REGIONAL IMMUNOLOGY USER MANUAL

June 2024

Additional Information & Cross References	
Replaces Document Number	**I-66 V26.0**
Change Management	
Related Documents	

Please ensure that this is the most up to date version of the Regional Immunology User Manual

Regional Immunology Service

Introduction

The Regional Immunology Service is based at the Immunology Day Centre and at the Kelvin Laboratory site, Royal Hospitals, Belfast Health & Social Care Trust.

Our full postal address is:

Clinical Service:
Regional Immunology Service
Immunology Day Centre,
Ward 34 Elliott Dynes Building
The Royal Hospitals
Belfast Health & Social Care Trust
Grosvenor Road
Belfast
BT12 6BA

Laboratory Service:
Regional Immunology Service
Kelvin Laboratories,
The Royal Hospitals
Belfast Health & Social Care Trust
Grosvenor Road
Belfast
BT12 6BA

Clinical Service.

The clinical Immunology service receives referral in the areas of allergy, immune deficiency and autoimmune disease.

The clinical service provided at the Immunology Day Centre includes infusion clinics for immunoglobulin replacement therapy (IRT) and biological drugs and an IRT home therapy service (IVIG / SCIG / FSCIG).

Allergy challenge testing and allergen desensitisation is also undertaken.

More information about Outpatient Clinics is available on the service website below.

www.regionalimmunologyservicenorthernireland.com

Laboratory Service.

Working hours: **Monday to Friday from 09:00 to 17:00** (excluding public holidays).

Any **out of hours** requests should be directed to the Royal Hospitals' telephone switchboard (02890 240503) who will then contact the appropriate staff.

For routine results: Test results are available via the **Northern Ireland Electronic Care Record (NIECR)** and **encompass**.
Please avoid telephoning wherever possible. Non-urgent telephone calls create a significant workload and cause unnecessary delay in processing samples.

Regional Immunology Laboratory Kelvin Building, Royal Victoria Hospital, Grosvenor Road, Belfast, BT12 6BA		
Laboratory Contacts:		
Laboratory Enquiries / Advice		028 96151569 028 96151566
Clinical Lead	Lisa Devlin	028 96150088
Discipline Manager	Mr Sean Conlan	028 96154863
Operational Manager	Mrs Denise Difallah	028 96151562
Clinical Scientist	Dr Lynn Maxwell	028 96151563
Quality and Health and Safety Officer	Mrs Christine Taylor	028 96151563
Medical Contacts:		
Immunology Consultant	Dr Lisa Devlin	028 96150088
Immunology Consultant	Dr Tanya Coulter	028 96150088
Immunology Consultant	Dr Cathal Steele	028 96150088
Immunology Consultant	Dr Jayne McGucken	028 96150088
Immunology Specialty Registrars	Dr Inas Makki Dr Vyanka Redenbaugh	028 96150088
Specialty Doctor	Dr Michael Zhang	028 96150088
Immunology secretaries		028 96150088
Out of Hours Contacts:		
Urgent Out of Hours	Contact RGH Switchboard who will notify the appropriate staff	028 90240503
Laboratory email address to request additional tests	immunologyaddons@belfasttrust.hscni.net	

All of the above staff can also be contacted via email using the address:
firstname.surname@belfasttrust.hscni.net

Test repertoire

The Immunology laboratory is a UKAS accredited testing laboratory No. 8612

The test schedule listing accredited tests can be found on the UKAS website:
https://www.ukas.com/wp-content/uploads/schedule_uploads/00007/8612-Medical-Single.pdf

Assays not currently UKAS accredited include:
Cellular Immunology: Lymphocyte Activation markers and TCR αβ γδ cells.
Allergy: Mast Cell Tryptase on plasma/lithium heparin samples.

Assays currently awaiting UKAS Extension to Scope accreditation:
Anti Intrinsic Factor Antibodies

We provide a comprehensive range of tests for the immunological investigation of patients. Our aim is to provide the highest quality of service with prompt delivery of accurate results, (backed up by specialist medical and scientific expertise). Where specific tests are not available locally, we will refer samples on to colleagues in other centres. Further information on the reference laboratories used can be obtained by contacting a Quality Lead.

The department is happy to assist in the interpretation of patient's test results. Interpretative comments will be added to reports where appropriate.
Comments on how our service could be improved are always welcomed.

A list of tests offered is described in the following pages and includes type and volume of specimen and, if appropriate, any special requirements. There is a brief summary of the clinical application of each test which is intended to be helpful but is not intended to replace discussion of individual patients. The final section is a "Disease Index" which is intended to assist in the selection of the most appropriate investigations.

Turnaround Time

Average test turnaround times (TAT's) in days are quoted for the various tests. The turnaround times for tests referred to other centres are closely monitored and are available upon request.

Urgent Samples:

The laboratory must be telephoned to arrange all urgent samples before the specimen is collected and sent to the laboratory. Instructions will be given. It is NOT sufficient to mark the sample and/or request form "urgent". The requesting clinician is responsible for arranging transport of urgent samples to the laboratory.

Consent for venepuncture and testing

All procedures carried out on a patient need the informed consent of the patient.
For most routine laboratory procedures, consent can be inferred when the patient presents and willingly submits to the usual collecting procedure, for example venepuncture. It is assumed that when a sample is sent to the laboratory, the clinician responsible for the care of the patient has obtained the appropriate and valid consent for the test, storage and sharing of the patient's information with the relevant Health Care Professionals to generate the result so that the laboratory is not required to confirm or document consent. In emergency situations, it is assumed that when a sample is sent to the laboratory, the decision to bleed and complete the relevant testing has been taken by the clinical team in the best interest of the patient.

Transportation of Samples

There is a legal responsibility and a duty of care on anyone who dispatches clinical material (diagnostic specimens) to the Belfast Trust Laboratories, (by whatever means, including hospital van, courier, taxi, post, internal portering, or pneumatic chute).
Samples from within the Royal Hospitals can be sent by hospital porter or via the pneumatic tube system (except Category 3 samples).
Samples from other hospitals / GPs may be sent by the relevant dispatch systems.
The following documents are available from the laboratory on request:

- Transport of Specimens to the Laboratory
- Health & Safety Rules for Porters & Couriers
- Pneumatic Tube Transport of Clinical Specimens

Postal samples must be sent in accordance with the guidelines issued by the Post Office in respect of postal transmission of pathological specimens.
For advice contact the laboratory.

https://belfasttrust.hscni.net/service/laboratory-services/laboratories-user-manual

High risk samples

The laboratory must be informed of any known or potential hazards associated with samples sent.
Specimens of blood, serum and other body fluids from suspected or known carriers of Category 3 pathogens (hepatitis B or C, HIV, CJD, COVID-19) must be clearly marked with hazard stickers and enclosed in a sealed plastic bag. Request forms should also have a hazard sticker.

For some types of sample, and specific categories of hazard, a restricted range of services may be offered.

Unsuitable Samples

If a sample is unsuitable for testing a report will be sent to the requestor giving the reason and requesting another sample. Samples unsuitable for testing include pleural effusion for any test, inappropriate presence or absence of anticoagulant, delayed cellular/ functional assay samples, haemolysed and/ or lipaemic samples and unlabelled samples/forms.

Requesting additional examinations

Patient serum samples are held by the laboratory for approximately 3 weeks. During this time the laboratory may be contacted for discussion on the appropriateness of additional testing. Additional tests must be confirmed in writing by request form or electronic equivalent. An email address is available for email requests: ImmunologyAddons@belfasttrust.hscni.net

Frequency of requesting examinations

How often a test should be repeated, if at all, should be based on a number of criteria:

- The physiological properties
- Biological half-life
- Analytical aspects
- Treatment and monitoring requirements
- Established guidance

The Royal College of Pathologists have published advice on the minimum retesting intervals (MRI) in pathology:

https://www.rcpath.org/resourceLibrary/g147-minimum-retesting-intervals-in-pathology.html

Duplicate samples

For most tests, samples received within 7 days of a previous sample will not be tested. The following comment will be printed on the report:
'*Test Name* has not been tested on this sample as it has already been checked within the last 7 days. Please refer to Lab number (e.g.) *24B3000001* on *01/01/24'*.

Exceptions to this rule are:

TEST	**MRI**
ANCA	1 day
GBM Antibody	1 day
Anti CCP Antibody	90 days (3 months)
Anti IgA tTG Antibody	42 days (6 weeks)
Connective Tissue Disease screen	90 days (3 months)
ENA specificities (with a positive history)	1 year

Immunology request forms

Supplies Information The immunology request form has a light brown strip along the top, middle and bottom.
Request forms can be obtained by contacting BSO PaLS via the customer helpline 028 90667799. Product code WPH000012

The request form contains 3 sections, which refer to separate sections of the laboratory: Autoimmune serology, Allergy and Cellular immunology. Tests may be requested by ticking the appropriate box or writing the test required in the space provided. **Separate blood samples and request forms are needed for tests performed in separate sections of the laboratory.**

Please note, Immunochemistry tests are no longer performed by Immunology and should be sent to the Clinical Chemistry laboratory. Refer to the laboratory sections in this handbook for further details of their individual sample requirements.

encompass:

encompass is a Health and Social Care (HSC) wide initiative that will introduce a digital integrated care record to Northern Ireland.

https://encompassni.hscni.net/

Lab tests ordered via encompass will include all the essential criteria required.

Belfast Trust users:

https://bhsct.sharepoint.com/sites/pm/SitePages/Instructions-for-sending-samples-to-the-lab-post-Encompass-go-live.aspx

The importance of supplying the correct legible information cannot be over-stressed since specimens cannot be accepted for analysis where the identifying information on either the specimen or request form (if applicable) is inconsistent or inadequate.
Missing or illegible information on a sample request form raises a patient safety concern e.g. the wrong test may be carried out (and the right one not carried out); a critically important result may not be communicated in a timely manner because the source is not identifiable; or results may not be readily available to look up because the patient is not uniquely identifiable. If the location/source and consultant is not identified, laboratory staff cannot telephone critical results. Some tests are time-specific and if the date and time of sampling are not stated on the request form, the accuracy of such results cannot be assured.

The responsibility for requesting and following up on a laboratory test lies with a trained and authorised practitioner. Furthermore, it is the responsibility of the requester to

ensure that samples are correctly labelled and request forms are completed to agreed standards.

Minimum Acceptance Criteria (MAC 2.0)
The following standards for safe patient and sample identification ensure that the correct result will be available to guide management.

Requests which fail to fulfil MAC 2.0 will be rejected.
If the collection process is not completed on EPIC, the laboratory will not receive an electronic request as a result the request will not meet MAC 2.0 and therefore will not be processed.

MINIMUM ACCEPTANCE CRITERIA	
SAMPLE	**REQUEST FORM**
• H&C number [1] • Patient Official First Name • Surname • Date of Birth (dd/mm/yyyy) • Name / signature of staff member taking the sample [4]	• H&C number [1] • Patient Official First Name • Surname • Sex • Date of Birth (dd/mm/yyyy) • Date & Time of Sample Collection • Full Name of GP and GP Cypher code[2] • GP Practice Name and Practice code • Test Requested • Specimen type and Anatomical Site [3] *(where relevant)* • Name / signature of staff member taking the sample [4]

Footnote

1. The H&C Number must be used unless the patient is not registered with a GP in NI / is registered but does not yet have their H&C number (in which case, it must clearly state “No H&C number available” on the request form) or In an emergency situation when the identity of the patient is UNKNOWN (in which case, use the local hospital emergency numbering system)
2. Only cyphers for salaried GP’s /Partners issued and managed by BSO FPS are acceptable. Locally derived Cyphers for locums, trainee’s etc. , are not acceptable and will result in sample being rejected under MAC 2.0
3. Mandatory for Microbiology and Virology only
4. Mandatory for Blood Transfusion only

- If the location/source and requesting practitioner is not specified, laboratory staff cannot telephone critical results.
- Some tests are time-specific and if the date and time of sampling are not stated on the request form, the accuracy of such results cannot be assured.

NOTE: It is recommended that all categories listed as desirable are completed to ensure a more comprehensive service.

- Clinical information should be provided on the request form for all requests but is essential for specific IgE & ANCA requests. For vaccine studies, it is essential to state on the request form whether the sample is pre or post vaccination.

All specimens from known or suspected carriers of Category III pathogens, e.g. Hepatitis B, Hepatitis C, HIV, CJD or COVID-19 MUST be clearly marked with hazard labels on the request form and the specimen tube.

Samples with inadequate identifying information will be rejected.

Referral Tests

Specialised tests which are not available in the Belfast Trust may be sent to selected referral laboratories for analysis by arrangement. The referral centre names are provided with the laboratory reports. Further details are available upon request.

Data Protection

The legal requirement for the Trust and its staff to treat personal information confidentially and hold it securely is set out in the UK General Data Protection Regulation (UK GDPR) and the UK Data Protection Act 2018.

The Belfast Health & Social Care Trust has the following document in place and it is available via the BHSCT Intranet site or from the laboratory on request:

Policy code BHSCT/PPI (06): Policy on the Data Protection and Protection of Personal Information

Comments/Complaints

The Regional Immunology Service adheres to the Belfast Trust 'Policy and procedure for the management of complaints and compliments'. A copy is available from the laboratory upon request. Comments or compliments should be directed to the Immunology Laboratory Services Manager, Mr Sean Conlan by post, email or telephone.

Tel: 02896 154863 or RGH ext 54863 sean.conlan@belfasttrust.hscni.net

Service Agreement

Each request accepted by the Regional Immunology Laboratory for examination(s) shall be deemed to be an agreement by the user for the Belfast Health & Social Care Laboratory services, or other accredited laboratories as may be used to perform testing outside repertoire, to carry out the necessary testing and reporting function. It also implies an acceptance of the conditions of preparation and transport as outlined in this manual.

Please Note: Tests and specimen types listed below are for guidance only. For tests not listed below, or specimen types not listed within a particular test please contact the laboratory to discuss clinical requirements.

Measurement Uncertainty

The uncertainty of measurement for each test listed in the repertoire table below can be obtained on request from the Quality Officer. See contact details at the start of this manual.

AUTOIMMUNE SEROLOGY

Sample requirements: One yellow topped gel sample tube required (4.0ml). Separate blood samples and request forms are needed for tests performed in separate sections of the laboratory. Please provide clinical details on the request form.

For specific disease associations please see antibody list below. All results should be interpreted in the context of the patient's clinical history.

If clinical advice regarding interpretation of results is required, please use the contact details listed above.

CONNECTIVE TISSUE DISEASE

Connective Tissue Disease screen (CTD)

CTD Screen is intended for the in vitro qualitative measurement of antinuclear IgG Antibodies (ANA) in human serum and plasma as an aid in the clinical diagnosis of systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), Sjögren's syndrome, scleroderma and polymyositis/dermatomyositis.
ANA may also occur in a number of other conditions including juvenile chronic arthritis, fibrosing alveolitis, autoimmune hepatitis, viral infections particularly EBV and CMV and in drug reactions.

CTD Screen includes U1RNP (RNP70, A, C,), SS-A/Ro (60kDa, 52kDa), SS-B/La, centromere B, Scl-70, Jo-1, Fibrillarin, RNA Pol III, Rib-P, PM-Scl, PCNA, Mi-2 proteins, Sm proteins and native purified DNA.

TAT for CTD screen: 5 days

Results reported as negative/equivocal/positive.

If CTD screen is negative, connective tissue disease is unlikely.

If CTD screen is positive or equivocal, follow on testing for anti-dsDNA and anti-ENA antibodies will be undertaken.

Anti ds DNA antibody

Anti dsDNA is intended for the in vitro quantitative measurement of IgG antibodies directed to dsDNA in human serum and plasma as an aid in the clinical diagnosis of systemic lupus erythematosus (SLE).
For the diagnosis of SLE, dsDNA antibodies are considered to be a highly specific marker representing one of the diagnostic criteria for SLE (ACR criteria). More than 90% of sera from patients with active SLE contain dsDNA antibodies. Additionally, the determination of dsDNA antibodies is a tool to monitor the clinical course of a defined SLE patient, because a clear-cut relationship exists between anti-dsDNA titre and disease activity, in particular renal involvement.
Reference *EliA™ dsDNA 250-5500-023 UK Issued Oct 2020*
Our present anti dsDNA profile includes two assays for dsDNA. Positive samples are also tested for anti dsDNA antibody by crithidia; see below.

TAT for dsDNA: 8 days
Anti dsDNA antibody: Results reported in IU/mL, 0-9 negative, 10-15 equivocal, >15 positive.

Extractable Nuclear Antigen Screen (ENA antibodies)

ENA screen contains SS-A/Ro (60kDa, 52kDa), SS-B/La, U1RNP (RNP70, A, C), Sm, Scl-70, Jo-1 and Centromere B antigens**.**
TAT for ENA screen: 8 days
Results reported as negative/equivocal/positive.
If ENA screen is positive or equivocal follow up testing for Anti ENA specificities will be undertaken.

Extractable Nuclear Antigen (ENA) specificities:

TAT: 14 days

Anti SS-A/Ro antibody

Detection of SS-A/Ro antibodies is of interest and significance for the clinical diagnosis of SLE (prevalence 40–50%) and Sjögren's syndrome (prevalence 60–75% for primary Sjögren's syndrome).
Reference *EliA™ Ro 250-5503-022 / UK Issued Oct 2020*
Anti Ro antibodies are also found in patients with subacute cutaneous lupus erythematosus (particularly photosensitivity), neonatal lupus, congenital complete heart block in babies born to SLE mothers (rare) and SLE with interstitial pneumonitis.
Anti-Ro (SS-A) contains Anti Ro60kDa and Anti Ro52kDa.
Results reported in EliA U/mL, 0-6 negative, 7-10 equivocal, >10 positive.

Anti SS-B/La antibody

SS-B/La antibodies are the serological hallmark of Sjögren's syndrome but a small proportion of patients remain anti-SS-B/La negative. Reported in 6–15% of sera from SLE patients, SS-B/La antibodies are associated with a lower prevalence of dsDNA antibodies and renal disease in these patients. Although a strong association of neonatal lupus erythematosus (NLE) with anti-SS-A/Ro was recognized first, the majority of mothers with babies with NLE are now known to have serum SS-B/La antibodies as well.
Reference *EliA™ La 250-5504-022 / UK Issued Oct 2020*
Results reported in EliA U/mL, 0-6 negative, 7-10 equivocal, >10 positive.

Anti U1RNP antibody

Antibodies to U1RNP (ribonucleoprotein) occur in patients with SLE and mixed connective tissue disease (MCTD).
Anti U1RNP contains RNP70, A, C.
Results reported in EliA U/mL, 0-4 negative, 5-10 equivocal, >10 positive.

Anti Sm antibody

Sm antibodies, and particularly those against the SmD component, offer a highly specific, but comparatively insensitive, clinical marker for SLE. Indeed, their presence constitutes one of the revised ACR criteria for diagnosis, even though their overall prevalence ranges from 20% to 30% in SLE.
Anti Sm contains SmD^{P}-S.
Reference *EliA™ SmDP-S 250-5672-021 / UK Issued Feb 2021*
Results reported in EliA U/mL, 0-6 negative, 7-10 equivocal, >10 positive.

Anti Scl-70 antibody

Antibodies against Scl-70 (topoisomerase-1) are characteristic and specific for scleroderma/systemic sclerosis (particularly the diffuse form; frequency up to 70%).
Reference *EliA™ Scl-70^{S} 250-5637-022 / UK Issued Oct 2020*
Results reported in EliA U/mL, 0-6 negative, 7-10 equivocal, >10 positive.

Anti Jo-1 antibody

Anti Jo-1 antibodies (histidyl tRNA synthetase antibodies) are found in 20-40% of patients with aggressive polymyositis usually in association with interstitial lung disease and arthralgia. Antibodies to other tRNA synthetases are also associated with variant myositis syndromes.
Results reported in EliA U/mL, 0-6 negative, 7-10 equivocal, >10 positive.

Anti centromere antibody

These antibodies are found in patients with the limited cutaneous form of systemic sclerosis and in the CREST variant (**C**alcinosis, **R**aynaud's, o**E**sophageal immotility, **S**clerodactyly, **T**elangiectasia). Also found in up to 12% of patients with primary biliary cirrhosis, over half of such patients have clinical signs of scleroderma.
Results reported in EliA U/mL, 0-6 negative, 7-10 equivocal, >10 positive.

Reference ranges provided by Phadia AB, Sweden.

Anti dsDNA antibody by crithidia. This assay is performed on samples which are dsDNA antibody positive by the EliA method (≥16 IU/ml). The assay has very high specificity but poor sensitivity for SLE.
Anti dsDNA antibody (Crithidia): Results reported as positive or negative.
TAT: 14 days.

Anti nuclear and anti centromere antibodies by indirect immunofluorescence (IIF) using HEp2 cells

A number of clinically relevant autoantibodies can be detected using human epithelial (HEp2) cells as antigen. In the Regional Immunology Laboratory, HEp-2 cells are only used for the detection of ANA and anti-centromere antibodies.
Results for these antibodies are reported as negative or a positive titre.
TAT: 14 days
For IIF tests, please note some additional patterns may be seen which have not been requested. In these instances further tests may be reflexed by the laboratory if appropriate.

Anti histone antibody

Anti histone antibodies are found in 18-50% of patients with SLE and in 95% of patients with drug induced SLE. *This assay is performed by the Supraregional Protein Reference Laboratory, Sheffield.*
Results reported as units / ml, positive >5 U/ml.

Anti phospholipid antibodies

One yellow topped gel sample tube required (4.0ml).
Anti-cardiolipin antibodies and anti-β2 glycoprotein 1 antibodies are used, in conjunction with clinical findings, to diagnosis of Anti-phospholipid Syndrome (APS). They are form part of a spectrum of anti-phospholipid antibodies. They may also be found in patients with a variety of diseases, such as infections, malignancies and autoimmune diseases.

Anti-phospholipid syndrome (APS) may be primary or secondary to systemic lupus erythematosus (SLE) or other connective tissue diseases.

The diagnosis of anti-phospholipid syndrome is based on the presence of clinical AND laboratory criteria. The major clinical features of APS are thromboses (arterial or venous) and recurrent spontaneous abortion and fetal loss. Thrombocytopenia and skin rash (livedo reticularis) may also be present. The laboratory features of APS include persistently positive anti-phospholipid antibodies (anti-cardiolipin antibodies and/or anti β2 glycoprotein 1 antibodies) and/or lupus anticoagulant. Anti-phospholipid antibodies should be present on 2 or more occasions, at moderate to high levels (>40 U/ml) at least 12 weeks apart.

A sample should also be sent to Haematology for coagulation (Lupus anticoagulant) studies.

Anti IgG and IgM cardiolipin antibody

Reported as U/ml: <20 Negative, ≥20 Positive.
TAT: 5 days.

Anti IgG and IgM β2 glycoprotein 1 antibody
Reported as U/ml: <20 Negative, ≥20 Positive.
TAT: 5 days

Reference ranges provided by Inova Diagnostics, Inc.

Antibodies in patients with myositis / dermatomyositis.
The Myositis panel (extended) includes antibodies to Ro52, OJ, EJ, PL-12, PL-7, SRP, Jo-1, PM/Scl-75, PM-Scl-100, KU, SAE, NXP2, MDA5, TIF1, Mi-2a & Mi-2b. *This assay is performed by the Rapid Response Laboratory, Royal Free Hospital, London.*
Results for these antibodies are reported as positive or negative.

Antibodies in patients with systemic sclerosis
Anti-RNA polymerase antibodies. *This assay is performed by the Rapid Response Laboratory, Royal Free Hospital, London.*
Results for these antibodies are reported as positive or negative.

RHEUMATIC DISEASE 
One yellow topped gel sample tube required (4.0ml).
Anti cyclic citrullinated peptide antibody (CCP)
Anti-cyclic citrullinated peptide (CCP) antibodies are present in early rheumatoid arthritis (RA) and appear to be a marker of more erosive disease. The sensitivity of anti-CCP is similar to that of RF but the test is more specific for RA.
Results reported in U/mL: Negative <5.3, Positive ≥ 5.3. TAT: 5 days
Reference ranges provided by Inova Diagnostics, Inc.

GASTROINTESTINAL DISEASE 
One yellow topped gel sample tube required (4.0ml).

Coeliac disease antibody screen
Requests for coeliac disease antibodies are screened for IgA anti tissue transglutaminase antibody (IgA tTG). Those positive are further tested for IgA anti endomysial antibody.

False negative Coeliac disease antibody testing can occur in patients with undetectable IgA (selective IgA deficiency; sIgAD) or if gluten has been avoided pre-test (normal quantities of gluten should be consumed for 6 weeks before testing). This assay detects some but not all patients with sIgAD. If sIgAD is detected by this assay IgG endomysial antibodies will be reflex tested. Parallel measurement of immunoglobulins (separate sample to Biochemistry) is recommended to exclude sIgAD. Ig IgA is detectable then IgA tTG result is valid.

Anti tissue transglutaminase antibodies (TGA)

Tissue transglutaminase is the antigenic target for anti endomysial antibody and these IgA class antibodies are tested in combination with anti endomysial antibodies bringing the sensitivity for coeliac disease to nearly 100%. Treatment with a gluten free diet leads to gradual disappearance of these antibodies. They can also be used to monitor dietary compliance. Approx 10% of coeliac patients are only positive for either endomysial or transglutaminase antibodies.

Results reported as Fluorescent Light Units (FLU), 0 – 4.9 FLU negative, ≥5 FLU positive. TAT: 10 days.

Reference ranges provided by Inova Diagnostics, Inc.

The analytical measuring range (AMR) of Ttg IgA is 1.02 FLU to 600.00 FLU.

Anti endomysial antibodies (EMA)

These IgA class antibodies are very specific (90-100%) for coeliac disease (CD) and dermatitis herpetiformis (DH). Treatment with a gluten free diet leads to gradual disappearance of these antibodies. They can also be used to monitor dietary compliance. IgG class anti endomysial antibodies may be detected in IgA deficient patients with coeliac disease.

Results reported as positive or negative. TAT: 14 days.

Interpretation of coeliac antibody results: IgA tissue transglutaminase (TTG) is a useful screening test for coeliac disease, whereas IgA endomysial antibodies (EMA) are more disease specific and will automatically be performed when the IgA TTG level is >4.9. Both tests may become negative in patients with coeliac disease on a gluten free diet. Duodenal biopsy remains the gold standard test for diagnosis.

Anti gastric parietal cell (GPC) antibodies

Anti GPC antibodies are present in 95% of patients with pernicious anaemia in the early stages and in patients with atrophic gastritis (type A). They are also associated with other organ specific autoimmune diseases especially autoimmune thyroid disease. Also found in the normal population (the incidence rising with increasing age). Anti-intrinsic factor antibody is a better confirmatory test for pernicious anaemia.

Results reported as negative or positive. TAT: 14 days.

Please note some additional patterns may be seen which have not been requested. In these instances, further tests may be reflexed by the laboratory if appropriate.

Anti intrinsic factor antibodies (IFA)

Anti IFA antibodies are highly specific for pernicious anaemia and are found in up to 75% of patients. Highly specific if found in combination with gastric parietal cell antibody. Anti-intrinsic factor antibody may be detected before anaemia develops.
Results reported in units, negative ≤20, equivocal 20.1-24.9, positive ≥25
Reference range provided by Inova Diagnostics.
TAT: 14 days

AUTOIMMUNE LIVER DISEASE 

One yellow topped gel sample tube required (4.0ml)

Antinuclear antibody

Please request ANA as a separate test and provide clinical details on the request form.

Liver associated antibodies **(the following three autoantibodies are detected as part of the liver associated autoantibody screen):**

Anti smooth muscle antibody

These antibodies can occur in high titres in patients with autoimmune hepatitis. Low titre antibodies may be detected after infection.
Results reported as titre, positive >40. TAT: 14 days.

Anti mitochondrial antibody

Anti mitochondrial antibodies are detected at high titre in 95% of patients with primary biliary cirrhosis. They can also be found in patients (usually lower titres) with chronic active hepatitis, autoimmune thyroiditis and Sjogren's syndrome.
Results reported as a titre, positive >40. TAT: 14 days.

Anti liver kidney antibodies (LKM)

Anti LKM-1 antibodies are associated in patients with type 2a and 2b autoimmune hepatitis. This is the most common form of autoimmune hepatitis in childhood and has a particularly poor prognosis and can be associated with hepatitis C infection. Anti LKM-2 antibody is associated with drug induced hepatitis and LKM-3 antibody is associated with hepatitis D infection.
Results reported as titre, positive >40. TAT: 14 days.

Please note some additional patterns may be seen which have not been requested. In these instances, further tests may be reflexed by the laboratory if appropriate.

Anti M2, anti LKM, anti Liver cytosol-1 (LC-1) and soluble liver antigen (SLA) antibodies (Liver antibodies line blot)

These antibodies are found in patients with primary biliary cirrhosis and autoimmune hepatitis 1and 2. Antibodies in the panel include Anti-SLA, M2, LKM, LC1, Ro-52,

GP210, PML, SP 100 & M2-3E. *This assay is performed by the Immunology Laboratory, King's College Hospital, London.*
Results for these types of antibody are reported as positive or negative.

ENDOCRINE DISEASE 
One yellow topped gel sample tube required (4.0ml).

Anti adrenal antibodies
These antibodies are detected in 60-70% of patients with idiopathic Addison's disease. *This assay is performed by the Supraregional Protein Reference Laboratory, Sheffield.*
Results reported as negative or positive.
Part of endocrine antibody panel- reported with ovary/testes antibodies.

Diabetic antibodies
This panel includes Anti IA-2, ZNT8 & GAD antibodies. If the diagnosis being queried is type 1 diabetes, then the presence of 1 or more of the diabetes autoantibodies is highly supportive. *This assay is performed by the Immunology Laboratory, King's College Hospital, London.*
Results reported in U/mL.
Reference range provided by Kings: IA-2 <7.5, ZNT8 <15 & GAD <5.0

Anti glutamic acid decarboxylase (GAD) antibodies.
These antibodies are found in >60% of patients with the stiff man syndrome (high titre) and also in patients with type 1 diabetes mellitus. *This assay is performed by The Immunology Department, Churchill Hospital,Oxford.*
Results reported as U/ml, normal range 0-5 U/ml.

Anti ovary/testes antibodies
A number of antibodies react with various cell types within the ovary and testes. Antibodies found in patients with Type 1 autoimmune polyendocrinopathy syndrome and premature gonadal and ovarian failure. *This assay is performed by the Supraregional Protein Reference Laboratory, Sheffield.*
Results reported as negative or positive.
Part of endocrine antibody panel- reported with adrenal antibodies.

NEUROLOGICAL DISEASE 
One yellow topped gel sample tube required (4.0ml).

Anti acetyl choline receptor antibody (AChR).
These antibodies are found in 85 – 90% of patients with myasthenia gravis. 10-15% of patients are sero-negative. *This assay is performed by The Immunology Department, Churchill Hospital,Oxford.*
Results reported as antibody concentration.
< 0.45 nmol/L: negative: ≥0.45 nmol/L: positive TAT: 21 days.

Anti ganglioside antibodies (GM1, GQ1b).

These antibodies are associated with a number of peripheral neuropathies. Anti GM1 antibodies are associated with Guillain Barré syndrome (GBS), chronic demyelinating polyneuropathy and multifocal motor neuropathy. Anti GQ1b antibodies are associated with Miller Fisher variant of GBS. *This assay is performed by The Immunology Department, Churchill Hospital,Oxford.*

Anti ganglioside GM1 antibody: Results reported in units, normal range 0-200.
Anti ganglioside GQ1b antibody. Results reported in units, normal range 0-25.

Anti muscle specific kinase antibody (MuSK).

These antibodies are found in approx 40% of patients with generalised myasthenia gravis who are negative for anti AChR antibody. *This assay is performed by The Immunology Department, Churchill Hospital,Oxford.*

Results are reported as positive or negative.

Anti paraneoplastic antibodies (neuronal nuclear and purkinje cell).

These antibodies are associated with paraneoplastic disorders with accompanying carcinomas. They include anti Yo (PCA), anti Hu (ANNA-1) and anti Ri (ANNA-2) antibodies. Further testing can include Western blot to Yo, Hu, Ri, Ma 2, CV2/CRMP5, amphiphysin, Zic-4, Sox-1, Tr, Titin and Recoverin antibodies.
This assay is performed by The Immunology Department, Churchill Hospital,Oxford.

Results are reported as positive or negative.

Anti NMDA (N-methyl D-aspartate) receptor antibody.

Associated with limbic encephalitis, SLE, ataxia, epilepsia partialis continua and prominent psychiatric symptoms such as behavioural and cognitive problems and seizures.
This assay is performed by The Immunology Department, Churchill Hospital,Oxford.

Results are reported as positive or negative.

Muscle antibodies:

Anti striated (skeletal) muscle antibody and Anti Cardiac Muscle antibody

Striated: These antibodies are present in some patients with myasthenia gravis and almost all (80 – 100%) patients with thymomatous myasthenia gravis. They can also occur in patients with hepatitis, acute viral infections and polymyositis.
Cardiac: Cardiac muscle antibodies are described in patients with Dressler's syndrome after myocardial infarction, cardiomyopathy, myocarditis and in patients who have undergone cardiac surgery or have had rheumatic fever.
These assays are performed by the Supraregional Protein Reference Laboratory, Sheffield.

Results reported as positive or negative.

Anti voltage gated calcium channel antibody (VGCC).

These antibodies are found in patients with the Lambert-Eaton myasthenic syndrome (LEMS). *This assay is performed by The Immunology Department, Churchill Hospital,Oxford.* **Results reported in pmol/L, positive >45pM.**

Anti voltage gated potassium channel antibody (anti VGKC ab)/ Anti-Caspr2 antibody and Anti-Lgi1 antibody.

These antibodies are associated with acquired neuromyotonia.
Please note requests for VGKC antibodies will be tested for Anti-Caspr2 and Anti-Lgi1 antibodies. This is the default screening pathway set by the referral centre.
https://www.ouh.nhs.uk/immunology/diagnostic-tests/tests-catalogue/potassium-channel-antibodies.aspx
These assays are performed by The Immunology Department, Churchill Hospital,Oxford.
Results reported as positive or negative.

Anti Aquaporin 4 antibody

Antibodies found in 80% of patients with neuromyelitis optica (NMO) or Devic's disease and approx 50% of patients with longitudinally extensive transverse myelitis. *This assay is performed by The Immunology Department, Churchill Hospital,Oxford.*
Results are reported as positive or negative.

Anti basal ganglia antibody (ABGA)

These antibodies have been associated with Sydenham's chorea, tic disorders and encephalitis lethargic like syndrome, all associated with streptococcal infections. *This assay is performed by The Neuroimmunology and CSF laboratory, Institute of Neurology, Queens Square, London .*
Results are reported as positive or negative.

Beta interferon neutralizing antibodies.

This assay is performed by The Neuroimmunology and CSF laboratory, Institute of Neurology, Queens Square, London.
Results are reported as positive or negative.

Pemphigoid and Pemphigus Antibodies. Skin Antibodies.

Diagnosis of autoimmune bullous skin diseases (pemphigus and pemphigoid).
This assay is performed by the Supraregional Protein Reference Laboratory, Sheffield.
Results are reported as weak positive, positive or negative.

RENAL DISEASE ASSOCIATED ANTIBODIES 
One yellow topped gel sample tube required (4.0ml).

Routine ANCA testing comprises of MPO-ANCA and PR3-ANCA only.
Samples positive for MPO-ANCA and/or PR3-ANCA will be automatically reflexed for indirect immunofluorescence. If indirect immunofluorescence is required on a sample which is negative for MPO-ANCA and/or PR3-ANCA please contact the laboratory.
Reference ranges provided by Inova Diagnostics, Inc.

Anti Myeloperoxidase antibody (MPO) TAT 2 days
Myeloperoxidase is the target antigen for the majority of P-ANCA and is associated with microscopic polyangiitis and Churg Strauss syndrome, but can also be found in some patients with GPA.
Results reported in IU/mL. Normal reference range 0 – 5.9 IU/mL
MPO: The reportable range of the assay is 1.0 to 221.9 IU/mL

Anti Proteinase-3 antibody (PR3) TAT 2 days
Proteinase 3 (PR3) is the major target antigen for C-ANCA. The detection of anti PR3-ANCA has a high predictive value for Granulomatosis with polyangitis .
Results reported in IU/mL. Normal reference range 0 – 4.9 IU/mL
PR3: The reportable range of the assay is 0.6 to 821.3 IU/mL
Anti neutrophil cytoplasmic antibodies (ANCA) TAT 2 days
Indicated in the investigation of ANCA associated vasculitis. Main patterns recognised, are cytoplasmic (C-ANCA) and perinuclear (P-ANCA).

C-ANCA with specificity for proteinase-3 (PR-3) has a high predictive value for active generalized Granulomatosis with polyangitis (GPA) and can also be found in patients with microscopic polyangiitis (MPA).

P-ANCA with anti-myeloperoxidase (MPO-ANCA) specificity is predictive for patients with active MPA and Churg Strauss syndrome (CSS), some patients with GPA also have this antibody. P-ANCA with specificities other than MPO-ANCA occur in some patients with inflammatory bowel disease, sclerosing cholangitis, rheumatoid arthritis, systemic lupus erythematosus, chronic active hepatitis and other autoimmune diseases. In such patients, ANCA levels are often low and of uncertain significance.

The presence of p-ANCA staining can be masked by ANA staining. If anti-nuclear antibody is detected during ANCA testing we cannot comment on the presence of p-ANCA.

The ANCA assay will only be performed on patients with clinical features associated with ANCA associated vasculitis (GPA, MPA, CSS).
Results reported as a titre, positive titre ≥20.

Anti glomerular basement membrane antibodies (GBM)

These antibodies are found in patients with Goodpasture's syndrome (>90% sensitivity).
Results reported in CU (chemiluminescent units).
Normal reference range 0 – 19 CU.
Reference ranges provided by Inova Diagnostics, Inc.
GBM: The reportable range of the assay is 2.9 to 1437.8 CU

The laboratory will endeavour to contact the requesting doctor upon the detection of a new positive GBM or ANCA with associated positive MPO/PR3 result providing that contact details have been specified on the request form.

Urgent MPO-ANCA & PR3-ANCA/GBM requests may be tested on the same day of sample arriving at laboratory if during normal working hours. All tests must be booked with the laboratory. The laboratory may not be able to process unbooked samples due to time and staffing constraints. Samples must be in the lab by 3pm on the day of testing. Please contact the laboratory prior to sending.

C3 nephritic factor (C3 Nef)

These antibodies, to the alternative pathway C3 convertase, are found in patients with membrano-proliferative glomerulonephritis (type II) and partial lipodystrophy. *These assays are performed by the Supraregional Protein Reference Laboratory, Sheffield.*
Results reported as detected or not detected.

OTHER AUTOANTIBODIES

Anti C1q antibodies

Antibodies to C1q may be associated with renal disease activity in patients with SLE. High levels are found in patients with hypocomplementaemic urticarial vasculitis syndrome (HUVS). *These assays are performed by the Supraregional Protein Reference Laboratory, Sheffield.*
Results reported as ELISA U/ml, positive value >15 U/ml.

Anti-IgA antibodies

These antibodies may occur in patients with selective IgA deficiency. They can cause blood product transfusion reactions**.** *This assay is performed by the NHS Blood and Transplant Centre, Barnsley.*
Results reported as negative or positive with titre.

Granulocyte Immunology

Anti Granulocyte Antibodies for the investigation of Autoimmune neutropenia, neonatal alloimmune neutropenia and drug induced antibody mediated neutropenia.
These assays are performed by NHS Blood and Transplant Histocompatibility & Immunogenetics service, Bristol.
For more information please see form
https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/14481/frm100131-hi-request-form-3e-granulocyte-immunology.pdf

Infliximab and Adalimumab therapeutic drug monitoring

Sample requirements: One 4mL yellow topped gel sample tube is sufficient for drug and antibody level measurements.

To aid interpretation of results, it is desirable that the following information is included on the request form:

- Infusion dosing interval
- Number of infusions to date
- Reason for request, i.e., poor response
- Primary diagnosis

This assay is performed by the Blood Sciences Department, Royal Devon & Exeter Hospital.

Therapeutic ranges
https://www.exeterlaboratory.com/test/anti-tnf-drug-and-antibody-testing-at-exeter-clinical-laboratory/

Other autoantibodies may be available on request: Please contact the laboratory.

IMMUNOCHEMISTRY

Sample requirements: One 4mL yellow topped gel sample tube is sufficient for all immunochemistry measurements, unless otherwise stated below.

IgD

Measurement of IgD is indicated in the investigation of hereditary periodic fever syndromes.
These assays are performed by the Supraregional Protein Reference Laboratory, Sheffield. **Results reported as kU/L, normal range 2-100 kU/L.**

IgE

See allergy section

Functional (specific) antibodies

Antibodies to pneumococcal specific antigens (PSSA) are available from *Clinical Immunology Laboratory Cambridge University Hospital.*

Antibodies to tetanus IgG, meningococcal C, haemophilus and diphtheria are available from *The Meningococcal Reference Unit, Manchester.*
Meningococcal reference unit (MRU): user manual - GOV.UK (www.gov.uk)

https://mft.nhs.uk/app/uploads/2021/11/MMMP_User-Manual-edition_18.pdf

Functional antibody tests are of limited value, and are used mainly in the investigation of primary immune deficiency. For advice, please contact immunology medical staff.

Results are reported as: Tetanus IgG: IU/mL, PSSA: ug/ml, MCA: rSBA titre, HIB: ug/ml, DIP: IU/ml
TAT: 35 days.

CH50 and AH50 Functional Assays.

Sample requirements: One yellow topped gel sample tube required (4.0ml). Samples must be received by the laboratory within 24 hours of venepuncture. Samples received >24 hours will be rejected.
Screening tests for classical (CH50) and alternate (AH50) complement activation pathways are indicated in the investigation of suspected immunodeficiency associated with recurrent pyogenic infections and atypical "immune complex disorders". Values within the normal range indicate that the classical and alternate pathway components are present. Quantitation of individual complement components should be undertaken in samples with sub-normal CH50 and AH50 levels.
Normal ranges CH50: 392-1019 units/ml APCH50: 64-128%. TAT: 21 days.
Reference ranges provided by The Binding Site group Ltd. UK.

C1 esterase inhibitor (functional)

Sample requirements: One yellow topped gel sample tube required (4.0ml). Samples must ideally be received in the laboratory within 24 hours of venepuncture. Samples that arrive in the laboratory within 24 to 48 hours after venepuncture may be tested, but low results must be interpreted with caution. Samples received >48 hours will be rejected.

In type I hereditary angioedema (HAE) (85% of patients), low levels of C1 esterase inhibitor (C1INH) are found by both the quantitative and functional assays. In type II HAE (15% of patients) normal or raised levels of functionally inactive C1INH are detected. Consequently, the functional C1INH assay is essential for this diagnosis. Both types of HAE are associated with low or absent C4 levels during an attack. Reduced levels of C1INH (quantitative and functional) and C1q are found in the rarer acquired form of C1INH deficiency. This condition generally occurs secondary to underlying disease, most frequently lymphoproliferative disorders.

C1INH functional will be tested on all samples. C1INH quantitative (see below) will only be tested on samples with a C1INH functional result that is below the normal range.

Normal range: C1INH (functional) 70 -130%

Reference range supplied by Technochrom®

TAT: functional – 14 days

C1 esterase inhibitor (quantitative)

Quantitative C1 inhibitor is no longer measured routinely. If the functional C1 inhibitor level is found to be low, quantitative C1 inhibitor will be measured automatically.

This assay is performed by the Molecular Immunology Service, Cardiff and Vale NHS Trust, Cardiff

Normal range 0.15 - 0.35 g/L

C1q

The primary indication for C1q measurement is in the differentiation of HAE (normal C1q levels) from acquired C1 esterase deficiency (reduced C1q levels). Levels are also decreased in conditions associated with immune complex mediated complement activation.

This assay is performed by the Molecular Immunology Service, Cardiff and Vale NHS Trust, Cardiff

Normal range 50-250 mg/L

Mannose Binding Lectin (MBL)

Mannose binding lectin (MBL) plays an important role in the innate immune system by facilitating complement activation. Measurement of MBL should be considered when immunodeficiency associated with the complement system is suspected, (recurrent infection, meningococcal disease).

The assay is performed by the Immunology Camelia Botnar Laboratories, Great Ormond Street, London.

Reference range:

<75 ng/ml correlates with homozygous variant alleles and non-functional MBL which is associated with the greatest risk of infection.

75 – 399.9 ng/ml correlates with functional MBL deficiency associated with increased risk of infection.

400 - 1300 ng/ml correlates with heterozygous variant alleles and may show mild deficiency associated with some increased risk of infection.

> 1300 ng/ml correlates with wild type alleles showing no deficiency.

Individual complement components are available on request: Please contact the laboratory.

ALLERGY

Sample requirements: One 4.0mL yellow topped gel sample tube is sufficient for all allergy testing, unless otherwise stated below.

Total IgE

Total serum IgE is usually elevated in patients with atopic disease. However, levels do not correlate with severity of disease and a raised IgE does not necessarily indicate the presence of allergic disease. Other conditions where serum IgE levels are raised include: parasitic diseases, some rare immunodeficiencies, atopic eczema, eosinophilia, bronchopulmonary aspergillosis and in some lymphoid malignancies.

Age | Normal range (KU/L)	
Newborn – 3 Months	**<5**
3 months – 1 year	**<11**
1 year – 5 years	**<29**
5 years – 10 years	**<52**
10 years – 15 years	**<63**
15 years - Adult	**<75**
Adult *	**<81**

*** Adult values are not stabilized until 15-20 years of age**

Reference ranges established by the PRU, Sheffield.
https://www.immqas.org.uk/pru.asp?ID=316

(Reference ranges were established using a population with demographics similar to Northern Ireland population).

TAT: 5 days.

Allergen specific IgE

Allergen specific IgE testing is of value where skin testing is difficult to perform, or contraindicated, ie.

- in very young children.
- in patients with severe/extensive eczema or dermographism.
- in patients taking anti-histamines which cannot be stopped.
- in patients in whom there is a significant risk of an anaphylactic reaction, the use of allergen specific IgE testing must be carefully considered and is not a substitute for careful clinical assessment.

High levels of specific IgE against a wide range of inhalant and food allergens are frequently found in patients with atopic eczema. The clinical significance of such sensitisation is often unclear.
Over 100 specific allergens are available for testing, however “screening“ for allergy using allergen specific IgE is not usually helpful.

If requesting allergen specific IgE testing, please provide as much clinical information as possible, and which specific allergens are required.

The detection of allergen specific IgE in serum is not diagnostic of clinical allergy, nor does the failure to detect allergen specific IgE exclude the diagnosis. Specific IgE concentrations (Ku/L) do not correlate with clinical severity of allergic reactions.
Reference range: 0 – 0.35 kUA/l
Reference ranges provided by Phadia AB, Sweden.
TAT: 5 days.

Booklets are available on House Dust Mite Allergy and Peanut Allergy. Copies may be obtained from the Immunology Secretaries in the Immunology Day Centre. Phone 02890 630003.

Extrinsic allergic alveolitis screen.
May be used in the investigation of patients with respiratory conditions in whom hypersensitivity reactions to inhaled organic material is suspected. These conditions are often associated with occupational exposure, for example, farmers' lung (thermophilic fungi) and bird fanciers' lung (pigeons, caged birds).
Assays, which may be requested individually or as a screen include:
IgG antibodies to aspergillus fumigatus. Results in mg antigen/litre, normal range <40.
IgG antibodies to micropolyspora faeni. Results in mg antigen/litre, normal range <10.
IgG antibodies to pigeon protein. Results in mg antigen/litre, normal range <32.
IgG antibodies to budgerigar protein. Results in mg antigen/litre, normal range <30.
TAT: 5 days.
Reference ranges established in-house.

Anaphylaxis

Please use the 'Anaesthetic allergy clinic referral form' (see below) when requesting investigations.

Sample requirements: Serial blood samples in 4mL yellow topped gel sample tubes are required at the following times. 1st sample: as soon as resuscitation has started, 2nd sample: 2 hours after reaction, 3rd sample: 24 hours after reaction.

Investigation of suspected anaphylactic reactions, in particular reactions occurring during anaesthesia.

Investigations are recommended for patients with Grade II (cardiovascular reaction: tachycardia, hypotension); Grade III (shock, life-threatening spasm of smooth muscles); Grade IV (cardiac and/or respiratory arrest).

The following are measured: mast cell tryptase and allergen specific IgE (as appropriate).

Notification of results will include interpretative comments and suggested arrangements for follow up skin testing as appropriate.

TAT: 14 days.

Mast cell tryptase

Raised levels are detected during anaphylaxis. Timing of blood sample is critical as maximum levels are observed within 3 hours post reaction. Elevated levels may also be found in patients with mastocytosis.

Post mortem samples should be taken within 48 hours from time of death.

Normal range: 1-11 µg/l. TAT: 7 days.
Reference ranges provided by Phadia AB, Sweden.

Due to the importance of the sample timings, plasma or lithium heparin samples may be accepted for this assay if no serum sample is available. However testing tryptase using these sample types is not UKAS validated.

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ANAESTHETIC ALLERGY CLINIC REFERRAL FORM

Serial blood samples in 4mL yellow topped gel sample tubes are required:

1st sample: as soon as resuscitation has started
2nd sample: 2 hours after reaction
3rd sample: 24 hours after reaction

Samples should be marked GENERAL ANAESTHETIC PANEL and sent to the Regional Immunology Laboratory, Royal Hospital, Belfast (02896151568)

Refer

- Fully completed form should be sent to AnaesAnaphylaxis@belfasttrust.hscni.net
- **Copy of anaesthetic chart, relevant drug kardex and surgical chart must be included**

Record

- A copy of this form should be filed in the patient's medical record
- Patient and GP must be informed (See Appendix 1 and 2)
- Anaesthetic alert to be added to patient's Electronic Care Record

Report

- Incident should be reported locally (DatexWeb)
- Report to MHRA via the Yellow Card scheme

Review

- Patient will be reviewed and added to the waiting list where appropriate
- For patients requiring further surgery prior to appointment advice can be sought via NAP6 link provided or see Appendix 3 Safe advice information

NAP6

Patient Details
Name
E-mail Address
Address
Contact Telephone Number

Date of reaction:
Time of onset of reaction:
Procedure/Surgery:

Was surgery/ procedure completed? YES ☐ NO ☐
If 'no', has another date for surgery been scheduled? YES ☐ NO ☐
Urgency/ date of future surgery

Known allergies prior to reaction YES ☐ NO ☐
If Yes:

Allergen	Type of Reaction

Was the patient exposed to the below during procedure/surgery?

Latex exposure	**YES**	**NO**
Chlorhexidine exposure	**YES**	**NO**
Iodine	**YES**	**NO**
Instillagel	**YES**	**NO**

Drugs administered IN THE HOUR BEFORE THE REACTION (including premedication)

Please include any other relevant events or exposures e.g. Patent Blue Dye, Bone cement, blood products, colloids

Drug or Event	Time	Route	Comments

Neuraxial Blockade/ Regional Anaesthesia

Procedure and drugs used	Time

Pre-operative Procedures Required

Outline any procedure which was undertaken prior to onset, such as placement of lines, arterial, CVC or urinary catheters, cardiopulmonary bypass or any other instrumentation.

Drugs and IV fluids given to treat the reaction

Drug/ IV Fluid	Time	Route

Was CPR Required? **Yes ☐** **No☐**

Duration of CPR

MHRA Reference Number

Description of reaction:

Symptom/ Sign	Time of onset	Description
Hypotension		
Tachycardia		
Bronchospasm		
Cyanosis/ desaturation		
Angioedema		
Urticaria		
Arrhythmia		
Other (specify)		

Grade of Reaction

Grade I	Grade II	Grade III	Grade IV

TABLE 1 Classification of clinical severity of perioperative immediate hypersensitivity: modified Ring and Messmer four-step grading scale[66,67]

Grade I Skin or mucosal signs only
- generalized erythema
- extensive urticaria
- with or without angio-oedema

Grade II Moderate signs from several organ systems
- skin or mucosal signs
- ± hypotension ± tachycardia
- ± bronchospasm
- ± gastrointestinal signs

Grade III Life-threatening signs from one or more organ systems
- cardiovascular collapse (life-threatening hypotension)
- tachycardia or bradycardia ± cardiac dysrhythmia
- ± bronchospasm
- ± skin or mucosal signs
- ± gastrointestinal signs

Grade IV circulatory and/or respiratory arrest

Check box before sending referral

Scanned copy of anaesthetic chart	
Scanned copy of surgical chart	
Letters sent to Patient and GP	
NIECR/MHRA/Yellow card updated	
Urgent surgery required using NAP6 avoidance guidelines	
Mast cell tryptase sent	

Appendix 1: Letter to Patient

Patient Name	
Patient Address	
Date of Birth	
H&C Number	
Hospital Number	

Dear

You had a suspected severe allergic reaction (anaphylaxis) during anaesthesia on:

To find out the cause of the reaction, I will refer you to the RVH Anaesthetic Allergy Clinic.
They will contact you with an appointment. If you have not received notification of this within 8 weeks, or if you have any queries, please contact me (details below).
It is important you attend the allergy clinic to prevent a further severe allergic reaction.

Until you have attended the allergy clinic, you should *avoid all the drugs and other potential causes you were exposed to during the hour prior to the suspected allergic reaction*. These include:

1. Latex
2. Chlorhexidine, including medical, dental and household products
3. Anaesthetic Drugs ---------- ----------
   ---------- ----------
   ---------- ----------
   ---------- ----------
4. Antibiotics ---------- ----------
   ---------- ----------
5. Analgesics ---------- ----------
   ---------- ----------
6. Other drugs/Substances

It is important that you show this letter if you have any medical appointments between now and the time of your clinic appointment.

I will write to your GP with this information.
Yours Sincerely,

---------- ----------
Consultant Anaesthetist Contact phone number/Date

Appendix 2: Letter to GP

Dear Dr ----------

Patient Name	
Patient Address	
Date of Birth	
H&C Number	
Hospital Number	

The above patient had **a suspected severe allergic reaction (anaphylaxis) during anaesthesia on:**
He/she has been referred to the RVH Anaesthetic Allergy Clinic.
Until the patient has attended the allergy clinic, they should avoid all drugs and other potential allergens to which they were exposed during the hour prior to the suspected allergic reaction.
These include:

1. Latex
2. Chlorhexidine, including medical, dental and household products
3. Anaesthetic Drugs ---------- ----------
   ---------- ----------
   ---------- ----------
   ---------- ----------
   ---------- ----------
4. Antibiotics ---------- ----------
   ---------- ----------
5. Analgesics ---------- ----------
6. Other drugs/Substances ---------- ----------

I have given the patient a letter providing the same information.
Yours sincerely,

---------- ----------
Consultant Anaesthetist Contact Phone Number/Date

Appendix 3: Safe Advice Information if Urgent Surgery required before Appointment

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Urgent surgical intervention after suspected perioperative anaphylaxis and prior to allergy investigations: suggested management plan

It is possible to provide safe anaesthesia in almost every case and unnecessary to postpone urgent surgery.

Discuss the case with the anaesthetic allergy team
AnaesAnaphylaxis@belfasttrust.hscni.net

Pre-warn the theatre team beforehand, and be prepared to diagnose and treat anaphylaxis promptly.

Consult appropriate guidelines in advance.

Premedication with antihistamines and steroids may reduce the severity of reactions caused by nonspecific histamine release but will not prevent anaphylaxis.

Avoid the following if administered/exposed during the 60 minutes prior to the suspected anaphylactic event:

• All drugs to which the patient was exposed, with the exception of inhalational anaesthetic agents
• All antibiotics of the same class that was administered. Discuss antibiotic choice with a microbiologist
• If an NMBA was administered, all NMBAs should be avoided unless it is absolutely impossible to do so, due to the risk of cross-sensitivity
• Chlorhexidine (including chlorhexidine antiseptic wipes, medical gel (e.g. used before catheter insertion) and chlorhexidine-coated intravascular lines/catheters)
• IV colloids
• Radiological contrast and dyes used for lymph node identification
• Latex
• Local anaesthetics of the same class (amides; esters)
• Histamine-releasing drugs (morphine and codeine) as the previous reaction may have been due to nonspecific histamine-release

If past anaesthetic records are not available, in addition to the above:

• Assume that the patient previously received an antibiotic. Discuss antibiotic prophylaxis with a microbiologist
• Assume that the patient was previously exposed to propofol, morphine, chlorhexidine, latex, IV colloid, and an NMBA

• If possible, use local or regional anaesthesia in patients who have had a previous suspected anaphylactic event during general anaesthesia, and vice versa

Alternative anaesthetic options

Regional anaesthesia, where practical

If propofol was used, alternatives include inhalational agents, thiopental, etomidate (non-lipid formulation) and ketamine.

If tracheal intubation is required and an NMBA is contra-indicated:

- A remifentanil infusion, magnesium sulphate and topical anaesthesia to facilitating laryngoscopy and intubation
- Alfentanil if remifentanil was used in the previous anaesthetic
- Awake intubation under topical anaesthesia
- If local anaesthetics are not contra-indicated, sufficient surgical muscle relaxation can usually be provided if necessary with an adequate depth of anaesthesia and adjunct neuraxial block, transversus abdominis blocks, rectus sheath blocks or other peripheral nerve block.

CELLULAR IMMUNOLOGY

Investigation of the cellular immune system should only be undertaken after discussion with Immunology medical staff. The appropriateness of testing and specimen requirements will be advised. Other assays may be available on request: Please contact the laboratory.

Lymphocyte subset phenotyping

Sample requirements: 5ml EDTA blood sample. Transport and store at room temperature.

Samples sent on a Friday must be received in the lab by 3pm. Any samples received after this time may not be tested due to time and staffing constraints.

Samples must be received by the laboratory within 48 hours of venepuncture. Please contact the laboratory for advice.

Indicated in diagnosis and monitoring of immunodeficiency and in leukaemia /lymphoma typing. Suspected cases of childhood T cell/combined immunodeficiency (SCID) should be regarded as URGENT and the laboratory contacted as soon as possible. Serial CD4 counts are of value in monitoring HIV disease, however measurement of CD4 cells has no place in the diagnosis of HIV infection, until serological status is established.

Requests for CD4 count as a “surrogate marker” of HIV infection will be refused.

Lymphocyte subset panel: CD3 (T cell), CD4 (T helper), CD8 (T cytotoxic), CD19 (B cell), CD16/56 (NK cell). Markers of maturation, activation, monoclonality available by arrangement.

Results given as percentage and absolute counts (see appendix 1).

TAT: 4 days

Lymphocyte activation marker/HLA-DR expression

Sample requirements: 5ml EDTA blood sample. Transport and store at room temperature.

Samples sent on a Friday must be received in the lab by 3pm.
Samples received after this time may not be tested due to time and staffing constraints.

Samples must be received by the laboratory within 48 hours of venepuncture. Please contact the laboratory for advice.

To rule out MHC class II deficiency (SCID) in paediatric patients, HLA-DR expression will now be performed on all infants less than 2 years old who have had lymphocyte subset phenotyping requested. This test will be a one off for each patient- repeat testing will not be performed on subsequent requests.

Results given as a percentage.

TAT 4 days

Lymphocyte Function

Sample requirements:4ml Lithium heparin blood sample
A normal control sample will be required with each request.
All tests must be booked with the laboratory in advance. The laboratory may not be able to process unbooked samples due to time and staffing constraints.
Samples can be sent on Monday, Tuesday and Wednesdays only.
Samples must arrive in the lab by 12 noon for same day referral.
Samples received on the wrong day will be rejected.
Indicated in further definition of humoral and/or cellular immunodeficiency.
Proliferative responses to mitogen.
Stimulants: Phytohaemagglutin (PHA, CD3, Candida).
Results reported as normal / impaired / absent PHA response.
The assay is performed by the Immunology Camelia Botnar Laboratories, Great Ormond Street, London.

Neutrophil Function Tests

Sample requirements: 5mL EDTA blood sample. Transport and store at room temperature.
A normal control sample will be required with each request.
Samples sent on a Friday must be received by the lab by 3pm. Samples received after this time may not be tested due to time and staffing constraints.
Samples must be received by the laboratory within 24 hours of venepuncture.
Please contact the laboratory for advice.
Indicated in investigation of recurrent skin infections, chronic gingivitis, recurrent deep seated bacterial and fungal infections.
The following functional assays are available: Neutrophil Respiratory Oxidative Burst.
Results given with reference to normal control value.
TAT: results within 24hr of sample receipt.

Lymphocyte subsets neutrophil (CD11a, CD18) (Integrins)

5ml EDTA (patient) and 5ml EDTA (control)
Assay by special request only.
The assay is performed by the Immunology Camelia Botnar Laboratories, Great Ormond Street, London.

Disease index

Disease	Investigations
Addison's disease	Anti-adrenal antibody
Allergy	IgE allergen specific IgE
Anaphylaxis	mast cell tryptase IgE allergen specific IgE
Angioedema	C1 esterase inhibitor C1q
Anti Phospholipid Syndrome (APS)	Anti-cardiolipin antibody (anti-beta2 glycoprotein I)
Chronic Active Hepatitis	Anti-smooth muscle antibody Anti-liver kidney microsomal antibody Anti-mitochondrial antibody Anti-nuclear antibody (CTD screen)
Chronic Granulomatous Disease	Neutrophil function test
Chronic Lymphocytic Leukaemia	Lymphocyte phenotyping
Coeliac Disease	Anti-transglutaminase antibody Anti-endomysial antibody
Congenital Heart Block	Anti-Ro antibody (CTD Screen)
Connective Tissue Diseases	Anti-nuclear antibody (CTD Screen) Anti dsDNA antibody Antibodies to ENA
CREST	Anti-centomere antibody (CTD Screen)
Dermatitis Herpetiformis	Anti transglutaminase antibody Anti-endomysial antibody
Dermatomyositis	Anti-Jo-1 antibody (CTD Screen)
Diabetes	Anti-IA2 antibody

	Anti GAD antibody
DLE	Anti-nuclear antibody (CTD Screen)
Dressler's Syndrome	Anti-cardiac muscle antibody
Extrinsic allergic alveolitis	IgG to aspergillus fumigatus. IgG to micropolyspora faeni IgG to avian proteins proteins (pigeon and budgerigar)
Fibrosing Alveolitis	Anti-nuclear antibody (CTD Screen)
Glomerulonephritis	Anti-neutrophil cytoplasmic antibody Anti-myeloperoxidase antibody Anti-proteinase 3 antibody Anti-GBM antibody
Goodpasture's Syndrome	Anti-GBM antibody
Guillain-Barre Syndrome	Anti-GM1 antibody Anti-GQ1 antibody
HIV Infection	Lymphocyte phenotyping
Immunodeficiency	Functional antibodies CH50 Cellular investigations
Juvenile Chronic Arthritis	Anti-nuclear antibody (CTD Screen)
Leukaemia/Lymphoma	Cellular studies
Lymphoproliferative disorders	Cellular studies
Mastocytosis	Mast cell tryptase
Membranoproliferative Glomerulonephritis (MPGN)	C3 nephritic factor
Microscopic Polyangiitis	Anti-neutrophil cytoplasmic antibody Anti-myeloperoxidase antibody Anti-proteinase 3 antibody
Mixed Connective Tissue Disease (MCTD)	Anti-nuclear antibody (CTD Screen) Anti-ENA antibody

	Anti-RNP antibody
Myasthenia Gravis	Anti-acetylcholine receptor antibody Anti MuSK antibody Anti skeletal muscle antibody
Non-Hodgkins Lymphoma	Cellular studies
Partial Lipodystrophy	C3 nephritic factor
Pernicious Anaemia	Anti-gastric parietal cell antibody Anti-intrinsic factor antibody
Polymyositis	Anti-Jo-1 antibody (CTD Screen)
Premature Ovarian Failure	Anti-adrenal antibody Anti-steroid producing cell antibodies
Primary Biliary Cirrhosis	Anti-mitochondrial antibody
Progressive Systemic Sclerosis	Anti-nucleolar antibody Anti-nuclear antibody (CTD Screen) Anti-Scl-70 antibody (CTD Screen)
Raynaud's Phenomenon	Anti-centromere antibody
Sjogren's Syndrome	Anti-nuclear antibody (CTD Screen) Anti-Ro antibody Anti-La antibody
Systemic Lupus Erythematosis	Anti-nuclear antibody (CTD Screen) Anti-dsDNA antibody Anti-ENA antibodies Anti-cardiolipin antibody
Vasculitis	Anti-neutrophil cytoplasmic antibody Anti-myeloperoxidase antibody Anti-proteinase 3 antibody
Granulomatosis with polyangitis	Anti-neutrophil cytoplasmic antibody Anti-PR3-ANCA antibody

Appendix 1: Lymphocyte Subset Reference Ranges

Table I. Relative size of lymphocyte subpopulations in blood

Lymphocyte subpopulations	Age groups									
	Neonatal (n = 20)	1 wk-2 mo (n = 13)	2-5 mo (n = 46)	5-9 mo (n = 105)	9-15 mo (n = 70)	15-24 mo (n = 33)	2-5 yr (n = 33)	5-10 yr (n = 35)	10-16 yr (n = 23)	Adults (n = 51)
$CD19^+$ B	12%	15%	24%	21%	25%	28%	24%	18%	16%	12%
lymphocytes	(5-22)	(4-26)	(14-39)	(13-35)	(15-39)	(17-41)	(14-44)	(10-31)	(8-24)	(6-19)
$CD3^+$ T	62%	72%	63%	66%	65%	64%	64%	69%	67%	72%
lymphocytes	(28-76)	(60-85)	(48-75)	(50-77)	(54-76)	(39-73)	(43-76)	(55-78)	(52-78)	(55-83)
$CD3^+/CD4^+$ T	41%	55%	45%	45%	44%	41%	37%	35%	39%	44%
lymphocytes	(17-52)	(41-68)	(33-58)	(33-58)	(31-54)	(25-50)	(23-48)	(27-53)	(25-48)	(28-57)
$CD3^+/CD8^+$ T	24%	16%	17%	18%	18%	20%	24%	28%	23%	24%
lymphocytes	(10-41)	(9-23)	(11-25)	(13-26)	(12-28)	(11-32)	(14-33)	(19-34)	(9-35)	(10-39)
CD4/CD8	1.8	3.8	2.7	2.5	2.4	1.9	1.6	1.2	1.7	1.9
ratio per $CD3^+$	(1.0-2.6)	(1.3-6.3)	(1.7-3.9)	(1.6-3.8)	(1.3-3.9)	(0.9-3.7)	(0.9-2.9)	(0.9-2.6)	(0.9-3.4)	(1.0-3.6)
$CD3^+/HLA\text{-}DR^+$	2%	5%	3%	3%	4%	6%	6%	7%	4%	5%
T lymphocytes	(1-6)	(1-38)	(1-9)	(1-7)	(2-8)	(3-12)	(3-13)	(3-14)	(1-8)	(2-12)
$CD3^-/CD16\text{-}56^+$	20%	8%	6%	5%	7%	8%	10%	12%	15%	13%
NK cells	(6-58)	(3-23)	(2-14)	(2-13)	(3-17)	(3-16)	(4-23)	(4-26)	(6-27)	(7-31)

The relative frequencies are expressed within the lymphocyte population: median and percentiles (5th to 95th percentiles).

Table II. Absolute size of lymphocyte subpopulations in blood

Lymphocyte subpopulations	Age groups									
	Neonatal (n = 20)	1 wk-2 mo (n = 13)	2-5 mo (n = 46)	5-9 mo (n = 105)	9-15 mo (n = 70)	15-24 mo (n = 33)	2-5 yr (n = 33)	5-10 yr (n = 35)	10-16 yr (n = 23)	Adults (n = 51)
Lymphocytes	4.8	6.7	5.9	6.0	5.5	5.6	3.3	2.8	2.2	1.8
	(0.7-7.3)	(3.5-13.1)	(3.7-9.6)	(3.8-9.9)	(2.6-10.4)	(2.7-11.9)	(1.7-6.9)	(1.1-5.9)	(1.0-5.3)	(1.0-2.8)
$CD19^+$ B	0.6	1.0	1.3	1.3	1.4	1.3	0.8	0.5	0.3	0.2
lymphocytes	(0.04-1.1)	(0.6-1.9)	(0.6-3.0)	(0.7-2.5)	(0.6-2.7)	(0.6-3.1)	(0.2-2.1)	(0.2-1.6)	(0.2-0.6)	(0.1-0.5)
$CD3^+$ T	2.8	4.6	3.6	3.8	3.4	3.5	2.3	1.9	1.5	1.2
lymphocytes	(0.6-5.0)	(2.3-7.0)	(2.3-6.5)	(2.4-6.9)	(1.6-6.7)	(1.4-8.0)	(0.9-4.5)	(0.7-4.2)	(0.8-3.5)	(0.7-2.1)
$CD3^+/CD4^+$ T	1.9	3.5	2.5	2.8	2.3	2.2	1.3	1.0	0.8	0.7
lymphocytes	(0.4-3.5)	(1.7-5.3)	(1.5-5.0)	(1.4-5.1)	(1.0-4.6)	(0.9-5.5)	(0.5-2.4)	(0.3-2.0)	(0.4-2.1)	(0.3-1.4)
$CD3^+/CD8^+$ T	1.1	1.0	1.0	1.1	1.1	1.2	0.8	0.8	0.4	0.4
lymphocytes	(0.2-1.9)	(0.4-1.7)	(0.5-1.6)	(0.6-2.2)	(0.4-2.1)	(0.4-2.3)	(0.3-1.6)	(0.3-1.8)	(0.2-1.2)	(0.2-0.9)
$CD3^+/HLA\text{-}DR^+$ T	0.09	0.3	0.2	0.2	0.2	0.3	0.2	0.2	0.06	0.09
lymphocytes	(0.03-0.4)	(0.03-3.4)	(0.07-0.5)	(0.07-0.5)	(0.1-0.6)	(0.1-0.7)	(0.08-0.4)	(0.05-0.7)	(0.02-0.2)	(0.03-0.2)
$CD3^-/CD16\text{-}56^+$	1.0	0.5	0.3	0.3	0.4	0.4	0.4	0.3	0.3	0.3
NK cells	(0.1-1.9)	(0.2-1.4)	(0.1-1.3)	(0.1-1.0)	(0.2-1.2)	(0.1-1.4)	(0.1-1.0)	(0.09-0.9)	(0.07-1.2)	(0.09-0.6)

Absolute counts ($\times 10^9$/L): median and percentiles (5th to 95th percentiles).

Reference

Immunophenotyping of blood lymphocytes in childhood

Reference values for lymphocyte subpopulations

Comans-Bitter et al. (The Journal of Pediatrics 1997; 130: 388-93).