Safe & compassionate care, every time

Buckinghamshire Healthcare NHS Trust

Pathology Services

Clinical Immunology Handbook

Covering services provided by the Stoke Mandeville Laboratory for Amersham, Wycombe and Stoke Mandeville Hospitals as part of the Buckinghamshire Healthcare NHS Trust

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1. Introduction

The Clinical Immunology Laboratory provides the clinical diagnostic service for the entire Buckinghamshire Hospitals NHS Trust since the restructuring of laboratory services in 2005. The laboratory receives and processes samples from the three main hospital sites within the trust as well as from GP patients living within a wide radius of the hospitals. The clinical lead for the laboratory is undertaken by the consultant medical staff at the John Radcliffe Hospital in Oxford who are available for clinical advice by telephone.

This handbook aims to provide answers to the questions most frequently asked by our users relating to accessing the laboratory service, required sample types and turnaround times for results. All initial enquiries should be directed towards the laboratory staff at Stoke Mandeville Hospital, who will refer callers to the medical staff as appropriate.

Immunology Laboratory Hours

The immunology laboratory is based at Stoke Mandeville Hospital and opening hours are as follow:

Monday – Friday	08.00 hrs – 17.30 hrs	Normal Laboratory Service
Saturday, Sunday	No Service	

Telephone contacts (prefix with 110 if phoning from sites other than Stoke Mandeville)

	Ext. numbers
Immunology Laboratory	5323
Ross Sadler (Consultant Clinical Scientist)	01865 225991
Anand Pancholi Head Biomedical Scientist Blood sciences	5355
Arshad Mahmood Lead Biomedical Scientist Blood sciences	5355
Pathology Reception	5591
Infection Prevention & Control	5337 (Bleep 762)
Ms Jules Hicken, General Manager Radiology and Pathology	6913
Mr D. Green, Pathology IT Manager	5013

(The majority of extension can be accessed directly 01296 31<ext.>)

For **immunology supplies** (i.e. forms, containers and swabs) please telephone the appropriate extension and leave a message on the answer phone for Wycombe and send an email with an attached supplies form to Stoke Mandeville.

Stoke Mandeville	bht.pathologysupplies@nhs.net
Wycombe	5248

2. The Department

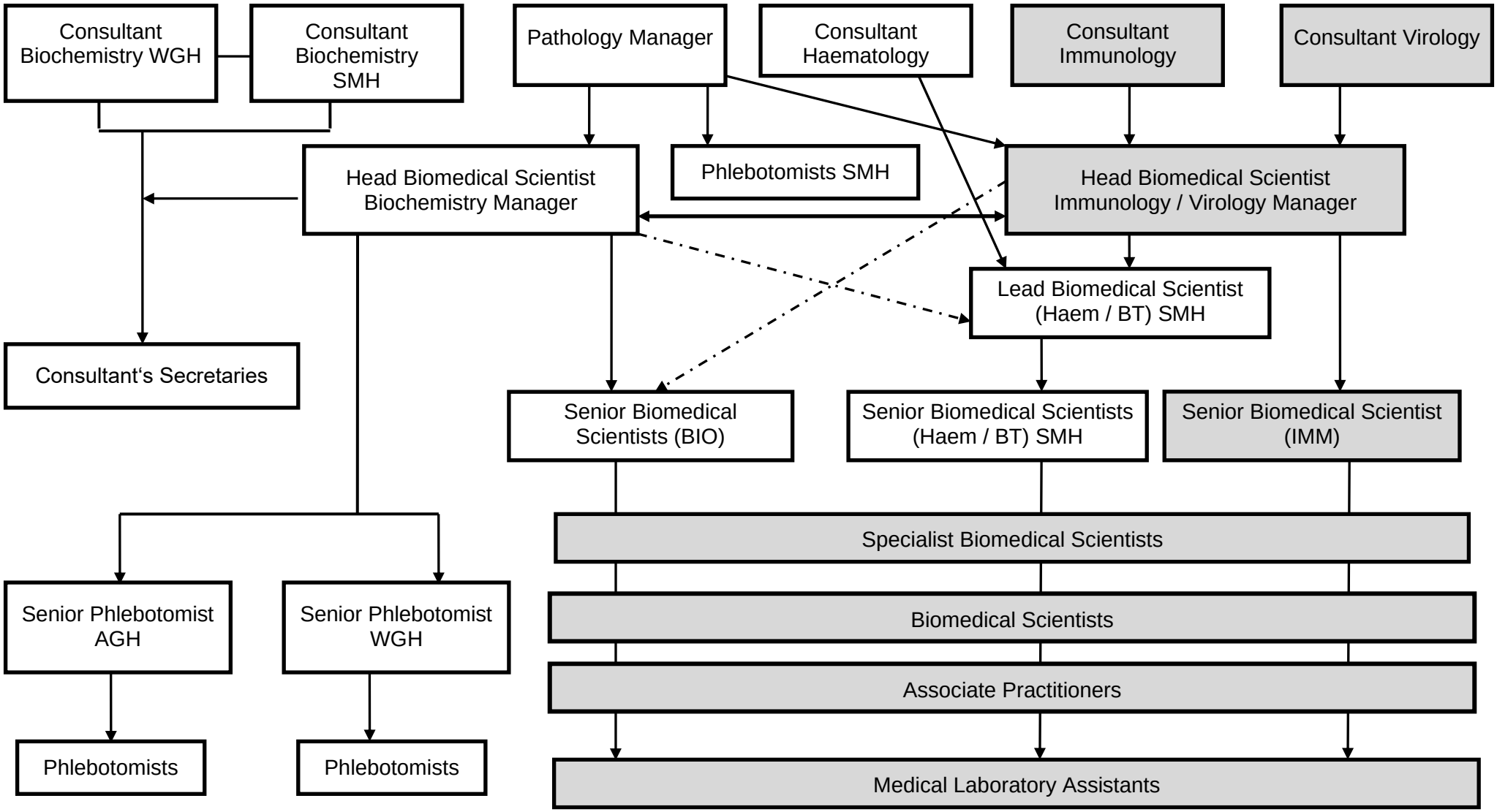
2.1 Departmental Overview

The Clinical Immunology Laboratory is located within the old part of the Stoke Mandeville site, just off of the rear access road, and can be accessed via entrance 3 or 4. The laboratory is combined with the Microbiology Department and undertakes the viral serology for the Buckinghamshire Hospitals Trust as well as the clinical immunology. This amalgamation of the Virology and Immunology workload provides the most sustainable and advantageous service available to the trust in terms of staff expertise and equipment utilisation.

2.2 Staffing and Establishment

The department employs a number of clinical, technical and non-technical staff thereby providing a full range of support for our users. Clinical support is provided by the Consultant Immunologist who is available to answer questions relating to the interpretation of results, patient management and other clinical queries. Technical and managerial support is provided by the Senior Biomedical Scientist and staff who, in conjunction with other biomedical scientists perform the analytical work. The laboratory is supported by Medical Laboratory Assistants who provide technical support and perform administrative tasks.

2.3 Immunology Relationship with Blood Sciences Organisational Chart



3. Specimen Information
3.1 Sample Types

It is essential that the correct specimen container is used to collect samples for testing. The sample requirements for some assays are very restrictive and the assay may not be possible if an inappropriate container is used. The majority of samples can be stored in a refrigerator before dispatch to the laboratory, but this is not possible for all sample types. Please contact the laboratory if in any doubt about sample storage.

For paediatric samples please contact the department if in doubt about the correct sample container **BEFORE** venepuncture. The small lithium heparin (orange tube) is not recommended for many of the commonly requested paediatric blood tests where serum, and not plasma, is the required sample type.

Samples being sent for lymphocyte markers, QuantiFERON-TB Gold or functional assays must **NOT** be placed into the refrigerator but kept at room temperature. It is not possible to process these samples if they are collected on a Friday due to the restrictions of the reference laboratory. CSF samples must be collected into sterile sample containers, as used for Microbiology samples, and not collected into blood tubes.

Below is a table of common BD Vacutainer™ tube types supplied by the laboratory.

BD Vacutainer Colour	Tube Name	Sample Produced	Routine Application
	SST II Advance	Serum	All routine Immunology Investigations.
	Potassium EDTA	Plasma or Whole Blood - EDTA	Lymphocyte markers, functional and genetic studies.
	Lithium Heparin	Heparinised Plasma or Heparinised Whole Blood	Functional assays and Cytogenetic studies

	Various	Various	QuantiFERON-TB Gold Plus for detection of *Mycobacterium tuberculosis*

3.2 Minimum Labelling requirements

In April 2003 the following minimum labelling policy was adopted by the Trust, in compliance with recommendations from the Royal College of pathologists. It is essential that samples received by the laboratory are correctly labelled and received with clearly completed request forms to minimise errors due to incorrect patient identification or clinically significant wrong results. The following table shows what the minimum labelling requirements are for all pathology samples:

Request Form	Sample
Full forename Surname Date of birth Hospital/NHS/A&E number	Forename Surname Date of birth

Labelling of samples

Samples which do not comply with the above minimum labelling policy **will be discarded** unless there is an exceptional reason as to why the sample cannot be repeated. Addressograph labels should not be used to label the specimens as this has been shown to be a significant source of labelling errors and they can interfere with some of the analysis equipment. Pre-labelling sample tubes, prior to sample collection, is an unsafe practice.

If the request is made using the ICE electronic requesting system then the printed sample labels can be used on the sample tubes.

The development of the Trust's Clinical Risk Policy has highlighted the importance of applying minimum standards to the labelling of pathology samples and the provision of information on pathology request forms. This has been reinforced by a recent

report on Serious Hazards in Blood Transfusion, where labelling errors have had fatal consequences.

Request form completion

Pre-printed adhesive labels from the patient notes can be used on Pathology request forms but these do not contain all the information required by the pathology department. Other information which should be added to the request form includes the requesting consultant, the location to which the report is to be sent, relevant clinical details and test(s) required. Illegible and incomplete request forms are a source of clinical risk and are time consuming for laboratory staff to decipher.

Where possible, please make all requests using the ICE electronic requesting system.

Inadequately labelled samples

Inadequately labelled samples will be rejected by the Pathology Laboratory unless the specimen is unrepeatable e.g. CSF or timed samples. In these instances, the samples will be processed at the discretion of the Pathology Laboratory and a comment placed upon the report stating that the sample did not meet the minimum labelling requirements.

3.3 Known High Risk Samples

The laboratory operates a policy of universal precautions applied to all samples received for testing. This means that all samples are treated with the degree of care necessary to minimise infection risks to staff. When a patient is known to be of high risk or has a confirmed infection with a blood borne virus we would ask that this extra risk be documented on the request form and high risk stickers used. This provides the laboratory staff with an opportunity to ensure that the samples are handled in the correct way to remove the possibility of any laboratory acquired infection. The laboratory is recognised as being a potential high risk area for occupational exposure to blood borne viruses and we would ask for your co-operation in helping to minimise these risks.

3.4 Storage and Retention of Samples

All initial laboratory testing, whenever possible, is undertaken from the primary sample tube which remains stored in the laboratory for the next 7-10 days.

The laboratory has a responsibility to store aliquots of antenatal booking bloods for 2 years to ensure that the samples remain available for additional testing if required during the pregnancy or post-natal period. A small volume of this original sample is transferred into a storage tube and is retained in the laboratory for 2 years, stored at -20°C.

The laboratory can be contacted at any time to request that additional tests are added to stored samples within the laboratory. The laboratory staff will confirm that a sample is available and then the additional requests can be made.

Contact the laboratory on 01296 315323 to discuss sample availability for additional testing.

3.5 Urgent Samples

The laboratory staff will make every effort to respond to requests for urgent results. Urgent requests are considered to be those which may impact directly on patient care or treatment, or when patient specific requirements need to be met.
If you have a requirement for an urgent result then please follow the instructions below:

- Contact the laboratory on 01296 315323 to inform them of the urgent request;
- Provide details of the patient to be tested and clinical indications for testing;
- Provide details of the patient location
- Expected time of arrival of the sample
- Contact details for telephoning the result

The laboratory will confirm whether the sample can be processed in the time frame requested and provide a guide as to when the result will be available.

Despite our best intentions, the laboratory may not always be able to respond in the time required due to the staffing constraints of a small laboratory and assays that we are already committed to running. Please be assured, we will always respond as quickly as we are able.

3.6 Key factors which may adversely affect test results

It is well accepted that the best possible results can only be achieved if the samples received are of the highest quality. The quality of the samples received remains a shared responsibility for both the laboratory and the requester to ensure that results are not adversely affected.

Acute Infections	It is not advisable to request auto-antibody testing on patients who have significant acute infections, bacterial or viral, as these patients will give weakly false positive reactions in many assays. This can lead to confusion and delay in diagnosis and treatment.
Cellular Assays	Cellular assays rely on the white blood cells remaining viable within the sample container, and so must be kept at room temperature and not refrigerated. These samples must reach the laboratory within 2 hours of collection and the laboratory should be contacted prior to sample collection to ensure that the collection plans are suitable.
Cryoglobulins	Samples for cryoglobulins can only be collected when a member of the laboratory staff is present to ensure that the temperature of the sample does not fall below 37°C. Samples that are not collected by the laboratory will not be processed as they will only provide very poor quality results. Please contact the laboratory to make an appointment for the sample to be collected, we are unable to provide this service without an appointment being pre-made.
Drips & Infusions	Do not collect samples from the same arm as any intravenous infusion as this will dilute the peripheral circulation and lead to a poor quality result.
EDTA	EDTA samples are only suitable for cellular phenotyping assays such as lymphocyte subsets, they should not be used for any other immunology assays.
Haemolysed Samples	Grossly haemolysed samples can adversely affect the immunology specific protein results and can make electrophoresis and paraprotein quantitation difficult to achieve.

Heterophile Antibodies	These are antibodies with non-specific binding properties which can cause confusion when looking for true auto-antibodies. They are particularly common in patients who have received multiple blood transfusions of in women with a history of multiple pregnancies.
Immune Deficiency	Patients receiving replacement IV immunoglobulin cannot be tested for IgG, IgA or IgM responses, as they do not have a normal humoral immune response. All results will be falsely negative.
Immunocompromised Patients	Patients who are significantly immunocompromised do not demonstrate normal antibody responses, and all of their results must be interpreted with caution. Please discuss the testing requirements for these patients with the laboratory before undertaking any testing.
Lipaemic Samples	Grossly lipaemic samples can interfere with antibody binding and either reduce the sensitivity of an assay or lead to weakly false positive results.
Lithium heparin	Lithium heparin samples should only be taken when specifically advised. They will not be accepted for routine immunology assays.
Post Transfusion	Results are unreliable on all patients who have recently received significant blood transfusions as the laboratory investigations will detect antibodies and antigens present in the transfused units.
Request Review	In order to maintain the confidence in the sensitivity and specificity of the laboratory results, all requests are reviewed by experience members of the laboratory staff. If the investigation is not indicated by the clinical information provided, then the request may be withdrawn and additional information requested. Samples are stored within the laboratory awaiting the information and are not discarded.
Results	All laboratory results are issued electronically either onto the hospital Review system, ICE electronic requesting or via GP links. Every effort should be made to obtain the required results from these locations before contacting the

	laboratory for a verbal result. This reduces the likelihood of a verbal transcription error.
Sample Type	If in any doubt, please contact the laboratory to confirm the specific sample type required for any specific investigations.
Subjective Results	Not all of the investigations performed in the laboratory produce numerical results which can be interpreted using fixed guidelines. The microscopic examination of immunofluorescence slides for autoantibodies and the interpretation of electrophoresis gels are performed manually and subject to individual interpretation. The inherent differences in interpretation has been minimised by adopting a double reading approach whenever possible and all staff training and competency with these tasks is closely monitored. It is expected that any variation in results due to differences between individual staff interpretations will be minimal and the user should have confidence in the reported results.
Tipped Samples	If a sample has been collected into the wrong tube, DO NOT tip it into the correct one. The sample will already have been adversely affected and then cause added complications as it appears to be in the correct sample tube.
Transport	All samples are transported to the laboratory as quickly as possible and in a way appropriate for the test request. There may be occasions when extreme or adverse conditions may affect sample integrity and this will be documented on any reports.

4. Laboratory Quality Assurance

It is essential that the users of the laboratory service have confidence in the data and results produced by the laboratory. Participation in External Quality Assurance (EQA) schemes as well as rigorous internal quality control programmes ensure that the laboratory performs at a high and sustainable standard. These external schemes are designed to provide a measure of the laboratory's performance compared to other laboratories nationwide performing a similar repertoire of investigations. Variations in results from users with the same analytical instrumentation can be identified and trends in poor performance resolved quickly.

The laboratories subscribe to the following EQA schemes. Performance data is available on request to the laboratory.

Scheme	Investigations
UKNEQAS Department of Immunology PO Box 894 Sheffield.S5 7YT	Cardiolipin, Thyroid Peroxidase, TTG IgA, TTG IgG, ANCA, SMA, Mitochondrial, Anti-GBM, Beta-2-Microglobulin, Monoclonal proteins, Rheumatoid Factor, anti-CCP and total IgE
UKNEQAS Wolfson EQA Laboratory PO Box 3909 Birmingham. B15 2UE	Immunoglobulins IgG, IgM, and IgA, Complement C3 and C4, alpha-1-antitrypsin and transferin

5. Reporting of Results

5.1 Laboratory Reporting

The Immunology Laboratory at Stoke Mandeville has the ability to deliver results to locations either in printed format, electronically via GP messaging systems or the Trust internal results viewing system (Review). Results are released for reporting after all the results appropriate to an individual report have been authorised by registered Biomedical Scientists or a Consultant Clinical Immunologist. Once results have been authorised they are released and held in queues for printing and electronic release as appropriate.

Electronic transmission of fully authorised results happens in a timely manner with deliveries to Review and GP Links occurring at 5 minute intervals. Print runs are

produced at various intervals throughout the day and the reports are sorted and then sent to the appropriate location for delivery with the next day's transport services. Abnormal results which the Immunology staff consider urgent are automatically phoned to the requesting clinician. Where the result may influence immediate management, results may be phoned to the Wards. If requesting results by telephone, please have the patient's date of birth and NHS/unit number available, and indicate when the sample was taken. Approximate test turnaround times, in working days, are given with each test listed below. If a test result is required more rapidly please contact the laboratory to discuss the result availability. The staff will do everything possible to provide the results in a timely manner.

5.2 Interpretation

Where possible, interpretations and appropriate comments are provided on the report, but we are pleased to answer any enquiries about the interpretation of test results. All enquiries will be passed to the relevant medical staff as required. If unsure of the most appropriate investigations, please discuss with the lab before taking the samples, as some tests may require special samples (clotted blood, EDTA etc).

Volumes of blood required are variable but, where possible, at least 5mls should be sent. Any special sample requirements are given with the test information below.

All requests are screened and any inappropriate or unnecessary tests will be altered or deleted. If inadequate clinical details are given and the tests do not appear appropriate, the request will be returned to the originator requesting the necessary information. The sample will be saved awaiting the requested details. It is not helpful to request "autoantibody screens" or "antibody profiles". Please be specific in the tests requested or ask the laboratory staff for guidance. Providing full and detailed clinical information will improve the quality of the result you receive and allow interpretative comments to be applied.

5.3 Turn Around Times

Expected turnaround times are provided for each assay and are expressed as whole working days. Samples sent to referral centres can take up to 28 or longer days for a report to be issued. If results are required urgently, laboratory staff can telephone the relevant reference laboratory to obtain a result more quickly.

6. Autoantibody Tests

6.1. Acetylcholine receptor antibodies (CHU)

Reported in titres:	<2 x 10^{-10}M	Negative
	2 – 5 x 10^{-10}M	Equivocal
	5 – 50 x 10^{-10}M	Positive
	50 – 500 x 10^{-10}M	Strongly positive

A highly sensitive and specific marker for patients with generalised myasthenia gravis (80 – 90% sensitivity). Up to 40% of patients with pure ocular myasthenia may be antibody negative.

Type of sample required: Serum

Turn-around time: 21 days

6.2 Adrenal antibodies (CHU)

Reported as: Positive or Negative

Present in 60% of patients with isolated autoimmune hypo-adrenalism. This prevalence rises to 90% in patients with hypo-adrenalism and primary ovarian failure.

Type of sample required: Serum

Turn-around time: 21 days

6.3. Anti-IgA antibodies (SHE)

Anti-IgA antibodies occur in IgA deficient patients who have received blood-products containing IgA. Their presence in high titres indicates increased risk of adverse reactions to blood products containing IgA.

Type of sample required: Serum

Turn-around time: 21 days

6.4. C3 Nephritic factor (CHU)

C3 nephritic factor is an IgG autoantibody which stabilises the alternative pathway C3 convertase and leads to continuous C3 breakdown. This is associated with type II Glomerular nephritis and also with partial lipo-dystrophy. This test will only be performed on patients who show a low C3 and normal C4 level.

A fresh serum sample is required, reaching the lab within 4 hours of venepuncture.

Type of sample required: Serum

Turn-around time: 28 days

6.5. Cardiolipin & Beta-2 glycoprotein 1 IgG and IgM antibodies (SMH)

Reported in: Units/ml (IgG reference range 0-10)

These antibodies are associated with thrombosis (arterial and venous) in patients with systemic lupus erythematosus, often in conjunction with a lupus anticoagulant. They may also occur in isolation in patients with thrombosis and no evidence of lupus. Antibody levels do not correlate with extent or severity of thrombosis. Moderate rises in anti-cardiolipin antibodies may occur transiently following infection. Lupus anticoagulant studies are performed by Haematology. Beta-2-Glycoprotein 1 IgG antibodies are also performed as part of this lupus antibody screen.

Type of sample required: Serum

Turn-around time: 3 days

6.6. Centromere antibodies – please request "ANA" (CHU)

Reported as: Positive or Negative

They are detected on standard HEp-2 tissue substrate used for screening antinuclear antibodies. They are a marker of the 'CREST' Syndrome (Calcinosis, Raynaud's, oEsophageal dysmotility, Sclerodactyly, Telangiectasia) and limited forms of Scleroderma.

Type of sample required: Serum

Turn-around time: 7 days

6.7. Coeliac antibodies (CHU)

Request Anti-Tissue transglutaminase (TTg) IgA

Reported as: Negative, <20 or as a numerical value in units/ml

Untreated coeliac disease in adults is characterised by the presence of IgA endomysial antibodies (EMA) (sensitivity 70 – 90%, specificity 90 – 100%) or anti-Tissue transglutaminase antibodies. There is a good correlation with disease activity, and relapse or poor compliance with a gluten free diet is often associated with a return to antibody positivity. IgA endomysial antibodies may be 'falsely negative' in patients with coeliac disease and total IgA deficiency. Simultaneous measurement of serum IgA ensures that these cases are not missed. Patients with positive TTg antibodies will be reflexed tested for EMA.

Type of sample required: Serum

Turn-around time: 7 days

6.8. DNA antibodies (Double stranded DNA) (CHU)

Reference range: IU/ml (0 – 30).

A highly sensitive and specific marker for SLE, with raised levels found in 70 – 90% of patients with SLE. Antibody levels tend to correlate with disease activity. Raised levels may also be seen in a small minority of patients with chronic active hepatitis.

Type of sample required: Serum

Turn-around time:7 days

6.9. Epidermal antibodies (CHU)

6.9.1. Pemphigus

Reported as: Positive (titres) or Negative

Serum antibodies directed against the cell surface of epidermal keratinocytes are found in 90% of patients with Pemphigus and correlate with disease activity. Direct immunofluorescence staining of skin biopsies reveals intra epidermal IgG & C3 deposition in 90% of patients.

6.9.2. Bullous Pemphigoid

Reported as: Positive or Negative

Serum antibodies directed against the basement membrane are present in 70% of patients. Immunofluorescence staining of skin biopsies reveals basement membrane IgG & C3 deposits in 90% of patients. Antibody levels do not correlate with disease activity.

Type of sample required: Serum

Turn-around time: 14 days

6.10. ENA antibodies (Extractable Nuclear Antigens: Ro (Ro52/Ro60), La, Sm, RNP (U1-snRNP, RNP70) , Scl – 70, Jo – 1 (CHU)

Reported as: Positive or Negative

Present in patients with SLE, Lupus overlap syndromes (Mixed Connective Tissue Disease), Sjogren's syndrome and Scleroderma. The sensitivity and specificity of individual antibodies for these diseases is variable e.g. Sm and Scl70 antibodies are specific for SLE and Scleroderma respectively, whilst Ro and La antibodies occur in lupus and Sjogren's syndrome. The isolated presence of anti-RNP antibodies is suggestive of MCTD. The presence of a speckled ANA (> 1/80) is often a clue but not specific to the presence of anti-ENA's. Conversely a negative ANA on HEP-2 cells precludes the presence of anti-ENA antibodies.

Type of sample required: Serum

Turn-around time: 7 days

6.11. Aspergillus IgG levels (CHU)

Reference range – 0-79mgA/L

Used to assist in investigations for allergic broncho-pulmonary aspergillosis (ABPA) and hypersensitivity pneumonitis. Conducted using Phadia 250 Immunocap method.

Type of sample required: Serum

Turn-around time: 14 days

6.12. Anti-GAD antibodies (CHU)

Reported as: Positive, sometimes with a numeric value, or Negative

Antibodies to glutamic acid decarboxylase in high titre are a reliable marker of the stiff-person syndrome (60% sensitivity), a rare neurological disease characterised by muscle rigidity and spasms.

Type of sample required: Serum

Turn-around time: 14 days

6.13. Ganglioside antibodies (CHU)

Anti-ganglioside antibodies are associated with several immunologically mediated peripheral neuropathies e.g. anti-GM1 (IgM) with multifocal motor neuropathy, GQ1b (IgG) with the Miller-Fisher syndrome.

Type of sample required: Serum

Turn-around time: 28 days

6.14. Gastric Parietal Cell antibodies (CHU)

Reported as: Positive or Negative.

An antibody marker of pernicious anaemia that is present in 90% of cases. It is highly sensitive, but not very specific. Testing for Pernicious anaemia should be primarily conducted with intrinsic factor antibodies (see anti-intrinsic factor antibodies).

Type of sample required: serum

Turn-around time: 14 days

6.15. Glomerular Basement Membrane antibodies (SMH)

Reported as: Positive or Negative U/ml.

This antibody is a highly sensitive and specific marker of Goodpasture's syndrome. Levels correlate well with disease activity and often predict clinical outcome.

If required urgently, please contact the laboratory.

Type of sample required: Serum

Turn-around time: 3 days

6.16. Intrinsic Factor antibodies (CHU)

Reported as: Negative or with a Numerical Value in U/ml

This is a highly specific marker for pernicious anaemia that is found in 75% of patients. The antibodies can prevent the uptake of vitamin B12 by either binding to the vitamin complex and preventing its uptake, or blocking the binding. It is an indicator of a disease process that requires further investigation.

Type of sample required: Serum

Turn-around time: 7 days

6.17. Jo-1 antibodies (see ENA antibodies) (CHU)

Reported as: Positive or Negative

Found in 25% of cases of polymyositis, it correlates well with the presence of interstitial lung disease. An extended panel of myositis specific antibodies is available following discussion with the laboratory.

Type of sample required: Serum

Turn-around time: 7 days

6.18. Liver Kidney Microsomal (LKM) antibodies (CHU)

Reported as: Positive or Negative

LKM antibodies are found only in a small number of patients with autoimmune chronic active hepatitis and drug induced hepatitis, but are highly specific for these diseases.

Type of sample required: Serum

Turn-around time: 7 days

6.19. Mitochondrial antibodies (CHU)

Reported as: Positive or Negative

A highly sensitive and specific marker of Primary Biliary Cirrhosis, it is also found in a small percentage of patients with autoimmune chronic active hepatitis.

Type of sample required: Serum

Turn-around time: 7 days

6.20. Neuronal antibodies (anti-Hu, anti-Yo,anti-Ri, Anti-Ma2, Anti-CV2/CRMP5, Anti-Amphiphysin, Anti-Zic-4, Anti-Sox-1, Anti-Tr, Anti-Titin & Anti-Recoverin) (CHU)

Reported as: Positive or Negative

These antibodies occur in a variety of para-neoplastic neurological syndromes associated with various malignancies e.g. small cell lung carcinoma, lymphoma, breast carcinoma & ovarian carcinoma. A wide range of additional neurological antibodies are available by special request.

Initial testing is performed by immunofluorescence, with follow-on testing by immunoblot panel if reactivity is seen. Requests for primary testing by the immunoblot method can also be made.

Type of sample required: Serum

Turnaround time: variable, but approximately 21 days

6.21. Neutrophil Cytoplasmic antibodies (ANCA) (CHU)

Reported as: Positive or Negative

If positive, the titre and staining pattern is also reported as: cytoplasmic (c-ANCA) or perinuclear (p-ANCA).

If required urgently, please contact the laboratory on ext 5323

The antigenic specificity of all positive ANCA samples is characterised by performing ELISA assays for anti-proteinase3 (PR3) and anti-myeloperoxidase (MPO) antibodies.

Presence of high titre (>1/80) c-ANCA in the appropriate clinical setting, directed against PR3, is highly suggestive of untreated necrotising vasculitis e.g. Wegener's granulomatosis or Microscopic polyarteritis. Antibody titres tend to correlate well with disease activity. Note 'false positives' may occur in diseases which may mimic systemic vasculitis e.g. infective endocarditis, tuberculosis, non-Hodgkin's lymphoma and acquired immune deficiency syndrome. P-ANCA (of anti-MPO specificity) are found in up to 50% of patients with microscopic polyarteritis and pauci-immune glomerulonephritis, in addition to rheumatoid arthritis, SLE and ulcerative colitis.

The laboratory operates a gating policy to reduce un-necessary requests or those with limited clinical value. Please contact the laboratory if you require further guidance.

Type of sample required: Serum

Turn-around time: 3 days

6.22. Nuclear antibodies (ANA) (CHU)

Reported as: Positive Titre or Negative

A sensitive marker of systemic lupus erythematosus; which occurs in virtually all patients with untreated disease. A negative ANA on HEp-2 cells effectively excludes untreated SLE. Presence of ANAs in significant titre (>1/80) is not specific for SLE, occurring in up to 15 – 50% of patients with other autoimmune diseases e.g. rheumatoid arthritis, scleroderma, Sjogren's, dermatomyositis. The ANA titre is of little value in monitoring SLE disease activity, as the antibodies have an *in-vivo* half - life of 3 – 4 weeks.

Clinically relevant, positive ANA results will have ENA screening and dsDNA ab screening reflexed on to them.

The laboratory operates a gating policy to reduce un-necessary requests or those with limited clinical value. Please contact the laboratory if you require further guidance.

Type of sample required: Serum

Turn-around time: 5 days

6.23. Ovarian antibodies (CHU)

Reported as: Positive or Negative

Present in patients with either isolated primary ovarian failure or it is associated with other autoimmune endocrinopathies such as Hypo-adrenalism, insulin dependent diabetes and pernicious anaemia.

Type of sample: Serum

Turn-around time: 21 days

6.24. Pancreatic Islet cell antibodies (PICA) (CHU)

Reported as: Positive or Negative.

Present in 90% of patients with insulin dependent diabetes mellitus at presentation. There is increased prevalence in relatives of patients with IDDM.

Type of sample required: Serum

Turn-around time: 21 days

6.25. Parathyroid antibodies (SHE)

Reported as: Positive or Negative

Present in up to 10% of patients with idiopathic hypo-parathyroidism. Many of these patients have multiple autoimmune poly-endocrinopathies with antibodies to both adrenal cortex and ovarian tissue.

Type of sample required: Serum

Turn-around time: 21 days

6.26. Rheumatoid Factor (SMH)

Reported as: Negative or Positive

Rheumatoid factor (usually IgM) is present in approximately 70% of patients with RA and correlates with more severe disease. The presence of RF is not essential for the diagnosis of RA. RF also occurs in other autoimmune diseases (SLE, scleroderma, Sjogren's), and chronic bacterial infection. Rheumatoid factors are present in low titre in 5% of the normal population.

Type of sample required: Serum

Turn-around time: 2 – 3 days for screen

6.27. Ribosomal antibodies (SHE)

Reported as: Positive or Negative.

Present in a minority of patients with SLE.

Type of sample required: Serum

Turn-around time: 2-5 days

6.28. Scl-70 (see ENA antibodies) (CHU)

Reported as: Positive or Negative

Present in 15 – 20% of cases of scleroderma, it forms a marker of systemic disease. Additional extended testing panels are available by special request.

Type of sample required: Serum

Turn-around time: 7 days

6.29. Smooth muscle antibodies (CHU)

Reported as: Positive (titre) or Negative

Present in up to 70% of patients with autoimmune chronic active hepatitis and in approximately 25 – 50% of patients with primary biliary cirrhosis. The antibody frequently occurs as a 'false positive' in patients with viral infections.

Type of sample required: Serum

Turn-around time: 5 days

6.30. Antibodies to Striated Muscle (CHU)

Reported as: Positive or Negative.

Found in 60% of patients with Myasthenia Gravis, the presence of striated muscle antibodies in myasthenia suggests the presence of a thymoma.

Type of sample required: Serum

Turn-around time: 21 days

7. Protein Immunochemistry

7.1. Alpha-1 anti-trypsin (1 Proteinase inhibitor) (SMH)

Reported as mg/dl (107 – 209mg/dl)

Measurement of α-1 anti-trypsin level is useful in the investigation of emphysema and unexplained liver disease in adults. In paediatric practice α -1 anti-trypsin deficiency is associated with neonatal jaundice. Patients with low α1-AT levels will be sent for Phenotyping to determine homo or heterozygosity.

Type of sample required: Serum

Turn-around time: 3 days

7.2. B2-Microglobulin (SMH)

Reported as mg/L NR <3.0mg/L

As an index of cellular turnover and renal tubular function, this test can be a useful prognostic marker in myeloma. Levels are raised in renal tubular dysfunction irrespective of cause.

Type of sample required: Serum

Turn-around time: 2-5 days

7.3. C1 inhibitor (formerly termed C1 esterase inhibitor) (CHU)

Quantitatively Result: as mg/dl (reference range 0.15 – 0.35)

Functional Activity (reference range 70 – 130u/ml)

C1 inhibitor deficiency (antigenic or functional) is transmitted as an autosomal dominant disorder resulting in hereditary angioneurotic oedema. Acquired C1 inhibitor deficiency may also occur with B-cell lymphomas. Samples collected during an acute attack of angioedema due to C1 inhibitor deficiency are characterised by a

low C4. Conversely, a normal C4 level virtually excludes all forms of C1 inhibitor deficiency.

Type of sample required: Serum

Turn-around time: 7 days

7.4. Complement levels (C3 & C4) (SMH)

Reported as: Unit value in mg/dl

C3 Range: 65 – 190 mg/dl

C4 Range: 15 – 50 mg/dl

Measurement of serum complement levels is useful in the diagnosis and monitoring of immune complex diseases e.g. SLE. Complement levels act as acute phase proteins and may be normal, despite complement consumption, in some inflammatory and infective disorders.

Type of sample required: Serum

Turn-around time: 1 – 2 days

7.5. Cerebro-spinal fluid Oligoclonal banding & IgG / Albumin (CHU)

Reported as: Oligoclonal bands Present or Absent

Oligoclonal bands, confined to the CSF, are indicative of intrathecal immunoglobulin synthesis. Oligoclonal bands are suggestive, but not pathognomonic of multiple sclerosis, being also found in infective and inflammatory diseases of the central nervous system e.g. viral encephalitis, bacterial meningitis, neurosyphilis, sarcoid and lupus.

Type of sample: Paired CSF & serum samples

Turn-around time: 21 days

7.6. Functional complement, CH100 (previously CH50) and AP100 (CHU)

Reported as: Normal or percentage of normal

Normal range is equal or >70% of control.

These investigations form a test of integrity of classical and alternate lytic pathways of complement. Low levels occur if any component is absent.

Sample needs to reach the laboratory within 1 hour of venepuncture. Patients with any form of meningococcal disease, particularly if recurrent, should be screened in convalescence with a CH100.

Type of sample: Serum

Turn-around time: 28 days

7.7. Cryoglobulins (CHU)

Reported as: Positive or Negative.

For Cryoglobulin testing, clotted blood (2 tubes) should be taken and immediately placed in a thermos flask containing water at 37 to 39°C. Please organise this sample collection with the laboratory who will arrange to be present when the samples are collected. If there are any questions or doubts about taking blood for this test, contact Immunology on 01296 315323.

Cryoglobulins are immunoglobulins which reversibly precipitate in the cold. Type I (monoclonal) cryoglobulins are associated with B cell lymphoma and myeloma whilst mixed cryoglobulins (type II & III) are associated with infective and inflammatory disorders. Type II cryoglobulins exhibit rheumatoid factor activity and are invariably associated with marked consumption of C4. Consider cryoglobulinaemia in any patient with unexplained renal or skin disease and a low C4.

Type of sample required: Serum **(Kept at 37°C)**

Turn-around time: 14 days

7.8. Serum electrophoresis (SMH)

Electrophoresis is essential in the investigation of suspected paraproteinaemia and immune deficiency. Characteristic patterns are also seen in the presence of an acute phase response, nephrotic syndrome and α1-antitrypsin deficiency. A polyclonal increase in the gamma region is seen in inflammatory and infective disorders, often with a concomitant acute phase response. A decrease in the gamma region indicates either primary or secondary hypogammaglobulinaemia. This test is performed in conjunction with immunoglobulins.

Type of sample required: Serum

Turn-around time: 2 – 3 days

7.9. Urine electrophoresis (SMH)

Urine electrophoresis is most useful in detecting the presence of Bence Jones proteins (monoclonal free light chains) in patients with suspected myeloma. Polyclonal free light chains may occur in the urine of healthy elderly people as well as in inflammatory disorders. All serum and urine samples with suspected paraprotein bands will be investigated by immunofixation electrophoresis.

Type of sample required: **Urine & Serum**

Turn-around time: 3 to 5 days

7.10. Immunofixation electrophoresis (SMH)

Result is reported descriptively as presence or absence of paraprotein.

All serum samples with suspected paraprotein bands on electrophoresis are typed by immunofixation and quantified by densitometry scanning.

7.11. Haemolytic complement activity – classical pathway / alternative pathway (CH100, AP100) (CHU)

See 7.6. Functional complement, CH100 (previously CH50) and AP100

7.12. Immunoglobulins (SMH)

NR: (Adult) IgG 6.0 – 13g/L
IgA 0.8 – 3.0g/L
IgM 0.4 – 2.5g/L

Paediatric ranges are applied to children's results.

This forms an essential investigation for 'failure to thrive', recurrent infections and lymphoproliferative diseases including myeloma. IgA deficiency occurs in 1 in 700 people and may not be associated with disease (but beware of transfusions). Polyclonally raised IgG occurs in chronic infection and inflammation, especially HIV infection, chronic liver disease and to a lesser extent in connective tissue diseases. Reduced immunoglobulins – predominantly IgG may be due to loss (protein – losing enteropathy, nephrotic syndrome), reduced synthesis (lymphoproliferative disorders; primary immune deficiency) and excessive catabolism. Low levels always warrant further investigation, as serious infective complications may occur.

Type of sample required: Serum

Turn-around time: 1 – 2 days

7.13. IgG subclasses (CHU)

Reported in g/L

Adult reference range: IgG1 3.2 – 10.2
IgG2 1.2 – 6.6
IgG3 0.2 – 1.9
IgG4 0.1 – 1.3

For children age matched reference ranges are provided. IgG subclass measurements are only useful in the investigation of selected cases of suspected immune deficiency. Levels vary with age. IgG2 levels are physiologically low in infancy and may not reach adult levels until 10 – 12yrs of age.

Type of sample required: Serum

Turn-around time: 21 days

7.14. Specific antibodies to Tetanus toxoid, Haemophilus type B and Pneumococcal Polysaccharide (CHU)

Reported quantitatively – levels vary with age.

Measurement of specific antibody production (spontaneous and post immunisation levels) is useful in the assessment of patients with suspected immune deficiency.

Assays are only undertaken following prior discussion with immunology medical staff.

Type of sample required: Serum

Turn-around time: 21 days

7.15. Total IgE (SMH)

Reported as: Units ku/L (adult reference range <120).

Total IgE levels are elevated in atopic eczema, allergic asthma bronchopulmonary aspergillosis, invasive helminthiasis and some forms of immunodeficiency.

Measurement of total IgE levels is not essential for the diagnosis of allergy.

Type of sample required: Serum

Turn-around time: 1-2 days

7.16. Specific IgE antibodies ('RAST' tests) (CHU, SHE)

Reported as: a numerical value, with descriptive interpretation of grade 0 – 6 reactivity

Specific IgE tests are available to a wide range of antigens. However, they are not a substitute for proper history taking and skin prick tests except in small children, those with extensive skin involvement that precludes skin testing or where there is a risk of anaphylaxis.

Blanket requests for 'RAST testing' will NOT be processed.

Please give details of suspected allergens and symptoms. Samples with insufficient details will be stored for 1 month awaiting the required information Tests for specific IgE to penicillin are not reliable in diagnosing immediate type hypersensitivity to this drug. If in doubt, please contact the laboratory on ext 5323 to discuss.

Type of sample required: Serum

Turn-around time: 21 days

7.17. Venom Specific IgE

Specific IgE to bee, wasp and hornet venom are available

Type of sample required: Serum

Turn-around time: 21 days

7.18. Component Resolved Specialist Allergens (CHU)

These assays are available for peanut, hazelnut, birch, insect venoms and egg antigens and may provide additional information regarding severity of any allergic response.

Type of sample required: Serum

Turn-around time: 21 days

7.19. Serum Free Light Chain Assay (CHU)

A specific measure of renal capacity to remove monoclonal free light chains from a patient's circulation as an indication of disease activity or myeloma relapse.

Requests are only processed from Haematology.

Type of sample required: Serum

Turn-around time: 21 days

8. Cellular Investigations

8.1. Lymphocyte surface marker analysis (CHU)

Please ensure that a full blood count is performed simultaneously. Indicated in the investigation of immunodeficiency and monitoring of HIV infection. The use of CD4 counts as a surrogate marker for the diagnosis of HIV infection is unhelpful and constitutes poor medical practice. A low CD4 count is not diagnostic of HIV infection, occurring in a wide variety of other conditions including primary immunodeficiency, viral and bacterial infection, lupus, steroid therapy. All requests for lymphocyte surface marker analysis, other than for HIV monitoring, should be discussed with the departments' medical staff.

It is not possible to process these samples if they are collected on a Friday due to the restrictions of the reference laboratory

Special samples required – discuss with Laboratory

Type of sample required: EDTA (5ml)

Turn-around time: 7 days

8.2. Neutrophil function (CHU)

Special samples required – discuss with Laboratory.

Neutrophil function tests are available through the department. These are useful in the diagnostic work-up of patients with suspected primary immunodeficiency, particularly those patients with recurrent deep-seated bacterial and fungal infections. Primary neutrophil defects are exceedingly rare. Tests are only available by special arrangement with the Laboratory's medical staff.

8.3. QuantiFERON-TB Gold Plus Testing (CHU)

Quantiferon is a gamma interferon stimulation assay that is used to look for latent TB in individuals who have visited areas with higher endemic risks or who have been in contact with known cases. The samples MUST be returned to the laboratory on the day of collection as the antigen stimulation stage of the assay must be initiated within 16 hours of collection.

Samples to be kept at Room Temperature after collection and NOT refrigerated

It is not possible to process these samples if they are collected on a Friday due to the restrictions of the reference laboratory

Special samples required – discuss with Laboratory

Type of sample required: Quantiferon collection kit available from immunology

Turn-around time: 7 - 10 days

9. Guidelines on the appropriate use of tests

9.1. Diagnosis and monitoring of Systemic Lupus Erythematosus

Diagnosis: ANA, antibodies to dsDNA, ENAs, antibodies to cardiolipin, C3, C4 and immunoglobulin levels should be requested at presentation. A negative ANA performed on HEp-2 cells effectively excludes the diagnosis of untreated SLE.

Monitoring: Since the half-life of antibodies is 3 weeks, serial measurement of antibodies at weekly or fortnightly intervals is unhelpful. At each visit measurement of C3, C4 and CRP is advised with intermittent measurement of ANA and dsDNA binding antibodies.

9.2. Systemic Vasculitides

At presentation, the following investigations are useful for baseline assessment:

ANA
ANCA
C3 and C4
CRP
RF
Immunoglobulins
Cryoglobulins

9.3. Diagnosis of Wegener's granulomatosis and Microscopic polyangiitis

Diagnosis: In patients with active untreated Wegener's granulomatosis, c-ANCA / p-ANCA (anti-PR3) is present in > 90% of cases. Although p-ANCA occurs in

microscopic polyangiitis, idiopathic pauci-immune glomerulonephritis and in a few patients with Wegener's, they are also present in a range of other autoimmune diseases as well e.g. SLE, RA and ulcerative colitis.

Monitoring: Wegener's, Microscopic polyangiitis – at each visit CRP and ANCA. In view of antibody half-life of 3 weeks, frequent ANCA measurement i.e. weekly / fortnightly is unlikely to provide clinically useful information. In patients in remission, a rising ANCA titre often heralds a relapse.

9.4. Investigation of renal failure

At presentation, the following investigations are useful for baseline assessment:

ANA
C3, C4
CRP
ANCA
Anti-GBM
Cryoglobulins
Serum immunoglobulins and electrophoresis
Urine electrophoresis

9.5. Suspected immunodeficiency

At presentation, the following investigations are useful for baseline assessment:

C3, C4
Immunoglobulins and Electrophoresis
Subclasses
Functional Antibody levels

Further investigations should be undertaken only after discussion with the laboratory staff.

9.6. Investigation of Allergy

Selective testing for specific IgE antibodies tailored to the clinical history. All encompassing 'blanket' screens for specific IgE antibodies cannot be accepted (see under specific IgE antibodies).

9.7. Investigation of Anaesthetic Reactions

Consult Laboratory as soon as possible.

Take 10ml clotted blood immediately after the onset of the reaction and collect 3 further samples over the next 24 hours and test for mast cell tryptase, i.e. 1hour, 6 hours and 24 hours post reaction.

Label each one clearly with the time taken.

Please send full details of the agents used and relevant previous drug history, type of operation, clinical manifestations, management and outcome. Patients with suspected anaesthetic allergy will need to be assessed in an allergy clinic during convalescence.

Type of sample required: Serum

Turn-around time: 10-14 days

10. Reference Laboratories

The majority of samples received in the laboratory are tested in-house, but investigations that are less commonly requested or those that require specialist equipment are referred to specialist laboratories for processing.

The laboratory in which the testing is undertaken is indicated by a three letter code in brackets, after the test description. SMH refers to the in-house laboratory. The other referral laboratories used routinely are listed below:

Code:	Reference Laboratory & Address
CHU	Department of Immunology Oxford University Hospitals NHS Trust Churchill Hospital Headington Oxford. OX3 7LE
SHE	Sheffield Protein Reference Unit Immunology Department P.O. Box 894 Sheffield S5 7YT
CAR	Cardiff Protein Reference Unit Medical Biochemistry & Immunology University Hospital of Wales Heath Park

	Cardiff. CF14 4XW
KIN	KingsPath Kings College Hospital 40 Denmark Hill, London
BAT	Bath Institute for Rheumatic Disease Allan Dixon Building 1 Trim Bridge Bath BA1 1HD
SJI	St John's Institute for Dermatology St Thomas' Hospital Lambeth Palace Road London