new positive mitochondrial antibodies are automatically tested for the M2, M4 and M9 PBC-associated subtypes. PBC develops very slowly, so long-term monitoring of liver function tests is important. Once M2 antibodies are confirmed, there is no need to continue to check anti-mitochondrial antibodies. Non-M2, M4, M9 subtypes are not associated with PBC, and various atypical patterns are also recognised – these will be identified on the report form. Previous studies suggested that having M2 anti-mitochondrial antibodies was highly predictive of developing PBC, but local data suggests around 11% at 5 years and 20% at 10 years will develop biochemical cholestasis (Fisher V, Sewell WA, Holding S. *Gastroenterology* 144.5 2013: S-9610.)

Sample

Plain serum sample (4 ml white topped tube filled to line)

Results

Expressed as negative, weak or positive.
M2, M4 or M9 subtypes are identified where relevant and reported as positive, equivocal, or negative.
Turnaround: negative within 5 working days; positive results within 13 working days.

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunofluorescence assay, so are subject to inter-operator variability, typically of ± 1 titre dilution step (i.e., 1/40 could be 1/20 – 1/80). Internal controls are always used, and the assay is subject to external quality assessment.

MPO antibodies

See myeloperoxidase antibodies.

Multiple nuclear dot antibodies

Background

This is not a test that can be requested directly but is sometimes detected when ordering an antinuclear antibody test. The 'multiple nuclear dot pattern' if found, can be seen in some cases of primary biliary cirrhosis, particularly those that have not yet developed anti-mitochondrial antibodies (see above). If the MND pattern is detected, we will go and check anti-mitochondrial antibodies for you if there is sufficient sample but suggest that you check the liver function tests and consider PBC as a possible diagnosis.

Sample

See nuclear antibodies.

Results

This test cannot be requested directly – it is an incidental finding on an anti-nuclear antibody test.

Muscle-specific kinase antibodies (MuSK antibodies)

Background

The classical autoantibody in myasthenia gravis is the anti-acetylcholine receptor antibody. In around 20% of cases of myasthenia gravis the AChR Ab is negative, and some of the remainder have an alternative autoantibody against muscle-specific kinase (MuSK). We do not automatically request MuSK antibodies, but if you strongly suspect myasthenia gravis and the AChR antibody is negative, please tell the laboratory and we will request this test from a reference laboratory.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Sent to a reference laboratory (**1**)
Reported as positive or negative.
Turnaround: within one month

Note: MuSK antibodies are tested alongside AChR antibodies.

Important factors affecting the result.

Immunosuppressive treatment and protein loss may reduce autoantibody levels; intravenous immunoglobulin preparations given therapeutically may themselves contain autoantibodies. Performance characteristics of the test are available on request.

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Myelin-associated glycoprotein antibodies (MAG antibodies)

Background

Antibodies again myelin-associated glycoprotein (MAG) are usually associated with sensory (rather than motor), usually distal, neuropathies in people typically over 50 years old. They are usually (over 85%) associated with paraproteins (often IgM) which should be excluded in new MAG antibody positive patients. These neuropathies often respond to immunosuppression.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Sent to a reference laboratory (**3**)
Reported as positive or negative.
Turnaround: within 21 days

Important factors affecting the result.

Immunosuppressive treatment and protein loss may reduce autoantibody levels; intravenous immunoglobulin preparations given therapeutically may themselves contain autoantibodies. Performance characteristics of the test are available on request.

Myeloperoxidase antibodies (MPO antibodies)

Background

Antibodies against myeloperoxidase (MPO) are the main pathogenic antibodies associated with pANCA, and are associated with Churg Strauss syndrome, microscopic polyarteritis and occasionally (about 10%) with Wegener's granulomatosis. Positive results are confirmed by ANCA IIF.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as concentration; normal 0-20 CU (units)
Turnaround: within one week

Important factors affecting the result.

Immunosuppressive treatment and protein loss may reduce autoantibody levels; intravenous immunoglobulin preparations given therapeutically may themselves contain autoantibodies. Performance characteristics of the test are available on request.

N

Neuronal antibodies

See Purkinje antibodies.

Neutrophil cytoplasm antibodies (ANCA, C-ANCA, P-ANCA)

Background

Anti-neutrophil cytoplasmic antibodies (ANCA) are associated with a wide range of vasculitic disorders, connective tissue diseases, infections, inflammatory disorders, and can be associated with several drugs. This means that the interpretation of ANCA results depends critically on the pre-test probability of an ANCA-associated disease being present. Clinical details are therefore essential. ANCA results cannot be interpreted in random screening of unselected patients. The laboratory approach to testing depends on whether the patient is already known to have ANCA, or whether ANCA is suspected, so please specify this on the request form.

The most important use of ANCA is in the diagnosis and monitoring of the ANCA-associated vasculitides (AAV). AAV include granulomatous polyarteritis (previously named granulomatosis), eosinophilic polyarteritis (previously named Churg Strauss).
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Levels of ANCA in these conditions are only roughly correlated with disease activity, but an increase >20% suggests that the monitoring interval should be reduced, and other features (such as CRP and creatinine) will also need monitoring.

Two main patterns of ANCA are significant, perinuclear (pANCA) staining patterns can be associated with antibodies to myeloperoxidase (MPO) (see above). However, many pANCA are not MPO antibodies, and the clinical significance of these other antibodies has not been established. Classical (cANCA) are usually associated with antibodies against proteinase-3 (see below). A range of other patterns are seen and are not clearly associated with any disorder and are termed atypical ANCA. The British Society for Rheumatology 2014 guidelines about using ANCA for both diagnosis and monitoring are available here: https://academic.oup.com/rheumatology/article/53/12/2306/1802843

Sample

Plain serum (4 ml white topped tube filled to line)
There is a protocol for urgent ANCA testing which must be followed. **All urgent ANCA must be discussed with lab staff in advance.** ANCA testing cannot be performed in the evenings or weekends.

Results

Reported as negative or positive with pattern. Only tested if positive MPO or PR3 measured, or by special request after discussion with the laboratory.
Turnaround: urgent – within 6 hours, routine within 5 working days

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunofluorescence assay, so are subject to inter-operator variability, typically of ± 1 titre dilution step (i.e., 1/40 could be 1/20 – 1/80). Internal controls are always used, and the assay is subject to external quality assessment.

NMDA-Receptor (N-methyl-1-D-aspartate receptor antibodies)

These rare antibodies are found in cases of limbic encephalitis, encephalitis, epilepsy partialis continua and ataxia. It is thought that these antibodies may play a role in the autoimmune pathogenesis of these syndromes. They are targeted against the NMDA receptor which is expressed on neuronal cell surfaces. As a paraneoplastic occurrence they are associated with both ovarian and mediastinal teratomas – both of which up-regulate and express the NMDA receptor.

Sample
Plain serum (4 ml white topped tube filled to line)

Results

Reported as Negative or positive.

Turnaround: this is a referral test to a specialist centre (5) - 12 days

Nuclear antibodies (ANA, ANF)

Background

Antinuclear antibodies (ANA) are found in a very wide range of disorders including connective tissue diseases (not just SLE), infections, inflammatory disorders and with various drugs. They are therefore not helpful as a screening test but should only be used to help confirm a strongly suspected rheumatological diagnosis. They are also useful in the diagnosis of autoimmune hepatitis type 1 but are not specific for that condition. There is **no** role for ANA testing in the investigation of back pain. The incidental finding of an ANA **does not mandate a referral to rheumatology** unless there are prominent clinical features of a relevant rheumatological diagnosis. ANA are detected using indirect immunofluorescence which shows a variety of patterns in positive samples; these are reported with positive results. **Homogeneous** ANA can be associated with antibodies to double-stranded DNA, as can the **peripheral** or **rim** patterns. **Speckled** ANA can be associated with antibodies to extractable nuclear antigens (ENA). **Centromere** antibodies are reported directly (see above). **Nucleolar** antibodies are occasionally seen in some subtypes of systemic sclerosis, but are very common non-specific findings, particularly in patients with hypertension, renal failure, liver disease or malignancy. When we identify a positive ANA, **providing the clinical details and other results** suggest a rheumatological disorder, we will automatically undertake additional identification of the autoantibody with ENA and dsDNA antibody tests. Our laboratory tests for ANA using a cell line called Hep-2 which is a very sensitive test and detects all relevant ENA antibodies – so samples negative for ANA are not usually tested further.

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Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as negative, weak positive, positive or strong positive and report of the pattern.
Turnaround: negative within 5 working days, positive within 10 working days

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunofluorescence assay, so are subject to inter-operator variability, typically of ±1 titre dilution step (i.e., 1/40 could be 1/20 – 1/80). Internal controls are always used, and the assay is subject to external quality assessment.

O

Oligoclonal bands

Background

Oligoclonal synthesis of IgG in the cerebrospinal fluid is detected by isoelectric focussing of CSF samples. Samples are sent to a reference laboratory. Reporting the pattern of oligoclonal bands detected requires comparison to a serum sample taken on the same day as the lumbar puncture, otherwise accurate interpretation is not possible. Oligoclonal bands are seen in numerous conditions including multiple sclerosis, infections, and connective tissue diseases.

Sample

At least 1 ml of CSF in a fluoride oxalate tube (glucose tube) or plain tube.
A plain serum sample (4 ml white topped tube filled to line) is also required.
Samples are despatched directly from local hospitals to the reference laboratory.

Results

Sent to a reference laboratory (**1**).
A clinical report is issued from the reference laboratory.
Turnaround: 18 days

Ovarian antibodies

Background

Antibodies against ovarian tissues can be seen in primary ovarian failure, and in the autoimmune polyglandular syndromes.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as positive or negative.
Turnaround: within one week

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunofluorescence assay, so are subject to inter-operator variability. Internal controls are always used, and the assay is subject to a sample share quality assessment.

P

Paraproteins (serum)

Background

Immunoglobulins produced by an expanded clone of B cells (a 'monoclone') are also known as paraproteins, although the term 'M-component' (for monoclonal), M-band or 'M-spike' is occasionally used in American textbooks. Although paraproteins are seen in myelomas, they are also found in other lymphoproliferative disorders (including chronic lymphocytic leukaemia and non-Hodgkin's lymphoma) and are common normal findings in the ageing population. However, all paraproteins have the potential for malignant transformation, so the term 'benign' paraprotein is now replaced by 'monoclonal gammopathy of undetermined significance' (MGUS). The differentiation of MGUS from a more serious paraprotein requires haematological advice and will be based on clinical and laboratory features including the presence of bone pain, hypercalcaemia, renal failure, abnormalities of the full blood count, bone marrow and urine examination. It is essential to recognise that 20% of myelomas are from B cell clones that produce only immunoglobulin light chain, so may not demonstrate any serum abnormality (although often reveal hypogammaglobulinaemia), making urine testing for monoclonal free light chains (Bence Jones proteins) essential if myeloma is suspected on clinical grounds.

Samples found to have a band on serum electrophoresis will undergo very sensitive immunofixation testing to confirm that the band is a paraprotein, rather than another form of serum protein. Immunofixation also identifies the type of paraprotein (e.g., IgG-kappa, IgM-lambda). Paraproteins are also quantified using a densitometric assay giving a result in g/L.

If monitoring a known paraprotein, please state this on the request form as it saves having to undertake time-consuming and expensive re-identification of the paraprotein – and a quantitative result only will be issued. However, if we detect a change in the electrophoretic pattern, we will go on to test for additional paraproteins, particularly to detect the presence of monoclonal free light chains in the serum which are extremely nephrotoxic.

Serum immunoglobulin levels of the same class of the paraprotein are not reported because paraproteins interfere with the test, giving unreliable results. The NICE Guidelines on Myeloma: diagnosis and management. the diagnosis of myeloma are available here: https://www.nice.org.uk/guidance/ng35 The 2014 BCSH Guidelines for diagnosing myeloma are available here: https://b-s-h.org.uk/guidelines/?search=M+protein and https://www.nice.org.uk/guidance/ng35

Sample

Plain serum (4 ml white topped tube filled to line). Other tubes are not suitable as they produce bands on electrophoresis.

Results

Reported as electrophoretic pattern for each component of a normal electrophoresis (alpha-1, alpha-2, beta and gamma zones). Paraproteins will be identified if new, and quantified in g/L.
Turnaround: within 5 working days

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect paraprotein levels. Plasma samples (i.e., anti-coagulated with EDTA) cause electrophoresis artefacts so serum should always be sent. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

Paraproteins (urine)

Background

Intact paraproteins (see serum paraproteins above) can enter the urine if there is any degree of renal damage. In addition, monoclonal free light chains either from a free light chain myeloma, or from excessive production in other myelomas, tend to accumulate in the urine rather than the serum as they are relatively small molecules. The presence of monoclonal free light chain (Bence Jones protein) in the urine is a significant factor in deciding whether a paraprotein is from a malignancy (myeloma) or from an MGUS. Monoclonal free light chain in the serum is a haematological emergency as it free light chain may be significantly nephrotoxic, so may result in renal failure. Around 20% of all myelomas only secrete monoclonal free light chain, so will not show up on serum tests, and require urine testing to be detected. Quantification of urine monoclonal free light chain is not generally helpful in patients with serum paraproteins but can be undertaken in patients with light chain myelomas; 24-hour urine samples can be used to quantify these, or random urines can be used with reference made to the paraprotein/creatinine ratio.

Sample

Plain universal container

Results

Reported as negative, or abnormality will be identified by type and quantified.
Turnaround: within 5 working days if negative, 10 working days if positive.

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Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect paraprotein levels. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

Pemphigus/pemphigoid antibodies

Background

Antibodies to various components of skin are associated with autoimmune blistering disorders. Antibodies to basement membrane zone antigens are associated with bullous pemphigoid, herpes gestationis and epidermolysis bullosa acquisita. Antibodies to the intercellular substance of the epidermis are associated with pemphigus vulgaris and pemphigus foliaceus. These tests are complementary to direct immunofluorescence examination of skin biopsies in these conditions.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as positive or negative.
Turnaround: within 8 working days

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunofluorescence assay, so are subject to inter-operator variability, typically of ±1 titre dilution step (i.e., 1/40 could be 1/20 – 1/80). Internal controls are always used, and the assay is subject to external quality assessment.

Phospholipase A2 receptor antibodies (PLA2R antibodies)

Background

PLA2R antibodies are positive in 70% of patients with primary membranous glomerulonephritis and are directed against a 185 kDa M-type phospholipase A2 receptor. Membranous glomerulonephritis presents with nephrotic syndrome or proteinuria

and is identified on renal biopsy by epithelial IgG and C3 deposition. The disease is twice as common in males than females and has a peak incidence age 40 - 50 years old. Secondary membranous GN is present in 25%, secondary to malignancy, autoimmunity, infection, and drugs. Primary membranous GN may have an autoimmune component with about 70% cases showing antibodies to M type phospholipase A2 receptor found in the lungs, placenta, leukocytes, and the podocytes of the kidney. Binding of antibody to PLA2R can cause subepithelial immune complex deposition and complement activation resulting in proteinuria and nephrotic syndrome.

One third of primary membranous cases will develop end stage renal disease, one third will have a persistent proteinuria and one third will have spontaneous remission. PLA2R antibodies are lower in patients in partial or complete remission compared to those with active disease. Spontaneous remission also occurs less frequently in patients with high antibody values. PLA2R antibodies can be used to monitoring the patient's response to immune suppression and can fall to normal range values 3-6 months before there are signs of clinical remission. Patients with membranous nephropathy undergoing renal transplantation have a disease recurrence of up to 45% but using APLA2R antibody levels to identify these individuals has not given conclusive results. PLA2RR antibodies have been described in patients with secondary membranous nephritis although it is suggested that these individuals also had concurrent primary membranous nephritis. PLA2R antibody measurement may have a role in identifying primary from secondary membranous nephropathy and in disease monitoring. However, they should not be viewed as a replacement for renal biopsy in the investigation of patients presenting with nephrotic syndrome.

Sample
Plain serum (4 mL white topped tube filled to line)

Results
Sent to a reference laboratory (**1**).
A clinical report is issued from the reference laboratory.
Turnaround: within three weeks

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect antibody levels.
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Phospholipid antibodies

See Cardiolipin antibodies.

Pneumococcal antibodies (pneumococcal IgG)

Background

Antibodies to the capsular polysaccharides of *S pneumoniae* ('pneumococcal' antibodies) are useful markers of the immune system's response to T-independent antigens. These are particularly important in diagnosing recurrent bacterial infections in patients who have normal total IgG, IgA, and IgM levels. The current pneumococcal antibody assay detects 23 different serotypes present within the Pneumovax II vaccine; it is unable to differentiate between levels against each serotype. In special situations, serotype-specific assays can be arranged with a reference laboratory (**6**). This assay has largely replaced IgG subclass determinations. Normal ranges are not well established, particularly in children. Diagnostic immunisation involves immunising with Pneumovax II and comparing the antibody levels taken 4 weeks post-vaccine with a pre-vaccine sample. Unfortunately, the variation within the assay is large, so, if possible, pre-, and post-vaccine samples are analysed together. Failure to respond to Pneumovax may indicate that the patient has Specific Antibody Deficiency (SpAD). Pneumococcal antibodies are useful in monitoring asplenic patients who may lose immunity to pneumococcus more rapidly than patients with a spleen.

Sample

Plain serum (4 ml white topped tube filled to line)
Please discuss diagnostic immunisation with the consultant immunologist **prior** to undertaking immunisation, particularly if conjugate vaccines such as Prevenar are being considered.
If Pneumovax or Prevenar have ever been given, please state the date on the request form, otherwise the results cannot be interpreted.

Results

Sent to a reference laboratory (**1**)
This parameter is not normally distributed; over 95% of normal adults have values ≥40 mg/L; over 75% of normal adults have values ≥60 mg/L.
Turnaround: within 28 working days

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect antibody levels. Internal controls are always used, and the assay is subject to external quality assessment. There can still be significant batch-to-batch variation, so for diagnostic pneumovax testing, if an earlier sample is available in the freezer, it will be run alongside for comparison. Performance characteristics of the test are available on request.

Proteinase-3 antibodies (Pr3 antibodies)

Background

Antibodies to proteinase-3 (PR3) are associated with Wegener's granulomatosis, and are usually seen with cANCA, although 10% are associated with pANCA. The level of antibody correlates approximately with disease activity, so increases in levels of >20% should indicate a reduction in monitoring interval and extra clinical vigilance. Positive results are confirmed by ANCA IIF.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as concentration; normal 0-20 CU (units)
Turnaround: within one week

Important factors affecting the result.

Immunosuppressive treatment and protein loss may reduce autoantibody levels; intravenous immunoglobulin preparations given therapeutically may themselves contain autoantibodies. Performance characteristics of the test are available on request.

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Purkinje cell antibodies

Background

Antibodies to various neuronal structures are associated with paraneoplastic neurological disorders. Antibodies to cerebellar Purkinje cells are well recognised, and patterns associated with the autoantibodies Hu, Yo and Ri can be detected. Although we undertake initial screening in the laboratory, confirmatory tests are sent to a reference laboratory (**5**) and results may take some time to produce. These antibodies cannot be used as screening tests but can be useful confirmatory tests. **Hu** antibodies are associated with small cell lung cancer and sensory neuropathies or encephalomyelitis. **Yo** antibodies are associated with paraneoplastic cerebellar degeneration and ovarian carcinoma, and sometimes breast cancer or Hodgkin's disease. **Ri** antibodies are associated with breast cancer, myoclonus, opsoclonus and ataxia. Other autoantibodies such as Ma-1, Ma2, CV2/CRMP, amphiphysin etc are also available after discussion.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as negative or positive.
Turnaround: negative within one-week, positive results are sent to a reference laboratory (**5**) for confirmation by sensitive immunoblotting method and may take several weeks depending on the complexity.

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunofluorescence assay, so are subject to inter-operator variability. Internal controls are always used, and the assay is subject to external quality assessment.
Q

R

RAST Tests

This is an outdated name for allergen-specific IgE tests, please see Immunoglobulin E (Allergen-specific)

Rheumatoid factor

Background

Rheumatoid factors are antibodies against the Fc region of other immunoglobulins. Rheumatoid factor, despite the name, **is not a test for rheumatoid arthritis**, but can be positive in most connective tissue disorders, many inflammatory disorders, with infections, and is frequently present in the healthy elderly. The only utility of rheumatoid factor is as a prognostic marker in patients with known rheumatoid arthritis. High concentrations of rheumatoid factor can be seen in vasculitis and in cryoglobulins and these conditions should be considered if very high levels are detected. The level of RF should not be used to monitor disease activity in rheumatoid arthritis.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Negative values < 14 IU/mL
Turnaround: within 3 working days

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

Ri antibodies

See Purkinje cell antibodies.

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RNP antibodies (ribonuclear protein antibodies, U1RNP antibodies)

Background

Antibodies against U1RNP are one of the ENA antibodies, and are associated with mixed connective tissue disease, although they can also be detected in systemic lupus erythematosus. This test is only undertaken if the antinuclear antibody is positive.

Sample

Plain serum (4 ml white topped tube filled to line).

Results

Reported as negative, equivocal, or positive.
Turnaround: within one week

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunoblot assay, so are subject to inter-operator variability. Internal controls are always used, and the assay is subject to external quality assessment.

Ro antibodies

Background

Ro antibodies are also known as SS-A antibodies and are one of the ENA antibodies. Ro antibodies are found in primary Sjogren's syndrome and in SLE. Ro antibodies can cross the placenta and cause disease in the neonate, particularly complete heart block, but also neonatal lupus. There is also an association with photosensitivity in lupus. As this laboratory uses a sensitive antinuclear antibody screening test, only samples positive for ANA are tested for Ro antibodies.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as negative, equivocal, or positive.
Turnaround: within one week

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunoblot assay, so are subject to inter-operator variability. Internal controls are always used, and the assay is subject to external quality assessment.

S

Scl-70 antibodies (topoisomerase antibodies)

Background

Anti-Scl-70 antibodies are an ENA antibody, also known as anti-topoisomerase I. They are associated with systemic sclerosis, particularly progressive systemic sclerosis (20-40%) but can also be seen in limited systemic sclerosis (20%). They are associated with musculoskeletal disease, skin disease and cardiopulmonary complications of systemic sclerosis. There may be an association with lung carcinoma in progressive systemic sclerosis. Scl-70 and centromere antibodies are mutually exclusive. However, 40% of patients with scleroderma do not have either centromere or Scl-70 antibodies. In patients with Raynaud's, the presence of Scl-70 predicts the development of progressive systemic sclerosis. Scl-70 screening is only undertaken on antinuclear antibody positive samples.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as negative, equivocal, or positive.
Turnaround: within one week

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunoblot assay, so are subject to inter-operator variability. Internal controls are always used, and the assay is subject to external quality assessment.

Serum Free Light Chains (sFLC)

See Free Light Chains

Sm antibodies

Background

Sm antibodies are an ENA antibody associated with systemic lupus erythematosus, particularly in Afro-Caribbean women. Sm screening is only undertaken on antinuclear antibody positive samples.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as negative, equivocal, or positive.
Turnaround: within one week

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunoblot assay, so are subject to inter-operator variability. Internal controls are always used, and the assay is subject to external quality assessment.

Smooth muscle antibodies (SMA)

Background

Antibodies against smooth muscle are the hallmark of autoimmune hepatitis type 1 (which may have either ANA, ASM or both), but are also seen in viral infections. High titre SMA are more indicative of autoimmune hepatitis.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Reported as negative or positive.
Turnaround: within one week

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunofluorescence assay, so are subject to inter-operator variability, typically of ±1 titre dilution step (i.e., 1/40 could be 1/20 – 1/80). Internal controls are always used, and the assay is subject to external quality assessment.

SSA antibodies

See Ro antibodies, ENA antibodies.

SSB antibodies

See La antibodies, ENA antibodies.

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Striated muscle antibodies

Background

Striated muscle antibodies can be associated with myasthenia gravis and is a significant finding because nearly all patients with these antibodies and myasthenia have a thymoma. They are not diagnostic of thymoma, and can be seen in patients on penicillamine, following a bone marrow transplant, and in graft-versus-host disease.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Sent to a reference laboratory (**1**)
Reported as negative or positive.
Turnaround: within one week

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Positive results are visually determined by comparison with known positive and negative controls in an immunofluorescence assay, so are subject to inter-operator variability. Internal controls are always used, and the assay.

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T

T cells

See lymphocyte subsets.

Tetanus antibodies (tetanus IgG)

Background

Antibodies against tetanus toxoid are used to assess the antibody response to T-dependent antigens, as most patients are immunised against tetanus during early childhood. Together with antibodies against *Haemophilus influenzae* b and pneumococcus, these make up the 'functional' or 'specific' antibody assessment of humoral immunity.

Sample

Plain serum (4 ml white topped tube filled to line).
Investigation of suspected primary immunodeficiency should be discussed with an immunologist prior to taking samples to ensure appropriate investigations are being undertaken.

Results

Sent to a reference laboratory (**1**)
Reported in iu/ml. Values >0.01 iu/ml indicate a response, but >0.1 iu/ml is required for optimal long-term protection.
Turnaround time: approximately 2 weeks

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect antibody levels. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

Thyroid peroxidase antibodies (TPO antibodies)

Background

Antibodies against thyroid peroxidase are associated with autoimmune thyroid conditions but are not specific to any condition. Low concentrations of TPO antibodies are seen in some healthy individuals, but high levels are commonly seen in Hashimoto's thyroiditis (95%), primary myxedema (90%) and Graves disease (50-80%).

Sample

Plain serum (4 ml white topped tube filled to line)

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Results

Reported as quantity.
Normal range 0-5.6 u/ml
Turnaround time: approximately 3 days

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

Tissue transglutaminase antibodies (tTG antibodies)

Background

IgA class antibodies against tTG are our screening test for coeliac disease. Positive results are seen in patients who have coeliac disease and are not excluding gluten from their diet, so in known coeliac disease this test can be used to assess dietary compliance. However, the timing of loss of tTG antibodies on gluten withdrawal is not fully established. Positive results are confirmed using an indirect immunofluorescence assay for endomysial antibodies. In selective IgA deficiency, we can also measure IgG endomysial antibodies, although the diagnostic specificity of these is not as firmly established as for the IgA antibodies. Please note that tTG antibodies are only a screening investigation, and formal diagnosis of coeliac disease requires endoscopic biopsy. Selective IgA deficiency is common in coeliac disease so if coeliac disease is strongly suspected, consider also screening for IgA deficiency.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Negative is 0-20 CU units (if positive, endomysial antibodies are also tested)

Turnaround: within 5 working days

Important factors affecting the result.

Immunosuppressive treatment and protein loss may affect autoantibody levels. For this assay, recent exposure to gluten can significantly affect the result, as can borderline low total serum IgA levels. Internal controls are always used, and the assay is subject to external quality assessment.

Topoisomerase antibodies

See Scl-70 antibodies.

Tryptase (mast cell tryptase)

Background

Tryptase is released from mast cells during anaphylaxis. It has a half-life of approximately 3 hours, and serum levels peak at approximately 2-4 hours after systemic anaphylaxis, and usually return to normal by 24 hours. Persistently elevated levels may be seen in mastocytosis and urticaria pigmentosum. Tryptase measurements are particularly helpful in patients with suspected atypical allergic reactions, and in anaphylaxis during general anaesthesia. **NICE Guidelines now recommend tryptase is measured immediately and 1-2 (certainly <4) hours after onset of all presentations of anaphylaxis.** It is **ESSENTIAL** to put the time of the sample and the time of the reaction on the request form. NICE Guidelines are available for the diagnosis and management of anaphylaxis here https://www.nice.org.uk/guidance/cg134 Note that urinary methylhistamine levels, which were used in the investigation of anaphylaxis, are no longer available.

Sample

Plain serum (4 ml white topped tube filled to line)
It is **essential** to mark the time of onset of the reaction on the request form as well as the time of sampling on the form and the tube.

Results

Reported as a concentration, normal 2-14 μg/L.
Turnaround: within one week

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Important factors affecting the result.

Sample storage and handling can affect tryptase levels; for post-mortem samples a peripheral sample is essential, and the case should be discussed with the consultant immunologist. Tryptase probably increases with body mass index, but correction factors for this phenomenon have not been produced yet. Rheumatoid factors and human-anti-mouse antibodies can cause falsely elevated results. Internal controls are always used, and the assay is subject to external quality assessment. Performance characteristics of the test are available on request.

TSH-Receptor antibodies (TSH-R Ab)

Background

The hyperthyroidism of Grave's disease is caused by the presence of stimulatory anti-TSH receptor IgG antibodies which bind to TSH receptors on the thyroid follicular cells and cause unregulated increased stimulation of thyroxine production. Such antibodies are detectable in the serum of >75% of patients with this condition. However, this radioimmunoassay test measures binding to TSH receptors only (both blocking and stimulatory) and thus cannot distinguish between stimulating antibodies in Grave's disease and inhibiting antibodies in myxoedema. In a patient with known hyperthyroidism where an autoimmune aetiology has been demonstrated by the presence of thyroid microsomal antibodies, measurement of TSH-R Ab is of little additional value.

Reports suggesting that high titres of these antibodies predict early relapse after treatment with antithyroid drugs have not been confirmed. The presence of high levels of this autoantibody in late pregnancy may predict a risk of neonatal hyperthyroidism but it is not clear that this is more informative than the clinical course of maternal thyroiditis in pregnancy and the anti-TPO titre. The test does not differentiate between stimulating and blocking antibodies to the TSH receptor and is positive in Grave's disease, Hashimoto's, and primary myxoedema. The test is sent to an external reference centre and takes

2-3 weeks. We only undertake this test following discussion with the patient's consultant, and only in maternal thyroiditis in pregnancy.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

This test is referred to a reference laboratory (**7**).
Reported in units (normal 0-20)
Turnaround: within three weeks

Important factors affecting the result.

Immunosuppressive treatment and protein loss may reduce autoantibody levels; intravenous immunoglobulin preparations given therapeutically may themselves contain autoantibodies. Performance characteristics of the test are available on request

U

U1RNP antibodies

See RNP antibodies.

V

Voltage-gated calcium channel antibodies (VGCC)

Background

VGCC antibodies are seen in Lambert-Eaton myasthenic syndrome. This is usually associated with small cell carcinoma of the lung (90% have VGCC Ab), but occasionally LEMS can develop before a carcinoma is apparent (40% have VGCC Ab). Very occasionally LEMS can develop without carcinoma. These tests are sent to a reference laboratory.

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Referred to a reference laboratory (**5**); Reported as negative or positive.

Turnaround: 20 days

Important factors affecting the result.

Immunosuppressive treatment and protein loss may reduce autoantibody levels; intravenous immunoglobulin preparations given therapeutically may themselves contain autoantibodies. The numerical imprecision of this assay is available on request from the reference laboratory.

Voltage-gated potassium channel antibodies (VGKC)

Background

The antibodies are associated with the rare condition of acquired neuromyotonia. These tests are sent to a reference laboratory (**5**).

Sample

Plain serum (4 ml white topped tube filled to line)

Results

Referred to a reference laboratory.
Reported as a concentration, <100 pmol/L negative, >200 pmol/L positive, with an equivocal region.
Turnaround: 17 days.

Important factors affecting the result.

Immunosuppressive treatment and protein loss may reduce autoantibody levels; intravenous immunoglobulin preparations given therapeutically may themselves contain autoantibodies. The numerical imprecision of this assay is available on request from the reference laboratory.

W

X

Y

Yo antibodies

See Purkinje cell antibodies.

Z

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Investigational strategies for particular situations

Rheumatological conditions and vasculitis

Autoimmune serology is useful as a confirmatory test for clinically suspected conditions but is not helpful as a screen in patients with vague aches and pains. Please see individual tests above for more information. In more serious rheumatological conditions such as vasculitis or pulmonary renal syndromes, urgent investigations will be required. It is **essential** that the laboratory is telephoned in advance to arrange these. In pulmonary renal syndromes urgent ANA, ANCA and GBM should be requested, and the clinical details must be discussed with the lab staff who will also discuss urgent sample transport arrangements with you.

Immunodeficiency

Over 300 primary immunodeficiencies have now been identified, so it is usually more helpful to discuss intended investigations with immunology staff in advance of taking a blood sample, as many of the tests need arranging in advance and/or special tubes and/or transport arrangements. It is not usually useful to take samples during episodes of acute sepsis.

Allergy

Allergy testing is available to both primary and secondary care practitioners. Blanket screening tests are not available, but we have useful panels of allergen tests for example in rhinitis and asthma. It is essential to specify what tests are needed in suspected food allergy. IgE testing is not helpful in chronic urticaria. Suspected drug allergy should be discussed with immunology clinical staff first as this is a specialist area.

Urticaria/angioedema

Chronic urticaria (wheals most days for >6 weeks) is usually not due to allergies so allergy testing is not usually relevant. Many cases are autoimmune, and there is a strong link with autoimmune thyroid disease. Pure angioedema (i.e., without co-existing urticaria) may be caused by ACE inhibitor drugs, even if taken without complication for several years. Pure angioedema always needs investigation. The term angioneurotic oedema has been abandoned as there is no neurosis involved! C1-inhibitor deficiency (hereditary angioedema) presents as isolated angioedema or recurrent abdominal pain. Please refer urticaria/angioedema to the immunology clinic for further investigation and management.

Back pain

Rheumatoid factor testing plays no role in the investigation of back pain, however investigations for myeloma may be relevant – send serum for electrophoresis.

Diabetes mellitus

Autoantibodies cannot be used to distinguish between type 1 and type 2 diabetes with complete reliability. The greatest utility of autoantibodies in diabetes mellitus is for prognostic purposes in assessing the risk of developing diabetes in currently unaffected first-degree relatives. Both GAD (and to a lesser extent ICA) antibodies may identify adults with latent autoimmune diabetes of adults (LADA).

HIV/AIDS

CD4 counts are only available in patients known to have HIV. CD4 counts have **no role** in 'suspected' HIV and may not be used as a 'surrogate HIV test'.

Severe combined immunodeficiency SCID

Suspected severe combined immunodeficiency in children is a medical emergency and must be discussed with immunological clinical staff immediately. Appropriate investigations will be arranged on an urgent basis.

Latex allergy

Latex allergy should be considered in all those who have reported symptoms of IgE-mediated allergy (dyspnoea, wheeze, rash, urticaria, angioedema) immediately (or within about 1 hour) of contact with latex. The specific IgE blood test is reasonably sensitive in individuals with total IgE >100 iu/ml but is less sensitive in subjects with lower total IgE levels. Skin prick tests may be required in cases where latex allergy is strongly suspected, but the latex specific IgE test is negative, particularly where the total IgE is <100 iu/ml. This skin test can be arranged by referring the patient to the clinical immunology/allergy service.

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Coeliac disease

The screening test for coeliac disease in this hospital is tissue transglutaminase (TTG) antibodies. Positive results are confirmed by testing for endomysial antibodies. Both are screening investigations – the definitive diagnosis relies on endoscopic duodenal biopsy, not serology. Both assays are based on IgA, so if the patient is IgA deficient, they will be ineffective. Consider screening for IgA deficiency, as this is not a part of our routine coeliac screen. IgG based assays are available but are less sensitive. Gliadin antibodies are not as sensitive/specific and reticulin antibodies have been superseded. Patients should not be excluding gluten when the test is being used for diagnostic purposes; the test will go negative in coeliac patients who are successfully excluding gluten, but the timing for exactly when patients will go TTG antibody negative has not been firmly established. NICE Guideline 86 suggests: gluten should be consumed in more than one meal every day for a minimum of 6 weeks before testing.

Anaesthetic reactions

See section on tryptase.

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SECTION 12	Andrology Service Handbook

12.1 General information

The Andrology Laboratories are a UKAS accredited service performing both fertility and post vasectomy testing on semen samples whilst adhering to latest guidelines. The Andrology laboratory is located within the Pathology Departments at Grimsby and Boston hospitals and are open Monday to Friday 9am until 5pm.

For a list of accredited tests provided by Path Links laboratories please refer to United Kingdom Accreditation Service (UKAS) Schedule of Accreditation; https://www.ukas.com

All laboratories maintain a comprehensive Quality Management System through Path Links and the Directorate and undertake regular internal quality control (IQC) and external quality assurance (EQA), participating in the United Kingdom National External Quality Assurance (UKNEQAS) schemes for semen analysis. Common SOPs and Internal Quality Assurance procedures ensure commonality of procedures and allow cross-site cover.

Please use the current version of the dedicated andrology form as it contains important up to date information for the patient. A dedicated andrology sample pot must be given to the patient which will be labelled with a red sticker stating that it has been toxicity tested by the laboratory. Please only use these sample pots as any other container given to the patient or used by the patient will be rejected at their appointment.

The sample must be taken after a minimum of 2 days (48hrs) and a maximum of 7 days of sexual abstinence and must be produced by masturbation and ejaculated directly into the toxicity tested container.

The form must be fully completed by the requesting clinician. The yellow box at the bottom of the form must be completed by the patient once the sample has been produced.

One of the questions within the yellow box on the form is asking if the laboratory can use any residual sample left over after reporting for quality control and training. We must maintain levels of quality by regularly assuring procedures are followed and to ensure that you can be confident of the results. Training is an important part of this procedure, and for new scientists working within the laboratory. All samples used for quality or training are anonymised and will not affect the patient's results in any way. We will not use the sample for anything else other than quality and training. Staff can advise if there are difficulties in understanding what we may use the sample for.

An appointment is required for all tests and samples must be delivered to the designated laboratory by the patient in a timely manner.

A room is available for patients who are unable to attend within the required time. Patients requiring the use of the room must highlight this to Andrology staff when making their appointment.

The policy of the Path Links Andrology service for those who do not attend on two consecutive occasions is that no third appointment will be made until the patient has had a further appointment with their GP.

For general enquires and to book appointments, the phone numbers are given on the reverse of the form (Boston: 01205 446314 or Grimsby: 03033 304494). If andrology staff are unable to take your call, then a message can be left on our dedicated answerphone, and we will call you back at our earliest opportunity.

We aim to report all results within 7 days to the requesting clinician either by electronic link or in paper format. Results are not telephoned, and patients are told that they cannot receive their results directly from the laboratory.

For more information, please refer to the relevant patient leaflet which can be found on the Trust's website (ULH and NLAG)

Measurement of uncertainty is available to view in the Path Links Pathology Services webpage under general information.

12.2 Fertility and vasectomy reversal

Some couples have difficulty conceiving and are referred for fertility investigations by their GP/clinician. One common cause of infertility is sperm dysfunction. The Andrology Laboratory assesses semen and sperm quality to provide important diagnostic information.

All fertility samples must be delivered to either Boston or Grimsby Andrology within 45-50 minutes of production. Alternatively, a room is available for patients who are unable to deliver their sample within this timeframe.

Here at the Andrology Laboratory, we assess the 'main' factors (sperm concentration, motility, and morphological appearance) as well as other parameters that are helpful in providing important diagnostic information.

Below is a table explaining these parameters and decision limits used (5^{th} centile, 95% confidence interval)

PARAMETER	**DESCRIPTION**	**DECISION LIMITS** **(**WHO Guidelines 2021)
Sperm concentration	Number of sperm per ml. Reported as millions per ml.	≥ 16 million per mL
Total Sperm number	Number of sperm in total ejaculate. Reported as millions.	≥39 million
Morphology	Shape and size of sperm. Samples containing sperm with poor morphology have a reduced chance of achieving a pregnancy. A high percentage of abnormally shaped or sized sperm is called teratozoospermia.	≥4%
Motility	Sperm are graded on their ability to move and the speed at which they do this. The fast forward swimming sperm are generally the most fertile. This is given as a percentage of sperm counted and divided into the following categories: a) Rapid Progressive a) Slow Progressive b) Non-progressively motile c)Immotile	Total progressive ≥30% Total motility ≥42%
pH	Measures the acidity or alkalinity of the semen using pH paper strips.	≥ 7.2
Round cells	This is the presence of cells that may be either germ cells and/or leukocytes and is reported within the comment section as being greater than 1 million per ml if detected as such. Erythrocytes are not classed as round cells, but we may comment on their presence if observed. These are non-sperm cells which may indicate an infection if seen in excess	
Vitality	Test to establish if sperm seen are dead or alive. This is then then reported as a percentage of live sperm. This test is only undertaken if the motility is severely reduced (≤40% total motility).	≥54%
Volume	The amount of semen produced	≥ 1.4 ml

Glossary of Terms that may be used (not exhaustive) -

AGGLUTINATION: Adherence of motile sperm to other motile sperm which is often associated with antisperm antibodies. Reduces the observed sperm count and motility.

AGGREGATION: Adherence of non-motile sperm to other non-motile sperm, other cells, or cell debris. Not to be confused with agglutination.

ANTISPERM ANTIBODY: Usually an autoantibody directed towards a man's own sperm but occasionally produced by the female to her partner's sperm. Men with high levels of usually of the IgG or IgA class often have sperm agglutination, reduced motility or even inability to bind and penetrate an egg. Screening for anti-sperm antibodies is not offered because there is no evidence of effective treatment to improve fertility (NICE guideline 2013, updated 2017) https://www.nice.org.uk/guidance/cg156

ART: Assisted Reproduction Technology. Procedures to bring about conception without sexual intercourse. Examples include IUI, ICSI and IVF.

ASPERMIA: Absence of an ejaculate.

ASTHENOZOOSPERMIA: Poor motility in sperm. World Health Organisation (WHO) definition is defined as <25% rapid motility or <50% progression in a semen sample.

AZOOSPERMIA: Complete absence of sperm, demonstrated by using large volume fixed depth (LVFD) slides.

CRYPTOSPERMIA: So, few sperm in the ejaculate that they are identified only after concentration and centrifugation of the sample. Germ cell failure; reversal of vasectomy.

GLOBOZOOSPERMIA: Often referred to as 'roundhead' defect. Sperm morphological defect where the acrosome is absent, and sperm usually have small round heads.

GONADOTROPHINS: Gonadotrophins are the follicle stimulating hormones (FSH) and luteinising hormones (LH). In women, they stimulate the ovaries; in men, they regulate spermatogenesis.

ICSI: Intracytoplasmic Sperm Injection. Procedure injecting a single sperm into a single egg and used mainly to treat male infertility.

IUI: Intrauterine Insemination. Artificial insemination of prepared sperm into the uterine cavity.

IVF: In Vitro Fertilization. The procedure where eggs are removed from the ovaries and mixed with sperm. Eggs that fertilize become embryos and are transferred to the uterus in the hopes that a pregnancy will result. Spare embryos are often cryopreserved and placed in storage.

LEUCOCYTOSPERMIA: Defined when the concentration of seminal leukocytes exceeds $1x10^6$/ml. Not thought to be of great clinical significance but may be indicative of infection.

LIQUEFACTION: Breakdown of the semen coagulum by prostatic secretions, usually within 30 minutes of the sample being produced.

OLIGOZOOSPERMIA: Used to describe semen samples with a sperm concentration of $<16x10^6$/ml or $<5x10^6$ in severe oligozoospermia.

POST-COITAL TEST: Test timed to ovulation where cervical mucus is harvested usually 8-12 hours after coitus and examined for the presence of motile sperm. Tests whether a man's sperm will penetrate the cervical mucus of his partner and whether the female partner is producing suitable mucus at the time of ovulation.

RETROGRADE EJACULATION: Semen that flows backwards into the bladder instead of forward through the urethra. It is caused by damage to the nerves closing the bladder neck and is often associated with conditions such as diabetes and spinal injuries.

SURGICAL SPERM RETRIEVAL: Therapeutic sperm retrieval for use in ART procedures.

VISCOSITY: Semen sample which forms long strands instead of droplets. Can make dilution, analysis, and preparation difficult.

12.3 Post vasectomy

Sperm tests to confirm the success (or failure) of a vasectomy operation are part of the post-operative management of that patient. It is essential to know whether the operation has been successful.

The first sample must be produced after a minimum of three months and 20 ejaculations post-surgery. Please ensure that the vasectomy date is stated on the request form.

This first sample can be delivered to either the analytical laboratories at Boston and Grimsby or our remote sites at Lincoln and Scunthorpe. Boston laboratory deals with our remote Lincoln site and Grimsby with our Scunthorpe remote site. Please bear this in mind when advising patients.

The assessment of a single sample, according to the 2016 PVSA guidelines, is sufficient to be considered by the surgeon for clearance if the following conditions are met:

- Minimum of three months and 20 ejaculations post-surgery
- Abstinence period is adhered to.
- Complete sample obtained.
- Analysed within 4 hours of production.
- Sperm absent

If any immotile sperm are seen in this first sample, then we will request a further sample. This second sample (PV-LIVE) will require the use of the on-site collection facilities at either Boston or Grimsby site. If this sample also contains immotile sperm, then a second confirmatory sample will be requested. If this second PV-LIVE sample confirms the findings of the first PV-LIVE sample, then the results meet the criteria for the surgeon to consider granting special clearance. Please contact the laboratory or clinical lead if advice is required.

In post vasectomy samples, an excess of round cells may obscure the detection of sperm and in some cases, we may request a repeat sample.

12.4 References

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3. National Institute for Clinical ExcellenceCG156 Fertility Problems: Assessment and Treatment (2013), reviewed Sept 2017 https://www.nice.org.uk/guidance/cg156

4. World Health Organization: WHO laboratory manual for the examination and processing of human semen. 6th edition, 2021.

5. P Hancock, B J Woodward, A Muneer, J C Kirkman-Brown Association of Biomedical Andrologists, the British Andrology Society and the British Association of Urological Surgeons. 2016 Laboratory guidelines for post-vasectomy semen analysis.

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