UNIVERSITY OF BIRMINGHAM

Clinical Immunology Services

College of Medicine & Health

School of Infection, Inflammation and Immunology

Clinical Test Handbook

A brief guide to immunology and haematology tests available for clinical use

Purpose
This handbook gives pre-analytical information and guidance to laboratory service users when requesting tests and includes:
- Details of services provided
- Laboratory contact details and opening hours
- Details of phlebotomy services
- Instructions for completing sample and request form information
- Arrangements for transporting samples to the laboratories

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Contents

1. Introduction

Clinical Immunology Services (CIS), are an ISO 15189 UKAS-accredited laboratory provides a comprehensive range of immunology and blood cancer cell phenotyping laboratory services.
The CIS is a department within the Institute of Infection, Inflammation and Immunology within the College of Medicine and Health at the University of Birmingham. The laboratory is based within the Birmingham Health Innovation Campus:

Clinical Immunology Services
3rd Floor, No.1 Birmingham Health Innovation Campus (BHIC)
Aston Webb Boulevard
Selly Oak
Birmingham, B29 6TH

This handbook provides details of test tests available and their clinical use, sample requirements and turnaround times, contact details for the laboratory.

1.1. Normal *working* hours and University Closed days

CIS normal working hours are 8:30am to 5:30pm from Monday to Friday. Clinical advice is available during working hours Monday to Friday via the laboratory contact details. On University closed days (additional university holidays outside of bank holidays around Christmas and Easter periods, see website for details www.birmingham.ac.uk/staff/employeebenefits/closed-days.aspx), only time critical and urgent assays will be performed by the laboratory which may affect turnaround time for other routine tests during these periods.

1.2. Quality

All services users can expect a commitment to quality and continued improvement from the CIS. The CIS will actively engage with service users and institutions that refer tests to the CIS and will notify them of any significant issues or changes in the service we provide. The CIS will inform users of issues that might significantly affect quality and impact the results or interpretations that are provided that may impact on patient and care.

The CIS is United Kingdom Accreditation Service (UKAS) accredited to ISO 15189 standard. The tests on scope can be reviewed through this link:

https://www.ukas.com/wp-content/uploads/schedule_uploads/00007/9556-Medical-Single.pdf.

Where we offer a clinical test that is currently not on scope (or schedule of Accreditation), usually due to when a test is changed or introduced in relation to the UKAS audit cycle, we will state this on the report. These tests are managed within the CIS Quality Management System.

The CIS monitors key quality indicators to monitor and evaluate performance throughout critical aspects of pre-examination, examination and post-examination processes. To ensure their continued appropriateness, we review the quality indicators at least twice yearly as part of our Laboratory Medicine Annual Management Review process.

Despite quality control measures it must be recognised that variation can occur in testing. The relevance of a particular result or a change in value must be considered in
light of both the reproducibility of the method and the biological variation within the
patient.

If in doubt concerning the significance of a result or a change in sequential results, a member of the laboratory or relevant clinical staff should be contacted and they can help guide interpretation or check the validity of the result.

Some of this variation or uncertainty in clinical laboratory testing there are potential “uncertainties” that can affect the test result. Examples include poor specimen collection or transport, patient related factors such as biological variation and the presence of drugs, or other interfering factors). In addition, the analytical process itself is subject to some degree of inherent variability and this is often referred to as the “reproducibility” or “imprecision” of the method. Laboratories regularly monitor this by the use of internal quality control samples within each batch of analysis and by comparing the results of external quality assurance schemes designed to ensure that results are comparable with other laboratories using similar methods.

If you have any issues about the quality of service that you receive, please contact the Laboratory or Quality Manager through the laboratory telephone or email (see contacts section).

1.3. Consent

Consent is implied by the receipt of the sample and request form to undertake the test required and any reflex testing recommended to facilitate patient care. Samples are usually destroyed following testing, but we will store samples which are useful to the laboratory for quality assurance purposes. These will be used in such circumstances in an entirely anonymous manner. An example of this might

be sending a sample to a different laboratory as external quality assurance for an assay where there is no NEQAS scheme.

1.4. Agreement with the service user

Each request for examination received by the laboratory is considered an agreement. However, receipt of a request does not constitute acceptance for diagnostic laboratory testing. On receipt of a sample, the laboratory will determine if the sample is suitable for performing the diagnostic test for which it was supplied. The Laboratory is under no obligation to carry out the examination if, in its opinion, the sample is of unsatisfactory quality, or if the minimum data set is not met as this could constitute a safety or quality issue. This information will be communicated to the end user.
For most routine laboratory procedures, patient consent can be inferred when the patient willingly submits to the sample collecting procedure, for example, venipuncture.

1.5. Data protection

The department is compliant with the Data Protection Principles, which are set out in the Data Protection Act 1998 and General Data Protection Regulation (EU) 2016/679 (GDPR). Staff processing personal information do so in accordance with the University's Data Protection Policy (https://www.birmingham.ac.uk/privacy/index.aspx), and training in data protection is mandatory for staff. To contact the University's Data Protection team or to make a complaint about how your data is or has been processed, email: dataprotection@contacts.bham.ac.uk or telephone +44 (0) 121 414 3916.

Where tests are not performed in this department but are referred to other laboratories, minimum necessary patient data is shared with these organisations to meet sample identification requirements. It is checked that these NHS laboratories have UKAS ISO15189 accreditation.

1.6. Complaints, suggestions and positive feedback.

All complaints, suggestions and positive feedback are recorded and investigated as part of our quality management system. Where appropriate we will update the end user with any change in our processes as a result of their feedback.
A complaint or concern is an expression of dissatisfaction about an act, omission or decision of the service, either verbal or written, and whether justified or not, which requires a response. Complaints should normally be made within 6 months of an incident or of the matter coming to the attention of the complainant. We will acknowledge all formal complaints within 5 working days. An appropriate manager will be assigned to investigate the complaint and a response will normally be provided within 20 working days. If the complaint is complex in nature and more time is required to investigate

thoroughly, we will keep the complainant informed until the matter is concluded. A complaint can be made by contacting the Laboratory Manager through the laboratory telephone or central email (see contacts section below).

Communication to the user can also be triggered by the CIS in circumstances whereby there is a foreseeable hazard and there remains a residual risk to the service provided. A letter informing the user of potential delay in TAT would be issued in the appropriate circumstance or notification of the need to refer samples to a different site to fulfil testing.

1.7. Contact details

Postal Address:

Clinical Immunology Service
3rd Floor,
No.1 Birmingham Health Innovation Campus (BHIC)
2 Bournbrook Way
Birmingham.
B29 6TH

Web address:

http://www.birmingham.ac.uk/facilities/clinical-immunology-services/index.aspx

or search the internet for: “Clinical Immunology Birmingham”

Key contact numbers:

General telephone enquiries/results: (0121) 414 4069

Laboratory Manager: (0121) 414 3092

Email enquiries:

For e-mails containing patient-sensitive information: UoBClin.Imm@uhb.nhs.uk

Non NHS Organisations e.g. COVID-19 Antibody testing: Clin.imm@contacts.bham.ac.uk

Non NHS individuals please contact Clin.imm@contacts.bham.ac.uk

Please note, for data protection reasons please use nhs.uk emails (or Trust emails with the same level of security) if queries involved patient identifiable information.

2. Specimen collection and test requesting

2.1. *General* specimen collection requirements

Different tests require different blood tubes. The colour of the tube (in the Vacutainer system) required for a test is indicated by the colour of the box in the request form and is also indicated for each test in section 3. If your site does not use the Vacutainer system, please contact the laboratory for guidance.

2.2. Test requesting

The CIS has two different request forms:

- General immunological and Neuroimmunology investigations Document code: REQ.G.
- Haemato-oncology requests Document code: RF001 MIRHO Request form

These forms (with integral specimen bags) can be obtained by contacting the laboratory or if bags are not required can be printed from the departmental website.

2.3. Requesting testing on a patient with Creutzfeldt Jacob Disease

All samples where there is a suspicion or risk that this may be from a patient with Creutzfeldt Jacob Disease (CJD) **MUST** clearly state this risk on the request form. Samples are not tested on site if they are from a patient with suspected CJD or variant CJD and are referred to University College London, Queen's square where appropriate biohazard protocols are in place. Please not this may affect turnaround times.

2.4. Minimum data set for requesting a test on a sample

Only correctly and clearly labelled samples with matching request forms will be accepted. We cannot receive a sample without a request form as minimum data requirements will not be fulfilled.

Where essential information is missing from a sample or request form, the laboratory will attempt to contact the requesting medical officer/practitioner identified on the request using the contact number, where this is given.

If the laboratory is unable to contact the requesting medical officer/practitioner or colleague, the sample will be rejected or analysis deferred until contact is made.

Failure to provide clinical information with the request may result in reporting delays or reduce the ability of reporters to interpret the result in the context of an individual patient. In some cases, assays will not be carried out without clinical justification of the work

When samples are rejected due to insufficient information, a report will be issued through the laboratory information system as soon as practicable, stating that the sample has not been processed and giving details.

The following table defines essential information which defines minimum data requirements.

	Essential	**Desirable**
Sample	1. Patient's first and surname 2. Date of birth and/ Patient's NHS/CHI number or other unique identifier (e.g. referring lab number) *(please use pre-printed patient labels where possible)*	1. Date and time of collection
Form	1. Patient's first and surname 2. Patient's NHS/CHI number or other unique identifier (e.g. referring lab number) 3. Patient's sex 4. Requesting consultant/GP or and Destination for report 5. Specimen type 6. Test(s) required *(please use pre-printed patient labels where possible)*	1. Clinician's telephone/bleep number or email (essential for urgent requests) 2. Patient's address 3. Requesting clinician's specialty Signature of person taking the sample 4. Date and time of collection 5. Relevant clinical information

2.5. Rejection of requests or samples

Rejection of requests will be made in circumstances where there is a failure to provide essential details as this may represent a risk to patient safety.

Samples may be rejected in the following circumstances:

- The minimum essential information is missing from the sample or request.
- The sample and request form information do not match.
- The sample is unlabelled or otherwise unsuitable (e.g. wrong tube type/temperature in transit in correct and undue delay in transport, sample integrity, quality or volume insufficient).
- The test has been re-run recently and retesting within the time period is not clinically indicated

Some assays are sensitive to interferences from icterus, haemolysis or lipaemia. If this is the case, the assay may not be possible, and the sample will be rejected. This will be indicated on the report issued through the laboratory information system, stating that the sample has not been processed and giving details.

Where repeat tests are requested within an inappropriate timescale the department will issue a report detailing the previous result and will store the sample in case other investigations are required. This includes:

Test	Timescale for intervention (days)
SFLC	2
ANCA, dsDNA	7
Complement C3/C4	30
MUSK, NMO, VGC, VGK	90
ANA	180
CCP, ENA, M2, mitochondrial, rheumatoid factor, TPO	330

Samples that have been rejected and not processed may be stored in the laboratory for up to one week to allow the requesting practitioner time to get in touch. This storage will be at the discretion of individual departments.

2.6. Urgent requests

Some assays are available with a reduced turnaround time on discussion with a member of the senior laboratory staff. Prior agreement by the laboratory for urgent requests is essential.
The request form must be clearly marked “Urgent” and with which member of staff the request was discussed. The sample must arrive before 2pm. Contact details (direct mobile phone number or email) for the requesting clinician must also be supplied to enable results to be communicated urgently.

2.7. Results reporting

To ensure rapid communication and accuracy of results to end users we strongly prefer electronic transmission of results to requestors. This can be through automatic transfer of results upon authorisation (if samples are from UHB/BSOL) or by automatic email (for other locations). If you require emailed reports, please contact the lab to provide your details, including requestors name/role, requesting location and secure email address.

2.8. Sample retention and additional requesting on samples

Most haematological malignancy immunophenotyping samples will be retained for one week, in case further tests are required. Other samples are routinely retained for >2 weeks. If you require additional tests, please contact the department and we will endeavour to assist wherever sufficient volume/correct sample type is available and storage requirements for the test have been met.

2.9. Sample delivery during work and out of hours

Please endeavour to ensure the sample is received in the laboratory between 0830 and 1600 to ensure the sample can be processed on the same day as receipt.

For samples arriving between 08.30 and 17.30

- Park in yellow hatched bay before the car park barriers outside the main entrance https://what3words.com/rugs.perky.jabs
- Inform reception that samples for Clinical immunology Services & take lifts to level 3
- Press specimen reception button on intercom for the Clinical immunology Services
- Someone will come to the lift area and receive and sign for the sample

For samples arriving out of hours between 17.30 and 08.30
Park in delivery area located to the left of the building when entering from the Roundabout. https://what3words.com/rabble.linen.sharp. Down the steps and turn to left. The CIS out of hours dropbox is just past delivery entrance.

2.10. Telephone requests for results

Our preference is for email requests for results if there is concern the result has not been communicated within the expected time as this provides an audit trail (Clin.imm@nhs.net). Only where electronic links are not available should the CIS be contacted by telephone. Prior to issuing a result by telephone, laboratory staff are required to establish the requester's identity. Enquiries should be made between 0900 and 17:00.

2.11. Tests currently referred to other UKAS accredited Laboratories

If we do not offer a specific test, as shown in the handbook or pre-agreed with the laboratory, we request that the sample is not sent to the CIS but sent directly to an appropriate laboratory. The exception to this is where we have tests that are related to other tests that we undertake and so we recognise that this facilitates the patient pathway. The following tests we will refer on from the CIS to other laboratories.

TEST	LOCATION	REFERRAL LAB TURN AROUND TIME
TCR/IGHV genetic studies	Southampton	21 days

3. Tests available

3.1. Tests on UKAS scope

The CIS is United Kingdom Accreditation Service (UKAS) accredited to ISO 15189 standard. The tests on scope can be reviewed through this link:

https://www.ukas.com/wp-content/uploads/schedule_uploads/00007/9556-Medical-Single.pdf.

Test name	Clinical indication and sample information
Adrenal Cortical Antibodies	**Description:** Cytochrome p450 enzymes are involved in steroid biosynthesis (e.g. 17-alpha-hydroxylase, 21-alpha-hydroxylase and cholesterol desmolase) in the adrenal gland, with the cells in the cortex able to produce cortisol and aldosterone. The enzymes and steroid-producing cells in the adrenal cortex are targeted by autoantibodies to these enzymes which can lead to destruction and adrenocortical insufficiency, named Addison's disease. These antibodies are also associated with Autoimmune Polyglandular Syndrome types 1, 2 and 3. **Indications for test:** Investigation of patients with adrenal insufficiency and polyglandular autoimmune disease. **Method:** Indirect immunofluorescence **Sample type and volume:** Serum (10 mL Red tube). Minimum volume 500 µL. Transport at ambient temperature via Royal Mail or courier. **Reference range:** Normal result shows no fluorescence **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Once every fortnight or before if enough samples to process. **Minimum request interval (if relevant):** Frequency to be determined by clinical context. **Factors affecting the test:** Incorrect storage of samples. **EQA scheme:** Currently no EQA scheme. A sample exchange programme is in place with Nottingham, Portsmouth and Wolverhampton Hospitals. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=2846 **See Also:** Endocrine antibodies

Test name	Clinical indication and sample information
Anti-Nuclear Antibodies (ANA Hep2)	**Description:** Anti-Nuclear Antibodies (ANA) are a type of autoantibody that targets the nucleus of cells. They are non-specific and are associated with various autoimmune conditions such as SLE, Rheumatoid Arthritis, connective tissue diseases, autoimmune hepatitis and primary biliary cholangitis. Of note, ANA levels increase with age with adults over 65 years more likely to have a positive ANA without any autoimmune conditions. ANA is measured by titre – which describes the highest dilution of blood where the antinuclear antibodies are still detected. Therefore, high titres (e.g. 1:160, 1:320, 1:640) are increasingly suggestive of clinical significance and low titres (1:80) are often not considered to be significant if asymptomatic and when taking patient age into account. ANA testing reveals different patterns by direct immunofluorescence – homogeneous, speckled, nucleolar – with different patterns suggestive of different conditions. **Indications for test:** Investigation of patients with suspected autoimmune conditions. **Method:** Indirect immunofluorescence **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum volume 500 µL). Transport at ambient temperature via Royal Mail or courier. **Reference range:** Normal result shows no fluorescence. Weakly positive ANA (1:80) may not be significant when taking patient age into account. **Turn-around time**: Up to 4 days **Testing frequency in laboratory:** Once every 4 days. **Minimum request interval (if relevant):** Once diagnosis is established, repeat testing is of limited value. **Factors affecting the test:** Incorrect storage of samples. Age of patient (as above). Medications can directly affect ANA levels (e.g. methyldopa, chlorpromazine) or indirectly through drug-induced Lupus. **EQA scheme:** Sheffield NEQAS Nuclear and Related Antigens Scheme **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=2929 https://medlineplus.gov/lab-tests/ana-antinuclear-antibody-test/ **See Also:** Rheumatoid factor, CCP antibodies

Test name	Clinical indication and sample information
Anti-C1q Antibodies	**Description:** Complement proteins are a key component of the innate immune system and play a fundamental role in inflammatory response. C1q is the initiation molecule for the classical complement cascade, as it forms the C1 complex with C1r and C1s. C1q antibodies lead to dysregulation in the complement pathway. They are associated with low C4 and often low C3. Clinically, C1q antibodies are associated with Hypocomplementaemic Urticarial Vasculitis (HUV), SLE and Lupus Nephritis. **Indications for test:** Investigation and diagnosis of HUV. Investigation and monitoring of SLE and Lupus Nephritis (C1q antibody levels increase in a lupus flare/suggest active glomerulonephritis). **Method:** INOVA ELISA Kit **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum volume 500 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** 0 – 20 units/mL **Turn-around time**: Up to 28 days **Testing frequency in laboratory:** Once every 4 weeks. **Minimum request interval (if relevant):** **Factors affecting the test:** Incorrect storage of samples. Highly haemolysed or lipaemic samples. **EQA scheme:** None. Currently a sample exchange scheme with Cardiff. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=2976 https://www.leedsth.nhs.uk/services/pathology/tests/anti-c1q-antibodies/ **See Also:** Complement C3 and C4, C1 inhibitor immunochemical levels and functional levels, ANA, dsDNA

Test name	Clinical indication and sample information
Neuromyelitis Optica IgG Antibodies / NMO Antibodies / Aquaporin 4 Antibodies	**Description:** Anti-NMO antibodies are associated with Neuromyelitis Optica (NMO), a demyelinating disease characterised by optic neuritis and transverse myelitis. Aquaporin 4 (AQP4) – a protein/channel expressed on certain cell surfaces – has been identified as the major NMO antigen, with high AQP4 expression in the optic nerve and cells in the spinal cord. This test distinguishes NMO from Multiple Sclerosis. NMO and MOG antibodies are run as a combined test. **Indications for test:** Diagnosis of Neuromyelitis Optica (NMO). **Method:** Indirect Immunofluorescence using Euroimmun Biochips. **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum volume 500 µL). Transport at ambient temperature via Royal Mail or courier. **Reference range:** Normal result = negative **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Every 14 days or before. **Minimum request interval (if relevant):** Repeat testing guided by clinical context. **Factors affecting the test:** None stated by the manufacturer. **EQA scheme:** None currently. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3329 **See also:** NMO Antibodies

Test name	Clinical indication and sample information
Aspergillus – Specific IgG Antibodies	**Description:** Specific Aspergillus antibodies (IgG) target the fungus Aspergillus fumigatus. Measurement is useful in the diagnosis of CPA (chronic pulmonary aspergillosis) or Aspergilloma. **Indications for test:** Investigation of suspected Chronic Pulmonary Aspergillosis (CPA) or Aspergilloma. Importantly, Aspergillus Precipitins would be a more appropriate investigation for Allergic Bronchopulmonary Aspergillosis (ABPA). **Method:** Immunocap 250 **Sample type and volume:** Serum or plasma. (10 mL Red tube). Preferred sample volume 2 mL (minimum volume 500 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Normal: <40 mgA/L **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** 3 times a week. **Minimum request interval (if relevant):** **Factors affecting the test:** None stated by the manufacturer. **EQA scheme:** NEQAS Sheffield Fungal and Related Antigens. **References or guidelines:** https://mft.nhs.uk/app/uploads/2023/07/Aspergillus-fumigatus-precipitins.pdf **See also:** Fungal antigens

<table>
<tr><th>Test name</th><th>Clinical indication and sample information</th></tr>
<tr><td>Autoimmune Encephalitis Screen

(NMDAR, CASPR2, LGI1, AMPAR1/2, GABABR1/2)</td><td>
Description: Some cases of encephalitis are due to autoimmune causes, with autoantibodies targeted at neuronal surface antigens. Diseases can be categorised by the presence of specific antibodies – including NMDAR, AMPAR1 and AMPAR2, CASPR2, GABABR1 and GABABR2 and LGI1.

These autoantibodies may be associated with paraneoplastic syndromes.
<table>
<tr><td>Receptor</td><td>Associated tumour</td></tr>
<tr><td>NMDAR</td><td>Ovarian teratoma</td></tr>
<tr><td>AMPAR1 and AMPAR2</td><td>Lung, breast, thymus</td></tr>
<tr><td>LGI1</td><td>Lung, thymus</td></tr>
<tr><td>CASPR2</td><td>Thymus</td></tr>
<tr><td>GABABR2/3</td><td>Lung (SCLC)</td></tr>
</table>
Indications for test: Investigation of suspected autoimmune encephalitis

Method: Indirect immunofluorescence using Euroimmun Biochips.

Sample type and volume:
CSF – 250 µL.
Serum (10 mL Red tube) or plasma (10 mL Green or Purple top) Transport at ambient temperature via Royal Mail or Courier.

Reference range: Normal result = negative.

Borderline = CASPR2 positive at 1:10 dilution

Positive = CASPR2 positivity at 1:100

Turn-around time: Up to 14 days

Testing frequency in laboratory: Twice a week.

Minimum request interval (if relevant): Frequency to be determined by clinical context.

Factors affecting the test: None stated by the manufacturer.

EQA scheme: Sheffield NEQAS Pilot scheme for NMDA antibodies.

References or guidelines:
https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3548
</td></tr>
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Test name	Clinical indication and sample information
Avian Antigens – Specific IgG Antibodies	**Description:** This detects and quantifies specific IgG antibodies directed against proteins in bird feathers, dander or droppings. These antibodies are associated with hypersensitivity pneumonitis (bird fancier's lung) which is a type of interstitial lung disease. **Indications for test:** Investigation of suspected hypersensitivity pneumonitis in individuals exposed to birds (e.g. bird breeding), or avian antigens (e.g. feathers in duvets/pillows). **Method:** Thermo Fisher Immunocap 250 **Sample type and volume:** Serum (10 mL Red tube) or Plasma (10 mL Green or Purple Top). Preferred sample volume 2 mL (minimum sample volume 500 μL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Budgie 0-8 mg/L. Pigeon 0-38 mg/L. **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** 3 times a week. **Minimum request interval (if relevant):** **Factors affecting the test:** None stated by the manufacturer. **EQA scheme:** Fungal and Related Antigens. **References or guidelines:** https://mft.nhs.uk/app/uploads/2023/07/Avian-precipitins.pdf

Test name	Clinical indication and sample information
Beta 2 Microglobulin (B2M)	**Description:** Beta-2-Microglobulin (B2M) is a polypeptide chain component of the class 1 Major Histocompatibility Complex, which is expressed on the surface of all nucleated cells but most abundantly on lymphocytes, monocytes and tumour cells. As B2M is eliminated by the kidneys, renal impairment can lead to raised B2M levels. **Indications for test:** Investigation and monitoring in patients with lymphoproliferative disorders including myeloma, HIV-related diseases, renal disease and in inflammatory conditions. **Method:** Turbidimetry. **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum volume 500 µL). Transport at ambient temperature via Royal Mail or courier. **Reference range:** Adult serum 0 – 4.0 mg/L **Turn-around time**: Up to 4 days **Testing frequency in laboratory:** Twice a week **Minimum request interval (if relevant):** **Factors affecting the test:** Incorrect storage of samples. Highly haemolysed or lipaemic samples. **EQA scheme:** Sheffield NEQAS B2M Scheme **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=2934

Test name	Clinical indication and sample information
B2GP1 Antibodies	**Description:** Beta 2 Glycoprotein 1 (B2GP1) inhibits the intrinsic coagulation cascade. Antibodies to B2GP1 are highly specific for Antiphospholipid Syndrome which can present with venous and arterial thromboses and recurrent miscarriages. Antiphospholipid Syndrome is also associated with other conditions like Systemic Lupus Erythematous (SLE). **Indications for test:** Investigation of suspected Antiphospholipid Syndrome **Method:** ELISA kit by INOVA. **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum sample volume 500 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Serum: 0 – 20 units/mL **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** Once a week. **Minimum request interval (if relevant):** Once diagnosis is confirmed using BCSH guidelines, repeat testing is of limited value. **Factors affecting the test:** Sodium azide may adversely affect the result if added to the sample. Grossly haemolysed or lipaemic samples should be avoided. **EQA scheme:** Sheffield NEQAS Anti Phospholipid. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=2950 **See also:** Cardiolipin antibodies

Test name	Clinical indication and sample information
Cardiolipin Antibodies	**Description:** Cardiolipin is phospholipid found on cell membranes. Antibodies against Cardiolipin (IgM and IgG) are associated with Antiphospholipid Syndrome, which can lead to venous and arterial thromboses and recurrent miscarriages. Antiphospholipid Syndrome is also associated with other conditions like Systemic Lupus Erythematous (SLE) and 30-40% of patients with SLE have detectable Cardiolipin antibodies. Some infections can lead to slight increase in Cardiolipin antibody levels (for example HIV, Hepatitis C, EBV, CMV). This may be considered clinically significant if antibody levels remain positive on repeat testing after 6 weeks. **Indications for test:** Investigation of suspected Antiphospholipid Syndrome, thrombosis associated with SLE, unexplained thrombocytopaenia **Method:** ELISA kit by INOVA. **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum sample volume 500 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** IgG 0-20 GPL U/ mL. IgM 0-20 MPL U/ mL. **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** Once a week. **Minimum request interval (if relevant):** Once diagnosis is confirmed using BCSH guidelines, repeat testing is of limited value. **Factors affecting the test:** Sodium azide may adversely affect the result if added to the sample. Grossly haemolysed or lipaemic samples should be avoided. **EQA scheme:** Sheffield NEQAS Anti Phospholipid. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3027 https://www.southtees.nhs.uk/services/pathology/tests/anti-cardiolipin-antibody-acl/#:~:text=They%20are%20also%20found%20in,transiently%20elevated%20in%20many%20infections. Uthman IW, Gharavi AE. Viral infections and antiphospholipid antibodies. Semin Arthritis Rheum. 2002 Feb;31(4):256-63. doi: 10.1053/sarh.2002.28303. PMID: 11836658. **See also:** B2GP1 antibodies, ANA, dsDNA

Test name	Clinical indication and sample information
C-Reactive Protein (CRP)	**Description:** C-Reactive Protein (CRP) is a non-specific acute phase protein, from the Pentraxin family. It detects and binds to molecules on damaged cell membranes and microbial polysaccharides, to aid phagocytosis. This is done by activating complement (binding C1q). As CRP production is driven by pro-inflammatory cytokines, CRP rises in the context of infection and inflammatory conditions. **Indications for test:** Investigation and monitoring in patients with suspected infection and inflammatory conditions (for example Rheumatoid Arthritis, Vasculitis). **Method:** Turbidimetry. **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum volume 500 µL). Transport at ambient temperature via Royal Mail or courier. **Reference range:** Adult serum 0 – 5mg/L **Turn-around time**: Up to 4 days **Testing frequency in laboratory:** Twice a week **Minimum request interval (if relevant):** **Factors affecting the test:** Incorrect storage of samples. Highly haemolysed or lipaemic samples. **EQA scheme:** Sheffield NEQAS CRP Scheme **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3079

Test name	Clinical indication and sample information
SARS-CoV-2 (COVID) anti-spike and anti-nucleocapsid antibodies	**Description:** This assay detects IgG antibodies in patients who have had prior exposure to SARS-CoV-2 spike glycoprotein and nucleocapsid protein. These include those recently infected or those with mild response. Advice and price on application. **Indications for test:** Detect antibody response to SARS-CoV-2. This tests IgG to spike protein and/or nucleocapsid. **Method:** Detection of COVID-19 antibodies using the Roche Cobas e411 Anti-SARS-CoV-2 Elecsys. **Sample type and volume:** Serum (2 mL tube). Dried blood spots aliquoted onto perforated filter cards. **Reference range:** 18-245 U/ mL (no dilution factor applied). Dilution factors applied when outside the accepted criteria. **Turn-around time**: Contact lab to discuss **Testing frequency in laboratory:** When required. Availability Monday to Friday. **Minimum request interval (if relevant):** Testing guided by project study protocols and cost per test quotations. **Factors affecting the test:** Incorrect storage of samples. Insufficient volume of sample. Insufficient number of dried blood spots. Delay in shipping of Roche kits / reagents. **EQA scheme:** UK NEQAS for SARS-CoV-2/COVID-19 Antibodies **References or guidelines:** https://www.immqas.org.uk/media/uo3hwxkw/43_participation-handbook-2023-2024-v2pub.pdf https://diagnostics.roche.com/gb/en/products/params/elecsys-anti-sars-cov-2-s.html

Test name	Clinical indication and sample information
CSF Tau Protein (Asialotransferrin)	**Description:** Cerebrospinal fluid (CSF) rhinorrhoea is clinically significant as it suggests basal skull fractures, which can increase the risk of serious infection including sinusitis or meningitis. CSF otorrhoea can result from skull base fractures, cholesteatoma or middle ear infections, which can increase the risk of meningitis or hearing loss. Therefore, it is important to clarify whether rhinorrhoea or otorrhoea is CSF. This assay identifies the presence of Tau protein (asialotransferrin) which is expressed only in CSF. **Indications for test:** Identification of CSF rhinorrhoea or otorrhoea **Method:** Agarose Gel Electrophoresis **Sample type and volume:** Suspected CSF (minimum 50 µL) with paired serum sample (red or gold top) **Reference range:** N/A – sample is positive if it is CSF **Turn-around time**: 7 days **Testing frequency in laboratory:** Weekly **Minimum request interval (if relevant):** **Factors affecting the test:** **EQA scheme:** UK-NEQAS B2 Transferrin **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3101

Test name	Clinical indication and sample information
Complement C3 and C4	**Description:** Complement proteins are a key component of the innate immune system and play a fundamental role in inflammatory response. Complement is rapidly synthesised following trauma or as part of an acute phase response. Therefore, low levels of C3 and/or C4 may indicate decreased synthesis (such as in gene defects, liver failure) or increased consumption (trauma, acute phase response). Measurement of C3/C4may also be helpful in the monitoring of multi-system disorders such as SLE (where C4 levels are low), cryoglobulinaemia, nephritis and angioedema. **Indications for test:** Investigation and monitoring of SLE, angioedema, renal disease, vasculitis, cryoglobulinaemia, C3 nephritic factor. **Method:** Turbidimetry. **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum volume 500 µL). Transport at ambient temperature via Royal Mail or courier. **Reference range:** Adult serum C3: 0.75 – 1.75 g/L Adult serum C4: 0.14 – 0.54 g/L **Turn-around time**: Up to 4 days **Testing frequency in laboratory:** Twice a week **Minimum request interval (if relevant):** **Factors affecting the test:** Incorrect storage of samples. Highly haemolysed or lipaemic samples. **EQA scheme:** Birmingham Quality Specific Proteins Scheme **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=2983 **See Also:** C1 inhibitor immunochemical levels, C1 inhibitor function activity, Anti-C1q Autoantibodies, ANA, dsDNA antibodies

Test name	Clinical indication and sample information
C1 (esterase) inhibitor - Immunochemical Levels	**Description:** C1 inhibitor is an important regulator of the classical complement pathway. C1 inhibitor also has a role in regulating the coagulation cascade and kinin systems. C1 inhibitor deficiency is a cause of Hereditary Angioedema (HAE) – either due to low immunochemical levels or due to functional defects in the protein. In HAE, often C4 levels are low and C1q levels are normal. C1 inhibitor deficiency is also associated with Acquired Angioedema which is often due to lymphoma or myeloma. **Indications for test:** Investigation and monitoring of Hereditary/Acquired Angioedema **Method:** Turbidimetry **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum volume 500 µL). Transport at ambient temperature via Royal Mail or courier. **Reference range:** Adult serum: 0.20 – 0.35 g/L **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Once every week/once a fortnight **Minimum request interval (if relevant):** **Factors affecting the test:** Incorrect storage of samples. Highly haemolysed or lipaemic samples. **EQA scheme:** Sheffield NEQAS Functional C1 Inhibitor Scheme **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=2974 **See Also:** C1 inhibitor functional activity, C1q levels, Complement C3 and C4

Test name	Clinical indication and sample information
C1 (esterase) inhibitor - Functional activity	**Description:** C1 inhibitor is an important regulator of the classical complement pathway and prevents over-activation of the complement cascade. C1 inhibitor also has a role in the coagulation cascade and kinin systems. C1 inhibitor deficiency is a cause of Hereditary Angioedema (HAE) – either due to low immunochemical levels or due to functional defects in the protein. In HAE, C4 levels are typically low and C1q levels normal. C1 inhibitor deficiency is also associated with Acquired Angioedema which is often due to lymphoma or myeloma. **Indications for test:** Investigation and monitoring of Hereditary/Acquired Angioedema **Method:** ELISA-style kinetic determination assay **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum volume 500 µL). Transport at ambient temperature via Royal Mail or courier. **Reference range:** 70 – 130% **Turn-around time**: Up to 21 days. If required urgently, please state on the request form, TAT for urgent requests 3 days. **Testing frequency in laboratory:** Once a fortnight (sample numbers permitting) **Minimum request interval (if relevant):** **Factors affecting the test:** Incorrect storage of samples. Samples must be frozen as soon as possible and transferred to the lab while still frozen. **EQA scheme:** Sheffield NEQAS Functional C1 Inhibitor Scheme **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=2974 **See Also:** C1 inhibitor immunochemical levels, Complement C3 and C4

Test name	Clinical indication and sample information
Cyclic Citrullinated Peptide Antibodies (CCP)	**Description:** Cyclic Citrullinated Peptide Antibodies (CCP Antibodies) are autoantibodies that are highly specific for Rheumatoid Arthritis. In Rheumatoid Arthritis, CCP-Antibodies may be positive even if Rheumatoid Factor negative. Also, patients may be negative for both CCP antibodies and RF but still have the disease, termed seronegative Rheumatoid Arthritis. **Indications for test:** Investigation in patients with suspected Rheumatoid Arthritis. **Method:** Turbidimetry. **Sample type and volume:** Serum (10mL Red tube) or Plasma (10 mL Purple or Green tube). Preferred sample volume 2mL (minimum volume 500 µL). Transport at ambient temperature via Royal Mail or courier. **Reference range:** Adult serum 0 – 7 U/mL **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** Three times a week. **Minimum request interval (if relevant):** Repeat testing once diagnosis is confirmed is of limited value. **Factors affecting the test:** Incorrect storage of samples. Highly haemolysed or lipaemic samples not to be used. **EQA scheme:** Sheffield NEQAS General Autoimmune Serology. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3105 **See Also:** Rheumatoid Factor

Test name	Clinical indication and sample information
Double stranded DNA (dsDNA) Antibodies ***Assay verified but awaiting addition to accredited scope***	**Description:** Autoantibodies directed against antigens in cell nuclei are common in connective tissue diseases – these are termed antinuclear antibodies (ANA). Double stranded DNA (dsDNA) is a specific autoantigen found in cell nuclei, whereby antibodies to dsDNA are associated with Systemic Lupus Erythematous (SLE). When serum for ANA testing is sent, if this is found to be positive then it is automatically tested for specific autoantibodies like dsDNA antibodies. However, dsDNA antibodies may be detected in the absence of ANA. The assay is performed initially with qualitative testing of dsDNA antibodies using indirect immunofluorescence using nuclei from Crithidia lucillae (protozoa), followed by quantitative testing by EIA (enzyme immunoassay). **Indications for test:** Investigation and monitoring of SLE. **Method:** Thermo Fisher EliA Immunocap 250 (Quantitative), INOVA Indirect Immunofluorescence for Crithidia screen. **Sample type and volume:** Serum (10 mL Red tube) only for Crithidia antibodies and Serum or Plasma (Purple or Green tube) for Quantitative value. Preferred sample volume 2 mL (minimum sample volume 500 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Indirect immunofluorescence using Crithidia – N/A (positive/negative) Serum EIA: • Negative <10 IU/ mL • Equivocal 10-15 IU/ mL • Positive >15 IU/ mL. **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** Twice a week. **Minimum request interval (if relevant):** Every 3-6 months. **Factors affecting the test:** Lipaemic or haemolysed samples should not be used. **EQA scheme:** Sheffield NEQAS Nuclear And Related Antigens. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3124 https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3122 **See also:** ANA, ENA

Test name	Clinical indication and sample information
Endocrine Antibodies **(Adrenal, Ovarian, Testes)**	**Description:** Cytochrome p450 enzymes are involved in steroid biosynthesis (e.g. 17-alpha-hydroxylase, 21-alpha-hydroxylase and cholesterol desmolase) in the adrenal gland, with the cells in the cortex able to produce cortisol and aldosterone. The enzymes and steroid-producing cells in the adrenal cortex are targeted by autoantibodies to these enzymes which can lead to destruction and adrenocortical insufficiency, named Addison's disease. These antibodies are also associated with premature ovarian failure, premature testicular failure and Autoimmune Polyglandular Syndrome types 1, 2 and 3. **Indications for test:** Investigation in patients with adrenal insufficiency, premature gonadal failure, and polyglandular autoimmune disease. **Method:** Indirect immunofluorescence. **Sample type and volume:** Serum (10 mL Red tube). Preferred volume 2 mL (minimum volume 500 μL). Transport at ambient temperature via Royal Mail or courier. **Reference range:** Normal result shows no fluorescence **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Once every fortnight or sooner. **Minimum request interval (if relevant):** Frequency to be determined by clinical context. **Factors affecting the test:** Incorrect storage of samples. **EQA scheme:** Currently no EQA scheme. A sample exchange programme is in place with Nottingham, Portsmouth and Wolverhampton Hospitals. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3138 **See Also:** Adrenal cortical antibodies

Test name	Clinical indication and sample information
Endomysial Antibodies (IgA)	**Description:** Endomysial antibodies are IgA isotype, which reacts with smooth muscle endomysium and are indicative of gluten-sensitive enteropathy (Coeliac disease). They are more specific for Coeliac than TTG antibodies. Decreasing titres of Endomysial antibodies correlate with adherence to a gluten-free diet. Endomysial antibodies can also be positive in patients with Dermatitis Herpetiformis. **Indications for test:** Investigation of suspected Coeliac disease and dermatitis herpetiformis. **Method:** Indirect Immunofluorescence. **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum sample volume 500 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Normal result - negative **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Once a week. **Minimum request interval (if relevant):** Only for confirmation of tTG positives. **Factors affecting the test:** Lipaemic or haemolysed samples should not be used. **EQA scheme:** Sheffield NEQAS Coeliac Antibodies Scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3140 **See also:** Tissue Transglutaminase (TTG) antibodies, Gliadin antibodies, Immunoglobulins

<table>
<tr><th>Test name</th><th>Clinical indication and sample information</th></tr>
<tr><td>ENA Antibodies

(ENA – Extractable Nuclear Antigen)

Assay verified but awaiting addition to accredited scope</td><td>
Description: Autoantibodies directed against antigens in cell nuclei are common in connective tissue diseases – these are termed antinuclear antibodies (ANA). Some of these antigens can be extracted and further identified– termed extractable nuclear antigens (ENA). Antibodies to specific ENAs can help pinpoint specific autoimmune diseases.
This assay can be used to identify the following ENA Antibodies:
<table>
<tr><th>ENA Antibodies</th><th>Clinical Disease Association</th></tr>
<tr><td>Anti-Sm (Smith)</td><td>SLE</td></tr>
<tr><td>Anti-RNP</td><td>Mixed connective tissue disease</td></tr>
<tr><td>Anti-Ro (SSA)</td><td>SLE, Sjogren’s, neonatal lupus, RA</td></tr>
<tr><td>Anti-La (SSB)</td><td>SLE, Sjogren’s</td></tr>
<tr><td>Anti-Jo1</td><td>Dermatomyositis, polymyositis</td></tr>
<tr><td>Anti-Scl70</td><td>Diffuse systemic sclerosis</td></tr>
<tr><td>Anti-centromere</td><td>Limited systemic sclerosis</td></tr>
<tr><td>Anti-histone</td><td>Drug induced SLE</td></tr>
</table>
Indications for test: Investigation of connective tissue disease or autoimmune conditions

Method: ELISA

Sample type and volume: Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum sample volume 500 µL). Transport at ambient temperature via Royal Mail or Courier.

Reference range: <20 units.

Turn-around time: Up to 7 days

Testing frequency in laboratory: Once a week.

Minimum request interval (if relevant): Frequency to be determined by clinical context.

Factors affecting the test: Lipaemic or haemolysed samples should not be used.

EQA scheme: NEQAS Sheffield Nuclear and Related Antigens.

References or guidelines:

https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3137

See also: ANA
</td></tr>
</table>

Test name	Clinical indication and sample information
Fungal antigens-specific IgG antibodies	**Description:** This assay quantifies specific IgG against Candida albicans, Aspergillus fumigatus and Micropolyspora faeni. N.B. Specific IgG to these fungal antigens can be seen in healthy individuals (and is seen in most adult females), due to exposure to commensal yeast flora. **Indications for test:** Investigation of fungal infections, including Candida and Chronic Mucocutaneous Candidiasis. **Method:** Thermo Fisher Immunocap 250. **Sample type and volume:** Serum (10 mL Red tube) or Plasma (Purple or Green tube). Preferred sample volume 2 mL (minimum sample volume 500 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** • Aspergillus 0-40 mg/l. • Candida 0-60 mg/l. • Micropolyspora faeni 0-22 mg/l. **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** Three times a week. **Minimum request interval (if relevant):** Frequency to be determined by clinical context. **Factors affecting the test:** None stated by the manufacturer. **EQA scheme:** NEQAS Sheffield Fungal and Related Antigens. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3005 **See also:** Aspergillus – specific IgG

Test name	Clinical indication and sample information
Ganglioside antibodies – GD1b	**Description:** Gangliosides are glycolipids found on the cell surface of neurons in the central and peripheral nervous systems. They play a role in cell signalling and cell to cell communication. Autoantibodies to gangliosides have been found in autoimmune neurological disorders. For example, GD1b Antibodies target the GD1b ganglioside, which is expressed on Schwann cells and oligodendrocytes. GD1b antibodies (IgM and IgG) are mainly associated with Guillain-Barre Syndrome – an acute, symmetrical, ascending demyelinating condition that can lead to sensorimotor polyneuropathy, loss of reflexes and respiratory failure. **Indications for test:** Investigation of sensorimotor neuropathy (normally peripheral neuropathy). **Method:** Enzyme Immunoassay (EIA) **Sample type and volume:** Serum (Red/Gold top tube). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** • <1:500 titre units – negative • 500-1000 titre units – equivocal, interpret within clinical context • >1000 titre units – positive **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Twice Weekly **Minimum request interval (if relevant):** **Factors affecting the test:** **EQA scheme:** Ganglioside, Sheffield NEQAS. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3038 Rinaldi, Simon; Willison, Hugh J. Ganglioside antibodies and neuropathies. Current Opinion in Neurology 21(5):p 540-546, October 2008. | DOI: 10.1097/WCO.0b013e32830b84b7 Hugh J. Willison, Nobuhiro Yuki, Peripheral neuropathies and anti-glycolipid antibodies, *Brain*, Volume 125, Issue 12, December 2002, Pages 2591–2625 **See also:** Ganglioside antibodies – GM1, GQ1b (Miller Fisher), Sulphatides

Test name	Clinical indication and sample information
Ganglioside antibodies – GM1	**Description:** Gangliosides are glycolipids found on the cell surface of neurons in the central and peripheral nervous systems. They play a role in cell signalling and cell to cell communication. Autoantibodies to gangliosides have been found in certain neurological disorders. For example, GM1 antibodies target the GM1 ganglioside, which is expressed on Schwann cells and oligodendrocytes. GM1 antibodies (IgM or IgG) are associated with neurological conditions such as Guillain-Barre Syndrome, multi-focal motor neuropathy and certain types of motor neurone diseases. **Indications for test:** Investigation of peripheral neuropathies (usually motor neuropathies). **Method:** Enzyme Immunoassay (EIA) **Sample type and volume:** Serum (Red/Gold top tube). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** • <1:500 titre units – negative • 500-1000 titre units – equivocal, interpret within clinical context • >1000 titre units – positive **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Twice Weekly **Minimum request interval (if relevant):** **Factors affecting the test:** **EQA scheme:** Ganglioside, Sheffield NEQAS. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3018 Hugh J. Willison, Nobuhiro Yuki, Peripheral neuropathies and anti-glycolipid antibodies, *Brain*, Volume 125, Issue 12, December 2002, Pages 2591–2625 Rinaldi, Simon; Willison, Hugh J. Ganglioside antibodies and neuropathies. Current Opinion in Neurology 21(5):p 540-546, October 2008. | DOI: 10.1097/WCO.0b013e32830b84b7 **See also:** Ganglioside antibodies – GD1b, GQ1b (Miller Fisher), Sulphatides

Test name	Clinical indication and sample information
Ganglioside antibodies – GQ1b (Miller Fisher Syndrome)	**Description:** Gangliosides are glycolipids found on the cell surface of neurons in the central and peripheral nervous systems. They play a role in cell signalling and cell to cell communication. Autoantibodies to gangliosides have been found in autoimmune neurological disorders. For example, GQ1b Antibodies target the GQ1b ganglioside, which is expressed in the peripheral nervous system and very highly expressed on the surface of cranial nerves. Autoantibodies to GQ1b (IgM and IgG) are associated with Miller Fisher Syndrome – a variant to Guillain-Barre Syndrome –characterised by ophthalmoplegia, ataxia and areflexia. **Indications for test:** Investigation of Miller Fisher Syndrome **Method:** Enzyme Immunoassay (EIA) **Sample type and volume:** Serum (Red/Gold top tube). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** • <1:500 titre units – negative • 500-1000 titre units – equivocal, interpret within clinical context • >1000 titre units – positive **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Twice Weekly **Minimum request interval (if relevant):** **Factors affecting the test:** **EQA scheme:** Ganglioside, Sheffield NEQAS. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=2998 Hugh J. Willison, Nobuhiro Yuki, Peripheral neuropathies and anti-glycolipid antibodies, *Brain*, Volume 125, Issue 12, December 2002, Pages 2591–2625 Rinaldi, Simon; Willison, Hugh J. Ganglioside antibodies and neuropathies. Current Opinion in Neurology 21(5):p 540-546, October 2008. | DOI: 10.1097/WCO.0b013e32830b84b7 **See also:** Ganglioside antibodies – GD1b, GM1, Sulphatides

Test name	Clinical indication and sample information
Ganglioside antibodies – Sulphatides	**Description:** Gangliosides are glycolipids found on the cell surface of neurons in the central and peripheral nervous systems. They play a role in cell signalling and cell to cell communication. Sulphatides are similar in structure to gangliosides (except with a sulphate group attached) and are also expressed on the cell surface of neurons. Autoantibodies to sulphatides have been associated with predominantly sensory neuropathies. **Indications for test:** Investigation of predominantly sensory neuropathies. **Method:** Enzyme Immunoassay (EIA) **Sample type and volume:** Serum (Red/Gold top tube). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** • <1:500 titre units – negative • 500-1000 titre units – equivocal, interpret within clinical context • >1000 titre units – positive **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Twice Weekly **Minimum request interval (if relevant):** **Factors affecting the test:** **EQA scheme:** Ganglioside, Sheffield NEQAS. **References or guidelines:** Hugh J. Willison, Nobuhiro Yuki, Peripheral neuropathies and anti-glycolipid antibodies, *Brain*, Volume 125, Issue 12, December 2002, Pages 2591–2625 **See also:** Ganglioside antibodies – GD1b, GM1, GQ1b (Miller Fisher)

Test name	Clinical indication and sample information
Gastric Parietal Cell Antibodies	**Description:** Gastric parietal cells are responsible for the production of Intrinsic Factor, a glycoprotein that is essential for the absorption of Vitamin B12 in the small intestine (vitamin B12 being essential in Haem synthesis). Antibodies to parietal cells are found in the majority (90%) of patients with Pernicious Anaemia and also in patients with chronic gastritis. The antibodies are directed against the hydrogen-potassium ATPase pump on the parietal cell surface. Parietal cell antibodies are more common in females and with increasing age. Parietal cell antibodies are also associated with autoimmune thyroid disease, Type 1 Diabetes and Sjogren's syndrome. **Indications for test:** Investigation of suspected Pernicious anaemia and chronic gastritis. N.B. Antibodies to Intrinsic Factor are carried out in conjunction with parietal cell antibodies. **Method:** Indirect Immunofluorescence. **Sample type and volume:** Serum. (10 mL Red tube). Preferred sample volume 2 mL (minimum sample volume 500 μL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Normal – negative **Turn-around time**: Up to 4 days **Testing frequency in laboratory:** Twice a week. **Minimum request interval (if relevant):** Frequency to be determined by clinical context. **Factors affecting the test:** Lipaemic or haemolysed samples should not be used. **EQA scheme:** NEQAS Sheffield General Autoimmune Serology Scheme. **References or guidelines:** https://www.ouh.nhs.uk/immunology/diagnostic-tests/tests-catalogue/gastric-parietal-cell-antibody.aspx#:~:text=Also%20known%20as%3A%20GPC&text=The%20auto%2Dantibody%20is%20found,sensitive%2C%20but%20not%20specific) https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3043 **See also:** Intrinsic Factor antibodies

Test name	Clinical indication and sample information
Gliadin Antibodies **(Gliadin deaminated peptide antibodies)**	**Description:** Gliadin are a class of proteins found in wheat, barley, rye and oat. Anti-gliadin antibodies (IgG) are found in patients with Coeliac disease and Dermatitis Herpetiformis, although not specific (also found in patients with Crohn's disease and Ulcerative Colitis). Measurement of anti-Gliadin antibodies can be useful in patients with IgA deficiency. IgA deficiency is common – found in about 1:400 healthy blood donors and 1:40 patients with Coeliac. **Indications for test:** Investigation of suspected Coeliac disease and dermatitis herpetiformis. N.B. First line tests for suspected Coeliac would be anti-TTG antibodies and anti-endomysial antibodies due to higher sensitivity and specificity. **Method:** Thermo Fisher Immunocap 250 **Sample type and volume:** Serum (10 mL Red tube) or Plasma (10 mL Green or Purple tube). Preferred sample volume 2 mL (minimum sample volume 500 μL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** • Negative <7 U/mL • Equivocal 7-10 U/mL • Positive >10 U/mL **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** Three times a week. **Minimum request interval (if relevant):** 6-12 months. **Factors affecting the test:** Lipaemic or haemolysed samples should not be used. **EQA scheme:** NEQAS Sheffield Coeliac Antibodies Scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3134 **See also:** Tissue Transglutaminase (TTG) antibodies, Endomysial antibodies IgA, Immunoglobulins

Test name	Clinical indication and sample information
Glomerular Basement Membrane (GBM) Antibodies	**Description:** Glomerular basement membrane (GBM) antibodies are directed against specific parts of Type IV Collagen (non-collagenous portion), which are expressed in the kidney (glomerulus) and lungs (alveolar basement membrane). Therefore, these autoantibodies are associated with disease involving kidneys and or lungs, including Anti-GBM Disease (glomerulonephritis) and Goodpasture's Syndrome. Antibody levels have been shown to correlate with the severity of disease. **Indications for test:** Investigation of possible glomerulonephritis or Goodpasture's Syndrome **Method:** Thermo Fisher Immunocap **Sample type and volume:** Serum (10 mL Red tube) or Plasma (10 mL Green or Purple tube). Preferred sample volume 2 mL (minimum sample volume 500 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Negative: <7 U/mL **Turn-around time**: Up to 4 days **Testing frequency in laboratory:** Three times a week. **Minimum request interval (if relevant):** Every 3-6 months while on treatment or more frequent if receiving plasma exchange therapy. **Factors affecting the test:** Haemolysed or lipaemic samples should not be used. **EQA scheme:** NEQAS Sheffield Anti Neutrophil Cytoplasmic Antibodies Scheme. **References or guidelines:** Joyita Bharati, Kenar D. Jhaveri, Alan D. Salama, Louise Oni. Anti–Glomerular Basement Membrane Disease: Recent Updates, Advances in Kidney Disease and Health, Volume 31, Issue 3, 2024, Pages 206-215, ISSN 2949-8139. https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3048

Test name	Clinical indication and sample information
Glutamic Acid Decarboxylase (GAD) Antibodies	**Description:** Glutamic acid decarboxylase (GAD) is an enzyme concentrated in neurons that controls muscle tone and spinal reflexes. Anti-GAD antibodies are associated autoimmune conditions, including Stiff Person Syndrome and Type 1 Diabetes Mellitus. GAD Index is available to determine CSF-specific GAD synthesis, which requires CSF sample and serum sample. **Indications for test:** Investigation of Type I Diabetes and Stiff Man Syndrome. **Method:** ELISA Euroimmun **Sample type and volume:** Serum (10 mL Red tube) or EDTA Plasma (10 mL Purple tube). Preferred sample volume 2 mL (minimum sample volume 500 μL). Transport at ambient temperature via Royal Mail or Courier. CSF sample needed for GAD Index calculation, to determine CSF-specific GAD synthesis. **Reference range:** 0 – 10 IU/mL **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Once a fortnight. **Minimum request interval (if relevant):** Not routinely required. **Factors affecting the test:** None stated by the manufacturer. **EQA scheme:** NEQAS Sheffield Diabetic Markers Scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3153

<table>
<tr><th>Test name</th><th>Clinical indication and sample information</th></tr>
<tr><td>Immunoglobulins (IgG,A,M) with electrophoresis</td><td>
Description: This is a combination test to enable the detection and quantification of IgG, IgA and IgM. The total amount of immunogloublin is measured, but to determine whether the immunoglobulin is polyclonal or monoclonal requires electrophoresis. Serum electrophoresis will determine the isotype of the light chain.

Indications for test:

Antibody deficiency: Patients with recurrent infection patients including those with lymphoproliferative diseases where hypogammaglobulinaemia may be present.

Monitoring of immunoglobulin replacement therapy.

Paraprotein detection in patients being investigated for possible myeloma.

Often undertaken as part of investigation of autoimmune, inflammatory and rheumatological diseases.

Method: Tubidimetry for immunoglobulin quantification. Zonal electrophoresis via capillary or gel.

Sample type and volume: Serum. 10 mL Red tube. Preferred sample volume 2 mL (minimum volume 500 µL). Transport at ambient temperature via Royal Mail or courier.

Reference range:

Reference ranges are age specific and may differ between ethnic groups (e.g. white patients have lower IgG than Asian and Black patients).

<table>
<tr><td>Ig type</td><td>Adult Normal Range (g/L)</td></tr>
<tr><td>IgG</td><td>6.0 – 16.0</td></tr>
<tr><td>IgA</td><td>0.8 – 4.0</td></tr>
<tr><td>IgM</td><td>0.5 – 2.0</td></tr>
</table>

Turn-around time: Up to 4 days

Testing frequency in laboratory: Three times per week

Minimum request interval (if relevant):

Factors affecting the test: Incorrect storage of samples. Highly haemolysed or lipaemic samples.

EQA scheme: Birmingham quality specific proteins scheme and Sheffield NEQAS Monoclonal proteins scheme.

References or guidelines:

Myeloma UK Laboratory Best Practice Tool – Myeloma Academy

See Also: Serum free light chains, Bence Jones protein/urine light chains, IgG subclasses, IgD, IgE,
</td></tr>
</table>

Test Name	Clinical Indication and sample information
Immunoglobulin D (IgD)	**Description:** This test enables quantification of IgD. IgD is an immunoglobulin expressed on the surface of circulating immature B-cells and in very small amounts in serum, comprising just 0.25% of serum immunoglobulins. It can be raised in certain types of myeloma, periodic fever syndromes (e.g. Hyper IgD Syndrome) and autoinflammatory syndromes. **Indications for test:** Patients with some forms of periodic fever syndromes (recurrent fevers, +/- lymphadenitis +/- arthritis may represent Hyper IgD Syndrome) and investigation of IgD Myeloma. **Method:** Turbidimetry **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum volume 500 µL). Transport at ambient temperature via Royal Mail or courier. **Reference range:** 0.05 – 0.2 g/L **Turn-around time**: Up to 14 days or within 4 days if a GP sample. **Testing frequency in laboratory:** Once a week/once every two weeks **Minimum request interval (if relevant):** **Factors affecting the test:** Incorrect storage of samples. Highly haemolysed or lipaemic samples. **EQA scheme:** Sheffield NEQAS IgD scheme (pilot) **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3245 Vladutiu AO. Immunoglobulin D: properties, measurement, and clinical relevance. Clin Diagn Lab Immunol. 2000 Mar;7(2):131-40. doi: 10.1128/CDLI.7.2.131-140.2000. PMID: 10702483; PMCID: PMC95839. **See Also:** Immunoglobulins and electrophoresis

Test Name

Immunoglobulin Subclasses (IgG1-4)

Clinical indication and sample information

Description: In normal adults, IgG constitutes approximately 75% of the total serum immunoglobulin. IgG1, IgG2, IgG3 and IgG4 are the subclass contributors to total IgG, with IgG1 being the major contributor. This test quantifies the IgG subclasses.

IgG1 may be raised in certain conditions like Sjogren's syndrome.
IgG2 and IgG4 are physiologically low in infancy and childhood.
IgG4 may be raised in certain conditions like atopy and parasitic infections.

Indications for test: Patients who may have primary or secondary immunodeficiency who suffer recurrent infections. IgG4 related diseases.

Method: Turbidimetry

Sample type and volume: Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum volume 1 mL). Transport at ambient temperature via Royal Mail or courier.

Reference range:

Reference ranges are age and gender specific.

Age	IgG1 (g/L)	IgG2 (g/L)	IgG3 (g/L)	IgG4 (g/L)
Cord blood	3.6 - 8.4	1.2 - 4.0	0.3 - 1.5	<0.5
6 months	1.5 - 3.0	0.3 - 0.5	0.1 - 0.6	<0.5
2 years	2.3 - 5.8	0.3 - 3.9	0.1 - 0.8	<0.5
5 years	2.3 - 6.4	0.7 - 4.5	0.1 - 1.1	<0.8
10 years	3.6 - 7.3	1.4 - 4.5	0.3 - 1.1	<1.0
15 years	3.8 - 7.73	1.3 - 4.6	0.2 - 1.2	<1.1
Adult	3.2 - 10.2	1.2 - 6.6	0.2 - 1.9	<1.3

Turn-around time: Up to 7 days

Testing frequency in laboratory: Once a week

Minimum request interval (if relevant):

Factors affecting the test: Incorrect storage of samples. Highly haemolysed or lipaemic samples.

EQA scheme: Sheffield NEQAS IgG subclass scheme,

References or guidelines:

https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3244

https://www.ouh.nhs.uk/immunology/diagnostic-tests/tests-catalogue/igg-subclasses.aspx

See Also: Immunoglobulins and electrophoresis

Immunoglobulin E (Total IgE)

Description: This quantifies total serum IgE, which may be commonly raised in atopic diseases (atopic eczema, allergic asthma, allergic bronchopulmonary aspergillosis) due to its role in Type I Hypersensitivity, parasitic infections, autoimmune diseases and in the primary immunodeficiency Hyper IgE Syndrome.

Very low levels of total IgE usually excludes atopic disorders.

Very high levels of total IgE can result in a false positive specific IgE results. Specific IgE can be measured ('RAST testing) against specific allergens.

Indications for test: Investigation of patients with atopic disorders, parasitic infections

Method: Immunocap 250 (ELISA)

Sample type and volume: Serum (10 mL red tube) or Plasma (10 mL Green or Purple tube). Preferred sample volume 2 mL (minimum volume 500 µL).

Reference range:
Reference ranges are age specific and may differ between ethnic groups.
Adult: 0 – 90 IU/mL

Age	Normal Range (KU/L)
0 – 3 months	<5
3 – 12 months	<11
1 year – 5 years	<29
5 – 10 years	<52
10 – 15 years	<63
15 years – Adult	<90

Turn-around time: Up to 7 days

Testing frequency in laboratory: Once every 3 days

Minimum request interval (if relevant): Not routinely required.

Factors affecting the test: None stated by the manufacturer.

EQA scheme: Sheffield NEQAS Total IgE Scheme

References or guidelines:

https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3239

See Also: Immunoglobulins, Specific IgE

Test name	Clinical indication and sample information
Intrinsic Factor Antibodies	**Description:** Gastric parietal cells are responsible for the production of Intrinsic Factor, a glycoprotein that is essential for the absorption of Vitamin B12 in the small intestine (vitamin B12 being essential in Haem synthesis). Antibodies to intrinsic factor are seen in 50-70% of patients with Pernicious Anaemia, which is characterised by atrophic gastritis and reduced Vitamin B12 absorption. **Indications for test:** Investigation of suspected Pernicious anaemia and chronic gastritis. N.B. Antibodies to Gastric Parietal Cells are carried out in conjunction with Intrinsic Factor antibodies. **Method:** ELISA **Sample type and volume:** Serum (10 mL Red tube) or Plasma (10 ml Green or Purple tube). Preferred sample volume 2 mL (minimum sample volume 500 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Normal: <6 units/m **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** Once a week. **Minimum request interval (if relevant):** Not routinely required. **Factors affecting the test:** Haemolysed or lipaemic samples to be avoided. **EQA scheme:** NEQAS Birmingham Intrinsic Factor Antibodies Scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3263 **See also:** Gastric Parietal Cell antibodies

Test name	Clinical indication and sample information
Islet cell antibodies for type 1 diabetes **3-Screen ELISA**	**Description:** Multiplex ELISA combining ZNT8, IA2 and GAD **Indications for test:** Glutamic acid decarboxylase 65 (GAD65) antibodies, protein tyrosine phosphatase 2 antibodies (IA2 antibodies) and Zinc Transport 8 (ZnT8, IA2 and GAD autoantibodies are islet cell antibodies known to target the pancreas and be associated with type 1 diabetes. Islet cell autoantibodies can be detected prior to stage 3 or clinical onset of T1D and the need for exogenous insulin treatment. Individuals with two or more of the autoantibodies have a 68% 5 year risk of developing type 1 diabetes. Those with 3 autoantibodies have a 100% 5 year risk of the disease [4]. Given autoantibodies are rare this test is most suitable for screening for type 1 autoantibodies as the assay cannot individually identify the autoantibodies. If the test is positive a follow up blood test can be requested. **Method:** RSR 3-Screen ELISA. 3 Screen Islet Cell Autoantibody ELISA kit from RSR – Instructions for use **Sample type and volume:** Serum and dried blood spot eluate **Reference range:** <20 u/ml negative, ≥ 20 u/ml positive **Turn-around time:** <21 days **Testing frequency in laboratory:** Weekly **Minimum request interval (if relevant):** **Factors affecting the test: N/A** **EQA scheme:** UK NEQAS for diabetic markers (Sheffield) **References or guidelines: Overview | Type 1 diabetes in adults: diagnosis and management | Guidance | NICE**

Test Name	Clinical indication and sample information
Islet cell antibodies for type 1 diabetes **Individual autoantibodies; IA2, Insulin, ZnT8, GAD**	**Description:** Individual ELISAs to detect insulin, ZNT8, IA2 and GAD autoantibodies. GAD autoantibodies measured as part of this panel but see above for detail on use of GAD in Stiff person syndrome. **Indications for test:** Detection of individual autoantibodies for the risk stratification for pre-T1D diabetes or the diagnosis of T1D when there is diagnostic uncertainty with type 2 diabetes. Pre-diabetes: Children with a single autoantibody have a 14% chance of developing type 1 diabetes within 10 years (22% risk over their lifetime). Children with two or more autoantibodies very early in life have >80% risk of developing type 1 diabetes within 10 years (Ziegler et al. 2013). If there is diagnostic uncertainty as to whether someone has T1D or T2D, the identification of an autoantibody confirms T1D. Autoantibodies do decline from time of diagnosis and so a negative autoantibody test does not exclude T1D and the result should be taken in context of other clinical signs and symptoms. **Method:** Insulin Organtec ELISA ZNT8 Euroimmun ELISA IA2 Euroimmun ELISA GAD Euroimmun ELISA **Sample type and volume:** 1x 10ml Red top serum for all four tests **Reference range:** <u>Insulin</u> <10 u/mL serum= Negative ≥10 u/mL serum=Positive <u>ZNT8</u> <15 RU/mL serum= Negative ≥15 RU/mL serum=Positive

	IA2 <10 IU/mL serum= Negative ≥10 IU/mL serum=Positive GAD <10 IU/mL serum= Negative ≥10 IU/mL serum=Positive **Turn-around time for all ELISAs:** <21 days **Testing frequency in laboratory:** Every 2 weeks **Minimum request interval (if relevant):** **Factors affecting the test:** Insulin autoantibodies can be non specifically detected if the patient is already on exogenous insulin. **EQA scheme:** UK NEQAS for diabetic markers (Sheffield) **References or guidelines:** Overview | Type 1 diabetes in adults: diagnosis and management | Guidance | NICE Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, Winkler C, Ilonen J, Veijola R, Knip M, Bonifacio E, Eisenbarth GS. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA. 2013 Jun 19;309(23):2473-9. doi: 10.1001/jama.2013.6285. PMID: 23780460; PMCID: PMC4878912.

Test name	Clinical indication and sample information
Isoelectric focusing (Oligoclonal bands) IgG	**Description:** This assay detects oligoclonal bands in CSF – which refers to discrete populations of immunoglobulin that are detected in CSF but not in serum from the same patient. Oligoclonal bands are typically present in Multiple Sclerosis. Therefore, this assay can be used as a confirmatory test in MS but it is not specific as oligoclonal bands can also be seen in patients with cerebrovascular accident, cerebral malignancy, CNS infections or processes involving an immune response (e.g. encephalitis, SLE, neurosarcoid). **Indications for test:** Investigation of suspected demyelinating disease (Multiple Sclerosis), CNS infections or conditions involving central nervous system immune response. **Method:** Isoelectric focusing **Sample type and volume:** Paired serum and CSF. Preferred volume 1-2 mL (minimum volume 250 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Positive or negative **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Twice weekly **Minimum request interval (if relevant):** **Factors affecting the test:** **EQA scheme:** Sheffield NEQAS Scheme CSF Oligoclonal Bands **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3343 **See also:** Immunoglobulins IgG, IgA, IgM

Test name	Clinical indication and sample information
Liver antigen antibodies (blot)	**Description:** This assay is used as a confirmatory qualitative test for the presence of liver autoantibodies that are commonly associated with Primary Biliary Cholangitis and Autoimmune Hepatitis. These antibodies include M2 (anti-mitochondrial antibodies), LKM, LC-1, SLA/LP, SP100, GP120, f-Actin. N.B. Antimitochondrial M2 antibodies can be quantified by ELISA. **Indications for test:** Investigation of suspected Primary Biliary Cholangitis or Autoimmune Hepatitis. **Method:** Enzyme Immunoassay (EIA) **Sample type and volume:** Serum (Red/Gold top tube). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** • <1:500 titre units – negative • 500-1000 titre units – equivocal, interpret within clinical context • >1000 titre units – positive **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** Twice Weekly **Minimum request interval (if relevant):** **Factors affecting the test:** **EQA scheme:** Sample exchange with Leeds **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3295 **See also:** Antimitochondrial antibodies, M2 antibodies, LKM antibodies, SLA/LP antibodies,

Test name	Clinical indication and sample information
LKM Antibodies (Liver Kidney Microsome)	**Description:** LKM (Liver-Kidney Microsome) antibodies are found in the cytoplasm of hepatocytes and renal tubules. They are found to be positive in some patients with Autoimmune Hepatitis (ANA-negative patients) or drug-induced hepatitis There are three isotypes of LKM antibodies – LKM-1, LKM-2, LKM-3. LKM-1 is positive in Chronic Active Hepatitis type 2, which is the most common autoimmune liver disease of childhood. **Indications for test:** Investigation of suspected autoimmune hepatitis. **Method:** Indirect immunofluorescence **Sample type and volume:** Serum (10 mL Red tube). Preferred volume 2 mL (minimum volume 500 μL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Negative/Positive **Turn-around time**: Up to 4 days **Testing frequency in laboratory:** Twice a week. **Minimum request interval (if relevant):** Frequency to be determined by clinical context. **Factors affecting the test:** Avoid using haemolysed or lipaemic samples. **EQA scheme:** NEQAS Sheffield General Autoimmune Serology Scheme. **References or guidelines:** https://www.ouh.nhs.uk/immunology/diagnostic-tests/tests-catalogue/liver-kidney-microsomal-antibodies.aspx https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3292 **See also:** Antinuclear antibodies

Test name	Clinical indication and sample information
Mast Cell Tryptase	**Description:** Mast cell tryptase is a marker of mast cell degranulation. Mast cells play a key role in IgE-mediated allergy through degranulation when activated, to release their mediators including tryptase (and histamine). This assay quantifies total tryptase levels to help in the assessment of IgE-mediated allergy including anaphylaxis or mast cell disorders such as Systemic Mastocytosis. N.B. All patients who have had anaphylaxis should be referred to a specialist Allergy clinic (as per NICE guidance as below). **Indications for test:** Investigation of suspected anaphylaxis (timings of tryptase samples should be taken as per the Resus Council guideline for Anaphylaxis management) and mast cell syndromes including systemic mastocytosis or hereditary alphatryptasaemia. **Method:** Thermo Fisher Immunocap 250 (Fluorescence enzyme linked immunoassay) **Sample type and volume:** Serum (10 mL Red tube) or Plasma (10 mL Green or Purple tube). Preferred sample volume 2 mL (minimum sample volume 500 µL). Transport at ambient temperature via Royal Mail or Courier if within 2 days. Specimens are stable for 1 week at 2-8°C, otherwise store at -20°C. **Reference range:** 0 – 13.5 ug/L. **Turn-around time**: Up to 5 days **Testing frequency in laboratory:** Three times a week. **Minimum request interval (if relevant):** Three samples over a period of 24 hours for anaphylaxis assessment. Repeat testing may be required in mastocytosis. Frequency to be determined by clinical context. **Factors affecting the test:** Incorrect storage of samples. **EQA scheme:** NEQAS Sheffield Tryptase Scheme. **References or guidelines:** https://www.nice.org.uk/guidance/cg134 https://www.resus.org.uk/sites/default/files/2021-05/Emergency%20Treatment%20of%20Anaphylaxis%20May%202021_0.pdf https://www.ouh.nhs.uk/immunology/diagnostic-tests/tests-catalogue/tryptase.aspx **See also:** Total IgE, Specific IgE

Test name	Clinical indication and sample information
Mitochondrial Antibodies	**Description:** This is a qualitative assay to detect Anti-Mitochondrial Antibodies (AMA), which are autoantibodies often present in chronic liver disease. They are strongly associated with Primary Biliary Cholangitis (PBC), present in >90% of patients. They are also associated with autoimmune hepatitis and autoimmune conditions (e.g. Rheumatoid arthritis, Sjogren's syndrome, Scleroderma). For quantitation of Anti-Mitochondrial Antibodies (M2 subtype), please request Anti-Mitochondrial M2 Antibodies **Indications for test:** Investigation of liver disease (strongly associated with Primary Biliary Cholangitis). **Method:** Indirect Immunofluorescence **Sample type and volume:** Serum (10 mL Red tube). Preferred volume 2 mL (minimum volume 500 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Negative/Positive. **Turn-around time**: Up to 4 days **Testing frequency in laboratory:** Twice a week. **Minimum request interval (if relevant):** Frequency to be determined by clinical context. **Factors affecting the test:** Avoid haemolysed or lipaemic samples. **EQA scheme:** NEQAS Sheffield General Autoimmune Serology Scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3313 **See also:** Mitochondrial M2 Antibodies

Test name	Clinical indication and sample information
Mitochondrial M2 Antibodies	**Description:** This is a quantitative assay for Anti-Mitochondrial M2-subtype Antibodies, which are autoantibodies that are strongly associated with Primary Biliary Cholangitis (PBC), present in >90% of patients. **Indications for test:** Investigation of chronic liver disease – strongly associated with Primary Biliary Cholangitis. **Method:** ELISA **Sample type and volume:** Serum (10 mL Red tube) or Plasma (10 mL Green or Purple tube). Preferred volume 2 mL (minimum volume 500 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** 0 – 10 U/mL **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** Once a week. **Minimum request interval (if relevant):** Frequency to be determined by clinical context. **Factors affecting the test:** None stated by the manufacturer. **EQA scheme:** NEQAS Sheffield General Autoimmune Serology Scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3313 **See also:** Mitochondrial Antibodies

Test name	Clinical indication and sample information
MOG Antibodies	**Description:** MOG (Myelin Oligodendrocyte Glycoprotein) is a glycoprotein expressed on the cell surface of the myeline sheath of nerve cells. MOG antibodies are implicated in MOG Antibody-Associated Disease (MOGAD) presenting with neurological symptoms such as optic neuritis, transverse myelitis, encephalitis and may even have similar clinical presentations to Multiple Sclerosis or Neuromyelitis Optica. MOG antibodies are also associated with Acute Disseminated Encephalomyelitis (ADEM). MOG Antibodies and NMO Antibodies are run as a combined test. **Indications for test:** Investigation of patients with optic neuritis, MOGAD or neurological symptoms consistent with a demyelinating disease. **Method:** Indirect Immunofluorescence **Sample type and volume:** Serum (10 mL Red tube), Plasma (10 mL Green or Purple tube) or CSF. Preferred sample volume 2 mL (minimum volume 500 μL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Negative/Positive **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Every two weeks. **Minimum request interval (if relevant):** Not routinely required. **Factors affecting the test:** None stated by the manufacturer. **EQA scheme:** None currently. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3470 Banwell B, Bennett JL, Marignier R, Kim HJ, Brilot F, Flanagan EP, Ramanathan S, Waters P, Tenembaum S, Graves JS, Chitnis T, Brandt AU, Hemingway C, Neuteboom R, Pandit L, Reindl M, Saiz A, Sato DK, Rostasy K, Paul F, Pittock SJ, Fujihara K, Palace J. Diagnosis of myelin oligodendrocyte glycoprotein antibody-associated disease: International MOGAD Panel proposed criteria. Lancet Neurol. 2023 Mar;22(3):268-282. doi: 10.1016/S1474-4422(22)00431-8. Epub 2023 Jan 24. PMID: 36706773. **See also:** NMO Antibodies/Aquaporin-4 Antibodies

Test name	Clinical indication and sample information
Myeloperoxidase (MPO) Antibodies	**Description:** This is a quantification assay (and confirmatory test) for MPO Antibodies in p-ANCA positive serum. Anti-Neutrophil Cytoplasmic Antibodies (ANCA) qualitative assay tests for the presence of antibodies to the constituents of neutrophil granules. Positive ANCA results are shown by immunofluorescence and the pattern of staining. p-ANCA (perinuclear ANCA) denotes a staining pattern around the nucleus of neutrophils and suggests autoantibodies against myeloperoxidase (MPO). Quantification of MPO antibodies is done by EIA (Enzyme Immunoassay) p-ANCA may be positive in conditions including Microscopic Polyangiitis, Granulomatosis with Polyangiitis, Eosinophilic Granulomatosis with Polyangiitis, rapidly progressive glomerulonephritis as well as other autoimmune diseases. **Indications for test:** Investigation of suspected small vessel vasculitis **Method:** Thermo Fisher Immunocap 250 FEIA. **Sample type and volume:** Serum (10 mL Red tube) or Plasma (10 mL Green or Purple tube). Preferred sample volume 2 mL (minimum sample volume 500 μL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** • Negative <3.5 IU/mL. • Equivocal 3.5 – 5.0 IU/mL. • Positive >5.0 IU/mL. **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** Three times a week. **Minimum request interval (if relevant):** On treatment: Six months or more frequent if receiving plasma exchange therapy. Off treatment: Annually. **Factors affecting the test:** Haemolysed or lipaemic samples should not be used. **EQA scheme:** NEQAS Sheffield Anti Neutrophil Cytoplasmic Antibodies Scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3321 https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=2913 **See also**: Anti-neutrophil cytoplasmic antibodies (ANCA), Proteinase 3 (PR3) antibodies

Test name	Clinical indication and sample information
Anti-Neutrophil Cytoplasmic Antibodies (ANCA)	**Description:** ANCA (Anti-Neutrophil Cytoplasmic Antibodies) is a qualitative assay to test for the presence of antibodies to the constituents of neutrophil granules. ANCA positive results are particularly associated with small vessel vasculitides, however, ACNA can be positive in other situations such as other autoimmune disorders, certain infections and cocaine use. As ANCA is non-specific, it should only be performed on patients with a high pre-test probability of small vessel vasculitis to avoid 'false positive' results. p-ANCA (perinuclear ANCA) denotes a staining pattern around the nucleus of neutrophils and suggests autoantibodies against myeloperoxidase (MPO). p-ANCA may be positive in conditions including Microscopic Polyangiitis, Granulomatosis with Polyangiitis, Eosinophilic Granulomatosis with Polyangiitis, rapidly progressive glomerulonephritis as well as other autoimmune diseases. c-ANCA (cytoplasmic or classical ANCA) is staining in the cytoplasm of the nucleus and suggests autoantibodies against proteinase 3 (PR3). c-ANCA may be positive in conditions including Granulomatosis with Polyangiitis and Microscopic Polyangiitis. **Indications for test:** Investigation of suspected small vessel vasculitis **Method:** Indirect Immunofluorescence **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum sample volume 500 μL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Normal result = negative. **Turn-around time**: Up to 4 days **Testing frequency in laboratory:** Twice a week. **Minimum request interval (if relevant):** On treatment: Six months or more frequent if receiving plasma exchange therapy. **Factors affecting the test:** Azide or other preservatives may adversely affect the result. Haemolysed or lipaemic samples should be avoided. **EQA scheme:** NEQAS Sheffield Anti Neutrophil Cytoplasmic Antibodies Scheme. **References or guidelines:** https://www.ouh.nhs.uk/immunology/diagnostic-tests/tests-catalogue/neutrophil-cytoplasmic-antibodies.aspx https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=2913 **See also**: Myeloperoxidase (MPO) antibodies, Proteinase 3 (PR3) antibodies

Test name	Clinical indication and sample information
Neuromyelitis Optica IgG Antibodies / NMO Antibodies / Aquaporin 4 Antibodies	**Description:** Anti-NMO antibodies are associated with Neuromyelitis Optica (NMO), a demyelinating disease characterised by optic neuritis and transverse myelitis. Aquaporin 4 (AQP4) – a protein/channel expressed on certain cell surfaces – has been identified as the major NMO antigen, with high AQP4 expression in the optic nerve and cells in the spinal cord. This test distinguishes NMO from Multiple Sclerosis. NMO and MOG antibodies are run as a combined test. **Indications for test:** Diagnosis of Neuromyelitis Optica (NMO). **Method:** Indirect Immunofluorescence. **Sample type and volume:** Serum (10 mL Red tube), Plasma (10 mL Green or Purple tube) or CSF. Preferred sample volume 2 mL (minimum volume 500 μL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Normal result = negative. **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Every 14 days. **Minimum request interval (if relevant):** Repeat testing guided by clinical context. **Factors affecting the test:** None stated by the manufacturer. **EQA scheme:** None currently. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3329 **See also:** Aquaporin 4 Antibodies

Test name	Clinical indication and sample information
Pancreatic Islet Cell Antibodies	**Description:** This assay detects autoantibodies to pancreatic islet cells that can be present in patients with Type 1 Diabetes Mellitus. Normally antibody levels are present in 90% of patients at the time of diagnosis, but wane over time. **Indications for test:** Diagnosis of Type 1 Diabetes Mellitus. **Method:** Indirect immunofluorescence. **Sample type and volume:** Serum. (10 mL Red tube). Minimum sample volume 500 µL (preferred sample volume 2 mL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Normal result = negative. **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Every 14 days or sooner. **Minimum request interval (if relevant):** Not routinely required. **Factors affecting the test:** The presence of antinuclear antibodies (ANA) or anti-mitochondrial antibodies (AMA) may mask islet cell antibodies. For this reason all suspected positives should be screened on LKS sections as a check. **EQA scheme:** NEQAS Sheffield Diabetic Markers Scheme. **References or guidelines:** https://www.southtees.nhs.uk/services/pathology/tests/anti-islet-cell-antibody/

Test name	Clinical indication and sample information
Paraprotein (Monoclonal protein, M-protein) quantitation	**Description:** Immunoglobulin (IgG, IgA, IgM, IgD and sometimes IgE) levels are measured immunochemically. If there is monoclonal immunoglobulin present (i.e. proliferation of one type of immunoglobulin with only one specificity) then electrophoresis and immunofixation can be used to define both the isotype and predominant free light chain type – this monoclonal protein is also called the M-protein or paraprotein. The presence of a paraprotein can indicate B-cell proliferation including MGUS or Myeloma, which would warrant further investigation and possible referral to Haematology. Importantly, MGUS is common in patients aged >50 years and these patients should be followed up to assess for transformation. **Indications for test:** Investigation and monitoring in patients with lymphoproliferative diseases including MGUS and myeloma and primary amyloidosis. **Method:** Zonal Electrophoresis via capillary and gel. Quantification of M-Protein via densitometry. **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum sample volume 500 µL). Transport at ambient temperature via Royal Mail or courier. **Reference range:** N/A **Turn-around time**: Up to 4 days **Testing frequency in laboratory:** 2-3 times per week **Minimum request interval (if relevant):** **Factors affecting the test:** Incorrect storage of samples. Highly haemolysed or lipaemic samples. **EQA scheme:** Sheffield NEQAS Monoclonal Proteins Scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3249 https://academy.myeloma.org.uk/resources/laboratory-best-practice-tool/ **See Also:** Immunoglobulins and electrophoresis, Bence Jones Protein/Urine Light Chains, Serum Free Light Chains, IgD

Test name	Clinical indication and sample information
Myelin Associated Glycoprotein (MAG) Antibodies	**Description:** Myelin Associated Glycoprotein (MAG) is a glycoprotein component of myelin in the cells (oligodendrocytes and Schwann cells) of the central and peripheral nervous system. Autoantibodies to MAG have been found in sensorimotor neuropathies, including in 50-75% of patients with IgM paraprotein-associated neuropathies. MAG antibodies have also been detected in other neurological conditions such as Multiple Sclerosis and Myasthenia Gravis. This assay is a quantitative assay to determine the presence of MAG antibodies. Any positive samples will be sent to Glasgow for quantitation. **Indications for test:** Investigation of sensorimotor neuropathies. **Method:** ELISA **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum sample volume 500 μL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** <1000 BTU **Turn-around time**: Currently sent to Oxford Immunology, so allow up to 28 days. **Testing frequency in laboratory:** Samples are sent to Oxford every week. **Minimum request interval (if relevant):** Frequency to be determined by clinical context. **Factors affecting the test:** None stated by the manufacturer. **EQA scheme:** NEQAS Sheffield Myelin Associated Glycoprotein IgM antibodies. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3320 **See also:** Ganglioside antibodies

Test name	Clinical indication and sample information
Phospholipase A2 Receptor (PLA2R) antibodies	**Description:** Phospholipase A2 receptor (PLA2R) is a glycoprotein expressed on the surface membrane of cells including podocytes in the kidney. Autoantibodies to PLA2R have been found in certain glomerulonephritides including primary Membranous Glomerulonephritis. **Indications for test:** Investigation and monitoring of suspected membranous glomerulonephritis **Method:** Indirect immunofluorescence **Sample type and volume:** Serum (10 mL Red tube) or Plasma (10 mL Green or Purple tube). Preferred sample volume 2 mL (minimum sample volume 500 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Normal result = negative. **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Twice a week. **Minimum request interval (if relevant):** Frequency to be determined by clinical context. **Factors affecting the test:** None stated by the manufacturer. **EQA scheme:** NEQAS Sheffield Phospholipase A2 Receptor Antibodies. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3362 **See also:** Glomerular Basement Membrane (GBM) Antibodies

Test name	Clinical indication and sample information
Proteinase 3 (PR3) Antibodies	**Description:** This is a quantification assay (and confirmatory test) for PR3 Antibodies in c-ANCA positive serum. Anti-Neutrophil Cytoplasmic Antibodies (ANCA) qualitative assay tests for the presence of antibodies to the constituents of neutrophil granules. Positive ANCA results are shown by immunofluorescence and the pattern of staining. c-ANCA (cytoplasmic or classical ANCA) denotes staining in the cytoplasm of the nucleus and suggests autoantibodies against proteinase 3 (PR3). Quantification of PR3 antibodies is done by EIA (Enzyme Immunoassay) c-ANCA may be positive in conditions including Granulomatosis with Polyangiitis and Microscopic Polyangiitis. **Indications for test:** Investigation of suspected small vessel vasculitis **Method:** Thermo Fisher Immunocap 250 FEIA. **Sample type and volume:** Serum (10 mL Red tube) or Plasma (10 mL Green or Purple tube). Preferred sample volume 2 mL (minimum sample volume 500 μL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** • Negative <2.0 IU/mL. • Equivocal 2.0 – 3.0 IU/mL. • Positive >3.0 IU/mL. **Turn-around time**: Up to 4 days **Testing frequency in laboratory:** Three times a week. **Minimum request interval (if relevant):** On treatment: six months or more frequent if receiving plasma exchange therapy. Off treatment: annually. **Factors affecting the test:** Haemolysed or lipaemic samples should be avoided. **EQA scheme:** NEQAS Sheffield Anti Neutrophil Cytoplasmic Antibodies Scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3421 https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=2913 **See also**: Anti-neutrophil cytoplasmic antibodies (ANCA), Myeloperoxidase (MPO) antibodies

<table>
<tr><th>Test name</th><th>Clinical indication and sample information</th></tr>
<tr><td>Paraneoplastic Neurological Antibodies</td><td>
Description: Paraneoplastic antibodies are autoantibodies targeting various antigens in the nervous system, that arise secondary to malignancy. These antibodies give rise to a plethora of neurological symptoms, termed paraneoplastic neurological syndromes.

Occasionally, patients present with paraneoplastic neurological syndromes before the primary malignancy is diagnosed.

Indications for test: Investigation of suspected paraneoplastic neurological syndromes.
<table>
<tr><th>Antibody</th><th>Neurological disorder</th><th>Commonly associated tumour(s)</th></tr>
<tr><td>Yo (PCA-1)</td><td>Paraneoplastic cerebellar degeneration</td><td>Ovarian, breast cancer</td></tr>
<tr><td>Ma (Ma1)</td><td>Paraneoplastic neurological disorder, brainstem encephalomyelitis</td><td>Various, lung cancer</td></tr>
<tr><td>Ta (Ma2)</td><td>Brainstem encephalomyelitis, limbic encephalomyelitis</td><td>Testicular cancer</td></tr>
<tr><td>Hu (ANNA1)</td><td>Paraneoplastic cerebellar degeneration, paraneoplastic encephalomyelitis, sensory neuropathy</td><td>Small cell lung carcinoma</td></tr>
<tr><td>Ri (ANNA2)</td><td>Opsoclonus/myoclonus, paraneoplastic cerebellar degeneration, brainstem encephalomyelitis</td><td>Breast, small cell lung carcinoma, gynaecological</td></tr>
<tr><td>GAD</td><td>Stiff person syndrome</td><td>Breast, colon, small cell lung carcinoma</td></tr>
<tr><td>CV2/CRMP5</td><td>Paraneoplastic encephalomyelitis/ sensory neuropathy</td><td>Small cell lung carcinoma, thymoma</td></tr>
<tr><td>Amphiphysin</td><td>Stiff person syndrome, paraneoplastic encephalomyelitis</td><td>Breast cancer, small cell lung carcinoma</td></tr>
<tr><td>SOX1</td><td>Lambert-Eaton myasthenic syndrome</td><td>Small cell lung carcinoma</td></tr>
<tr><td>Tr</td><td>Paraneoplastic cerebellar degeneration</td><td>Hodgkin’s lymphoma</td></tr>
<tr><td>Zic4</td><td>Paraneoplastic cerebellar degeneration</td><td>Small cell lung carcinoma</td></tr>
<tr><td>Anti-recoverin antibody (BB4)</td><td>Paraneoplastic retinopathy</td><td>Small cell lung carcinoma</td></tr>
</table>
</td></tr>
</table>

	Method: Indirect Immunofluorescence and confirmation by Western blot **Sample type and volume:** Serum (10 mL Red tube) and CSF. Preferred volume 2 mL (minimum volume 500 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Normal result = negative. **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** Twice a week. **Minimum request interval (if relevant):** Not routinely required. **Factors affecting the test:** Avoid haemolysed or lipaemic samples. **EQA scheme:** NEQAS Sheffield Paraneoplastic Antibodies Scheme. **References or guidelines:** https://www.ouh.nhs.uk/immunology/diagnostic-tests/tests-catalogue/neuronal-antibodies.aspx

Test name	Clinical indication and sample information
Rheumatoid Factor (RF)	**Description:** Rheumatoid Factor is an autoantibody directed against the Fc portion of IgG. It is mostly an IgM antibody, but there are also IgA and IgG forms. RF is non-specific – it is often used as a marker of Rheumatoid Arthritis and comprises part of the diagnostic criteria but can be positive in healthy patients over 75 years of age as well as other conditions such as SLE and Sjogren's syndrome. Alternatively, CCP Antibodies are highly specific for Rheumatoid Arthritis. It is important to note, 20-30% of patients with Rheumatoid Arthritis are not positive for RF, termed seronegative arthritis. **Indications for test:** Investigation in patients with suspected Rheumatoid Arthritis, connective tissue diseases, cryoglobulinaemic. **Method:** Turbidimetry. **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum sample volume 500 μL). Transport at ambient temperature via Royal Mail or courier. **Reference range:** Adult serum 0 – 14 IU/mL **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** Once a week **Minimum request interval (if relevant):** **Factors affecting the test:** Incorrect storage of samples. Highly haemolysed or lipaemic samples. RF is non-specific for Rheumatoid Arthritis (as above) and can be detected in individuals over 75 years. **EQA scheme:** Sheffield NEQAS Autoantibodies Scheme **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3105 **See Also:** CCP antibodies

Test name	Clinical indication and sample information
Serum specific IgE (allergen specific IgE / RAST testing)	**Description:** This assay quantifies IgE that is specific to various allergens. It is performed to screen for allergy (Type I hypersensitivity) to a specific substance. Specific allergens may include various foods, animal fur/dander, pollens and house dust mite. Of note, the specific IgE level does not correlate with the severity of allergic reaction. Alternatively, a positive specific IgE result does not necessarily mean a patient will have allergic symptoms. Interpretation of specific IgE results should be done by an Allergy specialist and interpreted alongside the clinical history. N.B. Total IgE level will be carried out on all samples where specific-IgE has been requested **Indications for test:** Investigation and assessment of allergy **Method:** Thermo Fisher Immunocap 250 FEIA. **Sample type and volume:** Serum (10 mL Red tube) or Plasma (10 mL Green or Purple tube). **Reference range:** Serum: 0 – 0.35 kU/L **Turn-around time**: Up to 7 days if reagents to specific allergens are in stock **Testing frequency in laboratory:** Three times a week. **Minimum request interval (if relevant):** Not routinely required. **Factors affecting the test:** None stated by the manufacturer. **EQA scheme:** NEQAS Sheffield Specific IgE Scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=2880 **See also:** Total IgE

Test Name	Clinical indication and sample information
Serum Free Light Chains (Serum FLC)	**Description:** Normal plasma cells produce more immunoglobulin light chains than heavy chains. Light chains are made of kappa or lambda molecules. Excess light chains (called free light chains (FLC) are secreted into the serum and cleared by the kidney in urine. Therefore, the serum concentration of FLC depends on both the amount produced and renal clearance. The relative concentration of kappa and lambda molecules should remain constant (kappa/lambda ratio). If there is increased polyclonal immunoglobulin production and/or renal impairment, the FLC concentrations can increase but the ratio is relatively unchanged. If there is a monoclonal immunoglobulin present, then there will be a skewed ratio of FLC – increased production of one light chain, often with bone marrow suppression of the other. Therefore, increased serum FLC (and thus increased urine light chains) can indicate and monitor monoclonal conditions such as MGUS, myeloma or amyloid. **Indications for test:** Investigation and monitoring in patients with lymphoproliferative diseases, plasma cell dyscrasias, MGUS, myeloma, primary amyloidosis. **Method:** Turbidimetry. **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum sample volume 500 μL). Transport at ambient temperature via Royal Mail or courier. **Reference range:** Reference ranges are age specific and may differ between ethnic groups.

Analyte	Normal Range (mg/L)
Kappa	3.30 – 19.40
Lambda	5.71 – 26.30
Kappa:Lambda Ratio	0.26 – 1.65

Turn-around time: Up to 4 days

Testing frequency in laboratory: Daily

Minimum request interval (if relevant):

Factors affecting the test:

Incorrect storage of samples. Highly haemolysed or lipaemic samples.

Assay may be inaccurate at FLC levels <0.9mg/L.

	False negative results can occur as a result of 'antigen excess' in patients with high SFLC levels. Skewing of light chain ratio can occur transiently with severe infection. Renal impairment can result in a rise in FLC but usually with normal ratio. **EQA scheme:** Sheffield NEQAS Monoclonal proteins scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3162 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4846284/ https://academy.myeloma.org.uk/resources/laboratory-best-practice-tool/ **See Also:** Immunoglobulins, Bence Jones Protein/Urine Light Chains

Test name	Clinical indication and sample information
Skin Antibodies	**Description:** Antibodies that are targeted against proteins expressed in the skin are responsible for blistering autoimmune skin diseases. Pemphigus vulgaris is an autoimmune blistering skin disease due to antibodies targeting desmoglein (Dsg1 and Dsg3) – a transmembrane protein on desmosomes (structures between keratinocytes). Antibodies to desmoglein may also be detected in severe burns or Trichophyton infection. Bullous pemphigoid is a blistering skin disease due to antibodies to target antigens on the basement membrane - BP180 is a transmembrane protein and BP230 a cytoplasmic protein. **Indications for test:** Investigation of suspected autoimmune skin disease **Method:** Indirect Immunofluorescence. **Sample type and volume:** Serum (10 mL Red tube). Preferred sample volume 2 mL (minimum sample volume 500 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Normal result = negative. **Turn-around time**: Up to 14 days **Testing frequency in laboratory:** Every 14 days or sooner. **Minimum request interval (if relevant):** On treatment: Every six months. Off treatment: Annually. **Factors affecting the test:** Avoid using haemolysed or lipaemic serum. **EQA scheme:** NEQAS Sheffield Bullous Dermatosis Scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3182

Test name	Clinical indication and sample information
Smooth muscle antibodies	**Description:** Smooth muscle antibodies (SMA) may be detected in patients with autoimmune hepatitis as well as Hepatitis B infection. Up to 70% of patients with autoimmune hepatitis may have SMA. Patients with autoimmune hepatitis may also be positive for ANA, dsDNA, mitochondrial and LKM antibodies. **Indications for test:** Investigation of possible autoimmune hepatitis **Method:** Indirect Immunofluorescence **Sample type and volume:** Serum (Red tube). Preferred sample volume 2 mL (minimum sample volume 500 µL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Normal result = negative. **Turn-around time**: Up to 4 days **Testing frequency in laboratory:** Twice a week **Minimum request interval (if relevant):** Frequency determined by clinical context. **Factors affecting the test:** Avoid haemolysed or lipaemic samples. **EQA scheme:** NEQAS Sheffield General Autoimmune Serology Scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3292 **See also:** ANA, dsDNA, Mitochondrial antibodies, LKM antibodies

Test name	Clinical indication and sample information
TPO Antibodies (Thyroid Peroxidase)	**Description:** Thyroid Peroxidase (TPO) is a protein present on the surface of thyroid follicular cells. Autoantibodies to TPO are seen in autoimmune thyroid conditions, including Hashimoto's thyroiditis (95% of patients with Hashimoto's have anti-TPO antibodies) and Grave's disease which causes hyperthyroidism (20% of patients with Grave's disease have anti-TPO antibodies). Patients with subclinical hypothyroidism may have anti-TPO antibodies and may go on to develop overt hypothyroidism. TPO antibodies should be checked prior to commencing on Amiodarone and appropriately monitored afterwards. **Indications for test:** Investigation of hypo- or hyperthyroidism **Method:** Thermo Fisher Immunocap 250 FEIA. **Sample type and volume:** Serum (10 mL Red tube) or Plasma (10 mL Green or Purple tube). Minimum sample volume 500 µL (preferred sample volume 2 mL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** • Negative <25 IU/mL. • Equivocal 25-35 IU/mL. • Positive >35 IU/mL. **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** Three times a week. **Minimum request interval (if relevant):** Not routinely required. **Factors affecting the test:** Haemolysed or lipaemic samples should be avoided. **EQA scheme:** NEQAS Sheffield General Autoimmune Serology Scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3472 **See also:** TSH Receptor Antibodies

Test name	Clinical indication and sample information
TSH Receptor Antibodies	**Description:** Thyroid Stimulating Hormone (TSH) stimulates the thyroid to produce Thyroxine (T3 and T4). It acts through binds to the receptor on thyroid cells – the TSH Receptor – which is a membrane glycoprotein. Autoantibodies to the TSH-Receptor therefore disrupt signalling in the thyroid, leading to autoimmune hyperthyroidism (Grave's disease). Pregnant women with Grave's disease or those previously treated for Grave's, are at risk of having a child with neonatal hypothyroidism which may need monitoring. **Indications for test:** Investigation of hyperthyroidism **Method:** This assay is currently sent to Immunology PRU, Northern General Hospital, Sheffield for testing **Sample type and volume:** Serum. (10 mL Red tube) or Plasma (10 mL Green or Purple tube). Minimum sample volume 500 µL (preferred sample volume 2 mL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** Normal result = negative **Turn-around time**: Up to 28 days **Testing frequency in laboratory:** Samples are sent to Sheffield every week. **Minimum request interval (if relevant):** **Factors affecting the test:** Haemolysed or lipaemic samples should not be used. **EQA scheme:** NEQAS Sheffield General Autoimmune Serology Scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3473 **See also:** TPO Antibodies (Thyroid Peroxidase)

Test name	Clinical indication and sample information
Tissue Transglutaminase (TTG) Antibodies	**Description:** Tissue transglutaminase (TTG) is an enzyme that is a major autoantigen in Coeliac disease. TTG antibodies can be either IgA or IgG. This assay performs anti-TTG IgA initially as a screening test. If the result is low, then the patient may have IgA deficiency. Therefore, we proceed to testing Gliadin antibodies (IgG Anti-Gliadin; which are more sensitive than anti-TTG IgG) and add Immunoglobulins to check for IgA deficiency. N.B. IgA deficiency is common – found in about 1:400 healthy blood donors and 1:40 patients with Coeliac. **Indications for test:** Investigation of suspected Coeliac disease and dermatitis herpetiformis. **Method:** Thermo Fisher Immunocap 250 FEIA **Sample type and volume:** Serum. (10 mL Red tube) or Plasma (10 mL Green or Purple tube). Minimum sample volume 500 µL (preferred sample volume 2 mL). Transport at ambient temperature via Royal Mail or Courier. **Reference range:** • Negative 0 – 7 units/mL • Equivocal 7 – 10 units/mL • Positive >10 units/mL **Turn-around time**: Up to 7 days **Testing frequency in laboratory:** Three times a week. **Minimum request interval (if relevant):** Every 6-12 months to monitor positive patients. **Factors affecting the test:** Haemolysed or lipaemic samples should not be used. **EQA scheme:** NEQAS Sheffield Coeliac Antibodies Scheme. **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=3364 **See also:** Gliadin antibodies, Endomysial antibodies IgA, Immunoglobulins

Test name	Clinical indication and sample information
Bence Jones Protein (BJP) or Urine Light Chains	**Description:** Normal plasma cells produce more immunoglobulin light chains than heavy chains. Light chains are made of kappa or lambda molecules. Excess light chains (called free light chains) are secreted into the serum and cleared by the kidney in urine. If there is increased monoclonal immunoglobulin, such as in conditions like MGUS or myeloma, there are increased free light chains that can be detected in urine – named urine light chains or Bence Jones protein (BJP). Immunofixation is used for the detection of the type of light chain in urine. **Indications for test:** Investigation of patients with MGUS, myeloma. **Method:** Immunofixation **Sample type and volume:** Urine (Universal container). Volume up to 20 mL, ideally early morning sample. **Reference range:** Normal result = no bands seen **Turn-around time**: Up to 5 days **Testing frequency in laboratory:** 2-3 times per week **Minimum request interval (if relevant):** **Factors affecting the test:** **EQA scheme:** Sheffield NEQAS Monoclonal Paraprotein Scheme **References or guidelines:** https://sheffieldlaboratorymedicine.nhs.uk/search-test.php?search=2949 **See Also:** Immunoglobulins and electrophoresis, Serum Free Light Chains

Cellular Immunophenotyping (haematological malignancy and immunodeficiency)

Urgent requests

All queries and requests for urgent investigations please contact the laboratory (0121 414 4069) and ask to speak to the Clinical Scientist or Senior BMS.

Urgent samples (which have been arranged and agreed with the lab in advance via telephone) will be processed and reported on the day of receipt (Monday to Friday) provided they reach the lab before 2.00pm. Results will be telephoned to the requesting clinician – please provide a mobile, or direct landline at the time of requesting. Turnaround time data for urgent requests will be available on request.

Immunodeficiency studies: please telephone for clinical discussion and advice regarding appropriate tests and samples required (0121 414 4069)

Specimen collection general information

All samples must arrive in the laboratory by 5.00 p.m. on the day of sampling accompanied by clinical details. Samples received on working day 1 will normally be processed working day 2 and reported working day 3 (except samples received on a Friday). At present there is no weekend or Bank Holiday service.

Specimen requirements

The following are optimal sample volumes: The minimum sample volume is individually described for each panel.

Bone marrow	* 4 mL bone marrow in **EDTA** and 2 unfixed marrow smear slides. Results from haemodilute bone marrow samples will be unreliable as not representative
Blood	5 mLs blood in **EDTA**
Effusions	At least 20 mLs in **EDTA**
C.S.F.	As much as possible in a universal bottle ideally containing tissue culture medium. Please discuss with the laboratory if only small volume as quality of testing is reduced.

Immunoglobulin/T cell receptor gene studies

Description and indication for test: Immunoglobulin heavy chain and T cell receptor gene studies are used to assess for clonality in blood or bone marrow samples.

Method: This assay is carried out by the University Hospitals Southampton (UHS) genetics laboratory and reported by the Clinical Immunology Service in conjunction with immunophenotyping results. The result will be sent directly to requestor from UHS. The result will also be sent to the CIS for integration into MIRHO reporting.

Sample type and volume: Blood or bone marrow drawn into an **EDTA** bottle (Heparinised material is unsuitable for the PCR process).

Turn-around time: 21 days

Test Name

Lymphoproliferative Disease (LPD) Screen

Haematological Malignancies Immunophenotyping

Clinical indication and sample information

Description: Flow cytometry panel including the following markers. A reduced panel is applied for CSF samples.

Bone Marrow / Peripheral Blood / Effusions: Kappa, Lambda, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11c, CD16, CD19, CD20, CD23, CD25, CD27, CD30, CD34, CD38, CD45, CD49d, CD56, CD79b, CD103, CD200, TCR-gamma/delta.

CSF: Kappa, Lambda, CD2, CD3, CD4, CD5, CD8, CD7, CD19, CD20, CD23, CD45, CD200, TCR-gamma/delta.

Indications for test: Appropriate for the investigation of unexplained lymphocytosis, mature B cell neoplasms and mature T cell neoplasms.

Method: Flow Cytometry

Sample type and volume: Transport at ambient temperature via Royal Mail or Courier.

Bone marrow	4 ml bone marrow in EDTA and 2 unfixed marrow smear slides
Blood	4 ml blood in EDTA
Effusions	At least 20 ml in EDTA or Universal bottle
C.S.F.	As much as possible in a universal bottle ideally containing tissue culture medium. Please discuss with the laboratory if only small volume as quality of testing is reduced.

Reference range: All include morphological appraisal and a written report with clinical interpretation.

Turn-around time: Within 3 working days (Mon-Fri). Most samples are processed and reported by the day after receipt. ***For urgent samples please contact the laboratory and ensure arrival before 2pm for same day processing.***

Testing frequency in laboratory: Daily (Mon-Fri)

Minimum request interval (if relevant): N/A

Factors affecting the test: Results from haemodilute bone marrow samples may be unreliable as not representative. Serous fluid, bone marrow or CSF contaminated with peripheral blood may yield non-representative results. Monoclonal biological treatments can affect the staining of certain markers. Failure to communicate the treatment on the request form will potentially lead to a misinterpretation of the staining profile.

	EQA scheme: UKNEQAS LI Immunophenotyping Scheme **References or guidelines:** WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

Myeloma Panel

Description: Flow cytometry panel including the following markers. A reduced panel is applied for CSF samples.

Cytoplasmic and surface Kappa and Lambda, CD3, CD5, CD10, CD11c, CD19, CD20, CD23, CD25, CD27, CD34, CD38, CD45, CD49d, CD56, CD79b, CD103, CD117, CD138, CD200

Indications for test: Appropriate for the investigation of known or suspected cases of myeloma, MGUS, lymphoplasmacytoid lymphoma and amyloid.

Method: Flow Cytometry

Sample type and volume: Transport at ambient temperature via Royal Mail or Courier.

Blood (for ?plasma cell leukaemia only)	4 ml blood in EDTA
Bone marrow	4 ml bone marrow in EDTA and 2 unfixed marrow smear slides
C.S.F.	As much as possible in a universal bottle ideally containing tissue culture medium. Please discuss with the laboratory if only small volume as quality of testing is reduced.

Reference range: All include morphological appraisal and a written report with clinical interpretation.

Turn-around time: Within 3 working days (Mon-Fri). Most samples are processed and reported by the day after receipt. ***For urgent samples please contact the laboratory and ensure arrival before 2pm for same day processing.***

Testing frequency in laboratory: Daily (Mon-Fri)

Minimum request interval (if relevant): N/A

Factors affecting the test: Results from haemodilute bone marrow samples may be unreliable as not representative. Bone marrow or CSF contaminated with peripheral blood may yield non-representative results. Monoclonal biological treatments can affect the staining of certain markers. Failure to communicate the treatment on the request form will potentially lead to a misinterpretation of the staining profile.

EQA scheme: UKNEQAS LI Immunophenotyping Scheme

References or guidelines: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

Myeloid Panel

Description: Flow cytometry panel including the following markers. A reduced panel is applied for CSF samples.

Kappa, Lambda, CD3, CD4, CD7, CD11b, CD13, CD14, CD16, CD19, CD33, CD34, CD45, CD56, CD71, CD117, CD123, CD235a, HLA-DR.

(Additional markers CD38, CD123, CD45RA, CLL-1 may be included)

Indications for test: Appropriate for ?MDS, ?MPD

Method: Flow Cytometry

Sample type and volume: Transport at ambient temperature via Royal Mail or Courier.

Bone marrow	4 mL bone marrow in EDTA and 2 unfixed marrow smear slides
Blood	4 mLs blood in EDTA
Effusions	At least 20 mLs in EDTA or Universal bottle
C.S.F.	As much as possible in a universal bottle ideally containing tissue culture medium. Please discuss with the laboratory if only small volume as quality of testing is reduced.

Reference range: All include morphological appraisal and a written report with clinical interpretation.

Turn-around time: Within 3 working days (Mon-Fri). Most samples are processed and reported by the day after receipt. ***For urgent samples please contact the laboratory and ensure arrival before 2pm for same day processing.***

Testing frequency in laboratory: Daily (Mon-Fri)

Minimum request interval (if relevant): N/A

Factors affecting the test: Results from haemodilute bone marrow samples may be unreliable as not representative. Serous fluid, bone marrow or CSF contaminated with peripheral blood may yield non-representative results. Monoclonal biological treatments can affect the staining of certain markers. Failure to communicate the treatment on the request form will potentially lead to a misinterpretation of the staining profile.

EQA scheme: UKNEQAS LI Immunophenotyping Scheme

References or guidelines: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

<table>
<tr>
<td>Acute Leukaemia Screen</td>
<td>
Description: Flow cytometry panel including the following markers.
Surface markers: Kappa, Lambda, CD3, CD4, CD7, CD13, CD19, CD33, CD34, CD45, CD117, HLA-DR

Cytoplasmic/ Intracellular markers: Kappa, Lambda, MPO, TdT, CD3, CD22, CD79a, CD38

Indications for test: This panel may be used for the diagnosis of a possible acute leukaemia including lineage determination. It can be processed to provide an urgent, telephoned report to the requesting clinician. The information derived will be used to select a more appropriate secondary panel / additional markers if appropriate.

Method: Flow Cytometry

Sample type and volume: Transport at ambient temperature via Royal Mail or Courier.

<table>
<tr><td>Bone marrow</td><td>4 mL bone marrow in EDTA and 2 unfixed marrow smear slides</td></tr>
<tr><td>Blood</td><td>4 mLs blood in EDTA</td></tr>
</table>

Reference range: All include morphological appraisal and a written report with clinical interpretation.

Turn-around time: Within 3 working days (Mon-Fri). Most samples are processed and reported by the day after receipt. For urgent samples please contact the laboratory and ensure arrival before 2pm for same day processing.

Testing frequency in laboratory: Daily (Mon-Fri)

Minimum request interval (if relevant): N/A

Factors affecting the test: Results from haemodilute or peripheral blood contaminated bone marrow samples may be unreliable as not representative. Monoclonal biological treatments can affect the staining of certain markers. Failure to communicate the treatment on the request form will potentially lead to a misinterpretation of the staining profile.

EQA scheme: UKNEQAS LI Immunophenotyping Scheme

References or guidelines: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues
</td>
</tr>
</table>

Acute Myeloid Leukaemia/ AML

Description: Flow cytometry panel including the following markers. A reduced panel is applied for CSF samples.

CD7, CD11b, CD13, CD14, CD19, CD33, CD34, CD38, CD45, CD56, CD117, HLA-DR.

The cytoplasmic/intracellular panel, and/or additional markers (CD38, CD123 and CD45RA) may also be included.

If AML M3 (APML) is suspected, a fixed cytospin may be stained for PML protein.

Indications for test: Appropriate for diagnosis or follow up AML patients

Method: Flow Cytometry

Sample type and volume: Transport at ambient temperature via Royal Mail or Courier.

Bone marrow	4 ml bone marrow in EDTA and 2 unfixed marrow smear slides
Blood	4 ml blood in EDTA
C.S.F.	As much as possible in a universal bottle ideally containing tissue culture medium. Please discuss with the laboratory if only small volume as quality of testing is reduced.

Reference range: All include morphological appraisal and a written report with clinical interpretation.

Turn-around time: Within 3 working days (Mon-Fri). Most samples are processed and reported by the day after receipt. ***For urgent samples please contact the laboratory and ensure arrival before 2pm for same day processing.***

Testing frequency in laboratory: Daily (Mon-Fri)

Minimum request interval (if relevant): N/A

Factors affecting the test: Results from haemodilute bone marrow samples may be unreliable as not representative. Bone marrow or CSF contaminated with peripheral blood may yield non-representative results. Monoclonal biological treatments can affect the staining of certain markers. Failure to communicate the treatment on the request form will potentially lead to a misinterpretation of the staining profile.

EQA scheme: UKNEQAS LI AML Scheme

References or guidelines: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

<table>
<tr>
<td>BALL panel</td>
<td>
Description: Flow cytometry panel including the following markers. A reduced panel is applied for CSF samples.

CD10, CD15, CD19, CD20, CD22, CD34, CD38, CD45, CD58, NG2

Indications for test: Appropriate for the diagnosis and follow-up of precursor B lineage neoplasms.

Method: Flow Cytometry

Sample type and volume: Transport at ambient temperature via Royal Mail or Courier.

<table>
<tr><td>Bone marrow</td><td>4 ml bone marrow in EDTA and 2 unfixed marrow smear slides</td></tr>
<tr><td>Blood</td><td>4 ml blood in EDTA</td></tr>
<tr><td>C.S.F.</td><td>As much as possible in a universal bottle ideally containing tissue culture medium. Please discuss with the laboratory if only small volume as quality of testing is reduced.</td></tr>
</table>
Reference range: All include morphological appraisal and a written report with clinical interpretation.

Turn-around time: Within 3 working days (Mon-Fri). Most samples are processed and reported by the day after receipt. For urgent samples please contact the laboratory and ensure arrival before 2pm for same day processing.

Testing frequency in laboratory: Daily (Mon-Fri)

Minimum request interval (if relevant): N/A

Factors affecting the test: Results from haemodilute bone marrow samples may be unreliable as not representative. Bone marrow or CSF contaminated with peripheral blood may yield non-representative results. Monoclonal biological treatments can affect the staining of certain markers. Failure to communicate the treatment on the request form will potentially lead to a misinterpretation of the staining profile.

EQA scheme: UKNEQAS LI BALL Scheme

References or guidelines: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues
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<tr>
<td>TALL Panel</td>
<td>
Description: Flow cytometry panel including the following markers. A reduced panel is applied for CSF samples.

CD1a, CD2, cytoCD3, surface CD3, CD4, CD5, CD7, CD8, CD10, CD13, CD33, CD45, CD56, CD99, CD117, TCRab, cyto TCRb, TDT.

Indications for test: Appropriate for the diagnosis and follow-up of precursor T lineage neoplasms

Method: Flow Cytometry

Sample type and volume: Transport at ambient temperature via Royal Mail or Courier.

<table>
<tr><td>Bone marrow</td><td>4 mL bone marrow in EDTA and 2 unfixed marrow smear slides</td></tr>
<tr><td>Blood</td><td>4 ml blood in EDTA</td></tr>
<tr><td>C.S.F.</td><td>As much as possible in a universal bottle ideally containing tissue culture medium. Please discuss with the laboratory if only small volume as quality of testing is reduced.</td></tr>
</table>
Reference range: All include morphological appraisal and a written report with clinical interpretation.

Turn-around time: Within 3 working days (Mon-Fri). Most samples are processed and reported by the day after receipt. For urgent samples please contact the laboratory and ensure arrival before 2pm for same day processing.

Testing frequency in laboratory: Daily (Mon-Fri)

Minimum request interval (if relevant): N/A

Factors affecting the test: Results from haemodilute bone marrow samples may be unreliable as not representative. Bone marrow or CSF contaminated with peripheral blood may yield non-representative results. Monoclonal biological treatments can affect the staining of certain markers. Failure to communicate the treatment on the request form will potentially lead to a misinterpretation of the staining profile.

EQA scheme: UKNEQAS LI Immunophenotyping Scheme

References or guidelines: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues
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</table>

<table>
<tr>
<td>Paroxysmal nocturnal Haemoglobinuria / PNH screen</td>
<td>
Description: Flow cytometry panel including the following markers.

WBC tube: CD15, CD24, FLAER on neutrophils CD64, CD14, FLAER on monocytes

RBC tube: CD59, CD235a on red blood cells (only added on if a known patient or a positive WBC tube result).

Indications for test: Appropriate for the investigation of suspected or known PNH cases.
Note: the PNH assay is a routine screening assay and not a high-sensitivity assay

Method: Flow Cytometry

Sample type and volume: Transport at ambient temperature via Royal Mail or Courier.

<table>
<tr><td>Blood</td><td>4 ml blood in EDTA. Requires fresh blood received within 48 hours of collection.</td></tr>
</table>

Reference range: All include morphological appraisal and a written report with clinical interpretation.

Turn-around time: Within 3 working days (Mon-Fri). Most samples are processed and reported by the day after receipt. For urgent samples please contact the laboratory and ensure arrival before 2pm for same day processing.

Testing frequency in laboratory: Daily (Mon-Fri)

Minimum request interval (if relevant): N/A

Factors affecting the test: Results on transfusion dependant patients will give a diluted RBC PNH clone. Grossly haemolysed samples will only be process for WBC and not RBC. Presence of undetected cold haemagglutinins or microclots may cause heterogeneous staining of cells. Clotted samples will not be processed.

EQA scheme: UKNEQAS LI PNH Scheme

References or guidelines: Cytometry B, Clinical Cytometry, 2010. Borowitz et al.; Cytometry B Clin Cytom. 2012. Sutherland et al.
</td>
</tr>
</table>

Test Name **T cell subset markers**	**Immunodeficiency Immunophenotyping** **Clinical indication and sample information** **Description:** Absolute and percentage values for the following markers CD45 (lymph), CD3, CD4, CD8 **Indications for test:** T cell (CD4) counts in known or suspected HIV+ patients. **Method:** Flow Cytometry **Sample type and volume:** Transport at ambient temperature via Royal Mail or Courier. Blood: 1 ml blood in EDTA **Reference range:** Included in report. Immunophenotyping of blood lymphocytes in childhood Reference values for lymphocyte subpopulations. Comans-Bitter, The Journal of Pediatrics: Volume 130, Issue 3, March 1997, Pages 388–393. **Turn-around time**: Within 3 working days (Mon-Fri). Most samples are processed and reported by the day after receipt. ***For urgent samples please contact the laboratory and ensure arrival before 2pm for same day processing.*** **Testing frequency in laboratory:** 2 times per week. **Minimum request interval (if relevant):** N/A **Factors affecting the test:** Samples must NOT have been refrigerated or centrifuged. Ideally <3 days post collection; samples >5 days will have a notification that results may not be reliable and repeat may be required. Anticoagulants other than EDTA have not been validated and will be rejected. Clots will give unrepresentative results, samples with apparent clots will be rejected. **EQA scheme:** UKNEQAS LI Immune Monitoring Scheme **References or guidelines:** British HIV Association guidelines for the routine investigation and monitoring of adult HIV-1-positive individuals 2016

<table>
<tr>
<td>Lymphocyte subset markers / TBNK</td>
<td>
Description: Absolute and percentage values for the following markers

CD45 (lymph), CD3, CD4, CD8, CD19, CD16, CD56

Indications for test: Investigation of cellular immunodeficiency, recurrence of B cells post rituximab.

Method: Flow Cytometry

Sample type and volume: Transport at ambient temperature via Royal Mail or Courier.

<table>
<tr><td>Blood</td><td>1 ml blood in EDTA</td></tr>
</table>

Reference range: Included in report. Immunophenotyping of blood lymphocytes in childhood Reference values for lymphocyte subpopulations. Comans-Bitter, The Journal of Pediatrics: Volume 130, Issue 3, March 1997, Pages 388–393.

Turn-around time: Within 3 working days (Mon-Fri). Most samples are processed and reported by the day after receipt. For urgent samples please contact the laboratory and ensure arrival before 2pm for same day processing.

Testing frequency in laboratory: 2 times per week.

Minimum request interval (if relevant): N/A

Factors affecting the test: Samples must NOT have been refrigerated or centrifuged. Ideally <3 days post collection; samples >5 days will have a notification that results may not be reliable and repeat may be required. Anticoagulants other than EDTA have not been validated and will be rejected. Clots will give unrepresentative results, samples with apparent clots will be rejected.

EQA scheme: UKNEQAS LI Immune Monitoring Scheme

References or guidelines: ESID Registry – Working Definitions for Clinical Diagnosis of PID
</td>
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</table>

<table>
<tr>
<td>B cell immunophenotyping, based on the EUROClass panel</td>
<td>
Description: Flow cytometry panel based on the EUROClass panel for B cell subsets with the following markers.

CD19, CD20, CD21, CD27, CD38, CD45, IgD, IgM.

An accompanying Lymphocyte subsets/TBNK result will be provide.

Indications for test: Assessment of immunodeficiency and immune competence in patients where B cell function is compromised. Suspected primary or secondary immunodeficency. Assessing B cell subset reconstitution after stem cell or bone marrow transplant. Assessing impact of B cell depleting immunotherapy.

Method: Flow Cytometry

Sample type and volume: Transport at ambient temperature via Royal Mail or Courier.

<table>
<tr><td>Blood</td><td>4 ml blood in EDTA</td></tr>
</table>

Reference range: A written report with clinical interpretation is provided. Reference values for B cell subpopulations from infancy to adulthood. Morbach et al. Clin Exp Immunol. 2010 Nov; 162(2): 271–279.

Turn-around time: Within 3 working days (Mon-Fri). Most samples are processed and reported by the day after receipt. For urgent samples please contact the laboratory and ensure arrival before 2pm for same day processing.

Testing frequency in laboratory: When received (Mon-Fri).

Minimum request interval (if relevant): N/A

Factors affecting the test: Clots will give unrepresentative results, samples with apparent clots will be rejected. Aged samples may give non-specific antibody binding and altered antigen expression. Monoclonal biological treatments can affect the staining of certain markers. Failure to communicate the treatment on the request form will potentially lead to a misinterpretation of the staining profile.

EQA scheme: Sample exchange with BCPS.

References or guidelines: Wehr et al Blood. 2008 Jan 1;111(1):77-85
</td>
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</table>

<table>
<tr>
<td>Autoimmune Lymphoproliferative disease / ALPS screening Panel</td>
<td>
Description: Flow cytometry panel to assess alpha beta double negative T cells with the following markers

CD3, CD4, CD8, CD45RA, TCR alpha/beta.

An accompanying T cell subset enumeration result will be provided.

Indications for test: Clinically ALPS is suspected. Expanded double negative T cell population identified on T cell subsets or other flow cytometry panel.

Method: Flow Cytometry

Sample type and volume: Transport at ambient temperature via Royal Mail or Courier.

<table>
<tr><td>Blood</td><td>4 ml blood in EDTA</td></tr>
</table>

Reference range: A written report with clinical interpretation is provided.

Turn-around time: Within 3 working days (Mon-Fri). Most samples are processed and reported by the day after receipt. For urgent samples please contact the laboratory and ensure arrival before 2pm for same day processing.

Testing frequency in laboratory: When received (Mon-Fri).

Minimum request interval (if relevant): N/A

Factors affecting the test: Clots will give unrepresentative results, samples with apparent clots will be rejected. Aged samples may give non-specific antibody binding and altered antigen expression. Monoclonal biological treatments can affect the staining of certain markers. Failure to communicate the treatment on the request form will potentially lead to a misinterpretation of the staining profile.

EQA scheme: Sample exchange with NBT.

References or guidelines: ESID Registry – Working Definitions for Clinical Diagnosis of PID: ALPS
</td>
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</table>

3.2 Tests not on UKAS scope

We also offer a number of tests that are not currently on UKAS scope but these tests have been verified for use in our laboratory. Where we issue a report on such a test we clearly state this on the report. If you require additional tests and verification of a test for a clinical study or trial, please contact the department via e-mail at Clin.imm@contacts.bham.ac.uk

Test name:	***Description and clinical relevance***
T memory cell immunophenotyping	Determining proportions of naïve, central memory and effector memory T cells in peripheral blood.
	Method
	Flow cytometry
	Application
	Supports the diagnosis of certain immunodeficiencies
	Sample type
	Peripheral blood (EDTA)

Test name:	***Description and clinical relevance***
CAR-T cell enumeration	Determining the proportion of anti-CD19 CAR-T cells in peripheral blood
	Method
	Flow cytometry
	Application
	Clinical applications for this test remain under evaluation
	Sample type
	Peripheral blood (EDTA)

Test name:	***Description and clinical relevance***
AML MRD by spectral cytometry	Assess measurable residual disease of AML patients.
	Method
	Spectral Cytometry
	Application
	High sensitivity monitoring of patients with AML.
	Sample type:
	Bone Marrow (EDTA) or Peripheral Blood (EDTA)

Test name:	**Description and clinical relevance**
CAR-T cell memory phenotyping	Determining the naïve, central memory and effector memory state of anti-CD19 CAR-T cells in peripheral blood
	Method Flow cytometry
	Application Clinical applications for this test remain under evaluation
	Sample type Peripheral blood (EDTA)

Test name:	**Description and clinical relevance**
High sensitivity IL-6	Interleukin 6 (IL-6) is an interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine. It is the principal driver of CRP production in humans.
	Method High sensitivity ELISA
	Application Quantification of low levels of serum IL-6 as a biomarker of other disease processes or therapies.
	Sample type Serum (SST)

Test name:	***Description and clinical relevance***
SARS-CoV-2 Serology	Measurement of anti-spike and anti-nucleocapsid IgG, IgA, IgM antibodies directed against ancestral and variants of SARS-CoV-2
	Method ELISA
	Application Assessment of humoral immunity to SARS-CoV-2 following infection or vaccination.
	Sample type Serum (SST)
Test name:	***Description and clinical relevance*** This assay is used to conjugate antibodies to metals, which allow protein detection and quantification by mass cytometry.

Mass cytometry antibody conjugations	***Method*** Standard BioTools protocol selected according to the metal of interest. Capacity to test the antibody by mass cytometry if required.
	Application In-depth phenotypic profiling of cell populations
	Sample type Unconjugated antibody and metal kit of interest (can be purchased upon request)

Test name: **Functional antibodies (specific microbial antibodies)**	***Description and clinical relevance*** This assay quantifies the antibody level to specific antigens that patients may have been vaccinated. The antigens tested are Pneumococcal antigens (Pn serotypes: 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 14, 18C, 19A, 19F, 23F), Meningococcus C (Men C), Tetanus, Haemophilus influenza B (Hib). Investigation of these functional antibodies is useful in the assessment of patients where immunodeficiency may be suspected (for example if they have had recurrent bacterial sepsis, invasive bacterial disease). This assay can also be useful in assessing immune reconstitution in patients following bone-marrow transplant or asplenic patients.
	Method Detection of antigen-specific antibodies using the Intelliflex or Luminex 200 machines.
	Application Assessment of humoral immunity to pneumococcal, meningococcal, tetanus, and Hib following infection or vaccination.
	Sample type Serum (SST)

Test name: **CMV-specific IgG**	***Description and clinical relevance*** This assay quantifies the IgG antibody level to human cytomegalovirus (CMV) to support the diagnosis of the infection with CMV.
	Method Quantitative Indirect ELISA detection of CMV-specific IgG antibodies using the Euroimmun Anti-CMV IgG ELISA.
	Application Investigation of prior CMV infection
	Sample type Serum (SST)

Test name:	*Description and clinical relevance*
Measles-specific IgG	This assay quantifies the IgG antibody level to human measles virus to determine immune status.
	Method Quantitative Indirect ELISA detection of Measles-specific IgG antibodies using the Euroimmun Anti-Measles Virus IgG ELISA.
	Application Investigation of prior measles infection or immune response to measles vaccination.
	Sample type Serum (SST)

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Test name:	*Description and clinical relevance*
Cytokines	This assay quantifies serum cytokine (IFN-γ, IL-1β/IL-1F2, IL-2, IL-4, IL-6, IL-8/CXCL8, IL-10, TNF-α) levels to determine immune status. Other cytokines available on request
	Method Quantitative xMAP INTELLIFLEX® DR-SE detection of IFN-gamma, IL-1beta, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-alpha cytokines using the Milliplex Human Cytokine Magnetic Bead Panel.
	Application To monitor the expression levels of cytokines in serum (IFN-γ, IL-1β/IL-1F2, IL-2, IL-4, IL-6, IL-8/CXCL8, IL-10, TNF-α) following treatment with ALETA-001 as part of the ALETA-001 trial.
	Sample type Serum (SST)

Test name:	*Description and clinical relevance*
Sezary Flow cytometry panel	This assay assesses T cell phenotype in the investigation of T cell lymphoma
	Method
	Flow cytometry: CD4, CD7, CD26
	Application
	For diagnosis of Sezary syndrome
	Sample type
	EDTA (4ml)

Test name:	*Description and clinical relevance*
HTLV1 panel	This assay assesses T cell phenotype in patients with HTLV1 infection
	Method
	Flow cytometry: CD4, CD8, DR, CD25
	Application
	Monitoring of T cell activity which is related to lung involvement.
	Sample type
	EDTA (4ml)