IMMUNOLOGY LABORATORY HANDBOOK

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Working in partnership — University Hospitals of North Midlands NHS Trust; Mid Cheshire Hospitals NHS Foundation Trust; East Cheshire NHS Trust

Amendment History

Date issued /Document Revision No.	Replaces document /revision	Summary of Changes	Page No.	Initial
V.10	V.09	Updated wording of UKAS accreditation to conform to GEN6	4	KAS
V10	V.09	Inserted reference to ELISA method for Cardiolipin and B2 Glycoprotein 1 abs as per new EULAR guideline 2023	12	KAS
V10	V.09	OTOblot 68kd inner ear protein no longer available (Oct 2023) .	22	KAS

INTRODUCTION TO CLINICAL IMMUNOLOGY SERVICES

The clinical immunology service for North Midlands and Cheshire Pathology Service (NMCPS) is based at the Royal Stoke University Hospital a division of the pathology directorate, providing a consultant led service specialising in autoimmunity, allergy and immunodeficiency. All tests are quality assured through the national scheme UKNEQAS.

The Laboratory is a UKAS accredited medical laboratory No. 9300
The Scope is accredited to ISO 15189:2012

To provide the best quality service we rely on feedback and day-to-day communication with GPs and hospital users. Dr Sarah Goddard and Dr Lavanya Diwakar are available for clinical advice on use of the laboratory for diagnosis and management of autoimmunity, allergy and immunodeficiency. The easiest way for GPs to access advice is via choose and book. This handbook is however designed to try and answer some of the more common problems.

There is also an outpatient clinical service to support the laboratory and provide diagnosis and management of primary immunodeficiency and allergy. There is an internal referral form for anaphylaxis and laryngeal oedema at UHNM (see intranet emergency medicine section: referral forms).

HOW TO CONTACT THE LABORATORY

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Pathology Directorate
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The laboratory is open Monday-Friday 09.00-17.30.
Dr Goddard is available at variable times throughout the week.
Dr Diwakar works Monday to Wednesday.

Rarely, urgent results are required and these should be discussed with the laboratory. New, MPO, PR3 and anti-GBM results will be phoned, and we will endeavour to phone any other results that appear to require particular attention.

USER FEEDBACK/COMMENTS/COMPLAINTS

We welcome user feedback and comments to help improve the service. However, if there is a problem and you are not happy with the service, in the first instance contact the departmental staff as above. Alternatively, contact the Pathology Quality Manager: Mrs Katie Berger (017826) 74234. Complaints are responded to in accordance with UHNM Trust policy 'Handling Complaints and Concerns'

CONSENT
There are no specific requirements for routine tests and staff should follow their local policies on consent
At UHNM (C43) Consent Policy states "The health professional undertaking the procedure is ultimately responsible for ensuring that the patient is genuinely consenting to what is being proposed" "Where verbal or implied consent is being sought at the point of the procedure being carried out, this will naturally be done by the healthcare worker at that time".

TESTS AND TUBES

The importance of providing good clinical information with requests cannot be overstated. The more information we are given, the better interpretation we can provide. The minimum requirements for accepting a sample state that the name, date of birth and either hospital or NHS number are on the request form and full name and another form of identification on the specimen, this is in line with Trust policy C49. Samples should be transported safely in clear specimen bag and extremes of temperature avoided. The following may lead to sample rejection:

- Insufficient information supplied with a sample
- Incorrect specimen (see below)
- Duplicate request which is either not necessary or falls within the minimum specified time period (national guidance on minimum re-testing intervals for immunology are provided in appendix)

Rejected samples will be reported with a reason through the normal reporting pathway.

Most Immunology tests require a serum sample using a gel specimen tube) Please see Table 1 for exceptions. Serum samples will be stored for approx. 2 weeks and additional tests can be requested.

For paediatrics and difficult to bleed patients, use the serum/clotted plain paediatric tubes.
The minimum sample size required is 1ml blood, although for multiple tests please contact laboratory for advice.

Table 1 Tests with specific requirements

CH50	Clotted specimen to be received in the laboratory within 2 hours.
Oligoclonal bands	Paired Serum and CSF samples taken within 24 hours of each other.
Serum tryptase	Tryptase has a short half-life, therefore several samples are required to detect a peak Immediately, 1-3 hours and 12-24 hours after the onset of suspected anaphylaxis.
Interferon gamma release assay IGRA	Specific 'Quantiferon plus' IGRA assay tubes are available from the Immunology laboratory at Royal Stoke Hospital and the Pathology Reception at Mid Cheshire and East Cheshire.
Lymphocyte subsets (TBNK)	EDTA (purple top). 2 samples generally needed (for FBC as well as subsets)
Other investigations of immunodeficiency	All other investigations e.g. lymphocyte proliferation and neutrophil function are sent to Heartlands laboratory. It is VERY IMPORTANT to liaise with the laboratory at Royal Stoke before taking blood to ensure correct samples and transport arrangements.

HLA typing for disease e.g coeliac, Behcets	EDTA (purple top)

PROTECTION OF PERSONAL INFORMATION

The recommendations of the Caldicott Report (1997) and the subsequent Information Governance Review (2013) have been adopted by the National Health Service as a whole. These recommendations relate to the security of patient identifying data (PID) and the uses to which they are put. Please refer to the UHNM NHS Trust policy No. IT02 Trust Policy for Information Security Management for further details.

USE OF THE IMMUNOLOGY LABORATORY FOR SOME COMMON CLINICAL SCENARIOS

An important principle in the use of immunology tests is to interpret the results in the clinical context. The tests will have a low positive predictive value if they are used indiscriminately- that is to say, if the tests are performed on patients who have little or no real clinical evidence of relevant disease, most of the positive results will be found in patients without disease.

Therefore, the sensible advice is**: *If there are no real clinical grounds for suspecting an autoimmune disease, autoantibody tests should not be requested. The result is unlikely to be useful.***

Rheumatoid arthritis (RA):

The decision to refer or treat arthritis is made primarily on clinical grounds. Referral should not be delayed for results of rheumatoid factor (RF) as it is unlikely to influence management. RF is absent in 30-40% of patients with RA and is seen in about 2% of normal population. High levels of RF are associated with complications such as systemic symptoms and more severe disease. However RF cannot be used to monitor disease activity. Anti-CCP antibodies are a more specific test for rheumatoid arthritis, but may be negative in 30% of patients and even more at presentation.

Connective tissue disease (CTD):

Anti-nuclear antibodies (ANA) are a good screen for connective tissue disease; a negative result suggests that the diagnosis is unlikely. Positive results are titrated, or diluted to find the level at which there is still staining. 1/80 is a low titre used for screening. However, low titre ANA is common especially in the elderly and those with infections and for this reason, positive ANAs are quantified. The higher the titre, the more likely will be the diagnosis of CTD. Specificity can be improved by testing for antibodies to extractable nuclear antigens. ANA titres greater than 1/320 are automatically tested for dsDNA and ENA antibodies in our laboratory.

Sm	High specificity for SLE
Ro	Sjogren's Syndrome, also subacute cutaneous lupus, neonatal lupus and SLE
La	Sjogren's Syndrome and SLE
RNP (no Sm)	one of the criteria for mixed connective tissue disease
Scl 70	scleroderma
Jo -1	myositis, often more aggressive, with lung involvement

Histone	drug-induced SLE
Ribosomal P	neuropsychiatric SLE
dsDNA	SLE (most specific test for dsDNA antibodies uses crithidia staining)

ANA antibodies are detected by staining of cells and some patterns of staining are associated with disease. Centromere pattern is associated with the limited form of scleroderma, also known as the CREST (Calcinosis, Raynaud's, Esophageal dymotility, Sclerodactyly, Telangiectasia) syndrome.
High titre nucleolar pattern is associated with scleroderma and related overlap disorders.
Speckled pattern is often associated with Ro or La antibodies, and homogenous staining is seen in the presence of dsDNA antibodies. Typically IgG is raised.
A diagnosis of anti-phospholipid syndrome should be excluded in pregnancy and planned pregnancy in individuals with autoimmune disease, see below. A very small proportion of pregnant patients with Ro positivity may deliver babies with neonatal SLE or heart block.
Once a diagnosis is established, repeat testing of ANA and ENA is not valuable unless the clinical features change. Repeat requests within 6 months will require prior discussion with the laboratory.

Initial Investigations: ANA, complement C3 & C4, immunoglobulins
Disease activity monitoring: 1) dsDNA (only if initially positive; do not repeat within 3 months)
2) complement C3 and C4(do not repeat within 3 months)

Anti-phospholipid Syndrome:

About half of patients have primary disease, and the other half have associated CTD. To make the diagnosis there must be positive laboratory findings associated with clinical features of thrombosis or foetal death or multiple miscarriages.
To satisfy the laboratory criteria there must be positive lupus anticoagulant (LA) or medium or high titre IgG anti-phospholipid abs (anti-cardiolipin abs or β2 glycoprotein abs) on two or more occasions at least 12 weeks apart. This is because transient non-specific antibodies are common, especially associated with infection. The LA test is less sensitive but more reliable as it is more specific.
Lupus anticoagulant test cannot be carried out on anticoagulated patients.
Investigations: IgG anti-cardiolipin and β2 glycoprotein antibodies on two or more occasions 12 weeks apart. Lupus anticoagulant (haematology)

Vasculitis

MPO (myeloperoxidase) and PR3(proteinase 3) antibody tests are used in the diagnosis and monitoring of individuals with ANCA-associated vasculitis e.g. Granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and Eosinophilic granulomatosis with polyangiitis (EGPA) (previously known as Churg-Strauss Syndrome) may present with rash, glomerulonephritis, pulmonary disease, and mononeuritis multiplex.
The immunology laboratory has a number of tests available to aid diagnosis; it may be useful to discuss patients before tests are requested.

In keeping with the revised 2017 international consensus on testing of ANCAs in GPA and MPA, the immunology laboratory has moved to using MPO and PR3 assays as the preferred screening method for diagnosis of ANCA associated vasculitis (effective from the 1st of April 2019).
ANCA indirect immunofluorescence is no longer routinely available

The presence of MPO/PR3 antibodies allows for classification of these conditions

Microscopic polyangiitis	usually MPO, may be PR3
Granulomatosis with polyangiitis (Wegener's granulomatosis)	PR3
Eosinophilic granulomatosis with polyangiitis (Churg-Strauss Syndrome)	usually MPO, may be PR3

Other conditions associated with MPO/PR3 positivity

Subacute bacterial endocarditis	may have PR3
Ulcerative colitis	PR3 positivity associated with more extensive disease

Please note that MPO/PR3 tests are not diagnostic of AAV in isolation but can support a diagnosis of AAV in the presence of clinical and histopathological features of the disease.

Other causes of vasculitis include Henoch-Schonlein purpura, connective tissue disease, rheumatoid arthritis, drugs, and cryoglobulinaemia. .MPO and PR3 testing is of no use in monitoring these conditions.
Cryoglobulinaemia may be associated with viral infection e.g. HepC and CTD or with a paraprotein (eg Myeloma). Great care must be taken in the collection of these samples, please contact the biochemistry laboratory for advice **prior** to taking samples.

Investigations for vasculitis: ANA, MPO/PR3, Immunoglobulins, complement C3 and C4, consider anti-GBM and cryoglobulins.

Disease monitoring: MPO and PR3 antibodies and complement C3, C4 may be used to monitor disease activity.

Acute kidney injury
The following investigations are indicated when glomerulonephritis is suspected, i.e. there is anuria or significant amount (not just a trace) of blood and, or protein in the urine. Please phone the laboratory and ask for testing to be done URGENTLY if necessary. Autoimmune serology may be indicated as a second line of investigation in patients in whom other causes have been excluded.
Please discuss new patients with positive results for anti-GBM, MPO or PR3 with the on call renal registrar.
Investigations: MPO/PR3, anti-GBM, ANA, Immunoglobulins, complement C3 & C4, consider cryoglobulins.

Coeliac Disease

NICE guidance (CG86 & NG20). IgA tTG is the first line of investigation for adults and children. Provided that the patient is on a gluten containing diet* and has detectable serum IgA, a negative IgA tTG test makes coeliac disease unlikely. IgA endomysial ab test is done by the laboratory to confirm all new positives and equivocal results.

The most recent ESPGHAN (European Society for Paediatric Gastroenterology, Hepatology and Nutrition) guidance (2020) suggests that children and adolescents will not routinely need the confirmatory IgA Endomysial antibody test done. More details can be obtained here.

Coeliac disease is associated with IgA deficiency**, and the IgA tTG will be falsely negative in these patients, therefore IgA levels should be checked. If there is IgA deficiency, i.e. undetectable IgA levels, then the sample will be tested for IgG deamidated gliadin peptide. Patients with positive tests should be referred to an adult or paediatric gastroenterologist . Gliadin antibodies have poor specificity and their use is not recommended

HLA typing is occasionally a useful second line investigation to exclude coeliac disease in patients without HLA-DQ2/DQ8 in a specialist setting (present in 25% of normal population, present in almost all patients with coeliac disease).

Diagnosis: IgA tTG (and IgA); IgG DGP in IgA deficient individuals

Monitoring: IgA tTG – as per NICE guidance, however please note the tTG assay is not recommended by the manufacturer for monitoring.
*A gluten-containing diet: gluten in more than 1 meal per day for at least 6 weeks prior to testing.
**IgA deficiency is defined as total IgA less than 0.07g/L.

Autoimmune liver disease

Half of patients with autoimmune hepatitis have other autoimmune disease e.g. thyroid disease. Some patients have ANA antibodies and high titre smooth muscle antibodies. Low titre smooth muscle antibodies are very common, and often associated with infection. Another group have negative ANA and LKM antibodies. Sometimes pANCA and mitochondrial antibodies may be present.
Almost all patients with primary biliary cirrhosis have anti-mitochondrial antibodies. There are a number of patterns of mitochondrial staining, but it is the M2 pattern which is specific for PBC. M2 specificity may also be confirmed by ELISA or blotting techniques, but this is not routinely carried out. Typically IgM is raised.
Additional liver autoantibodies can be detected by Immunoblot

Immunodeficiency

Although primary immunodeficiency (PID) is rare, it is important to consider this diagnosis in some patients, as delay in diagnosis is common and causes irreversible tissue damage e.g. bronchiectasis. Consider this diagnosis in patients with:

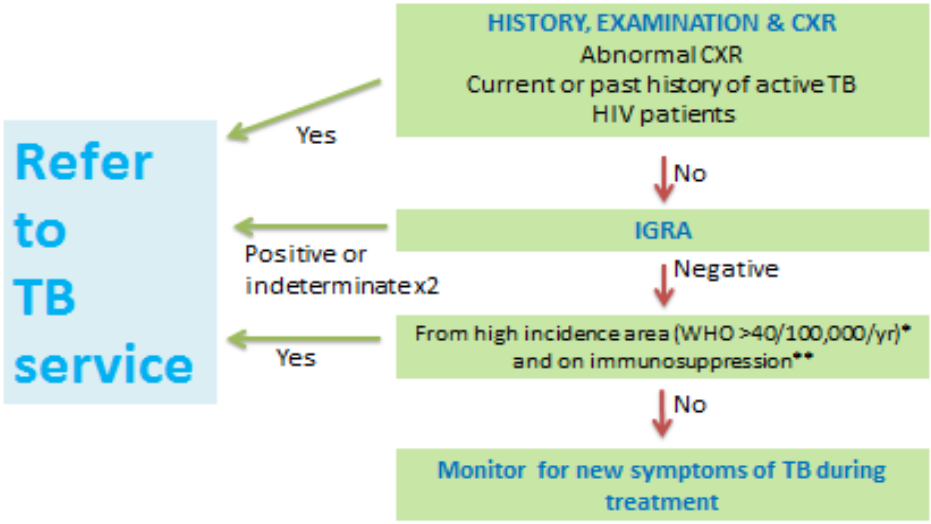
- **S**erious infection e.g. severe chicken pox
- **P**rolonged i.e. difficult to treat infection
- **U**nusual infection and opportunistic infection e.g. staphylococcal liver abscess or pneumonia, atypical TB, pneumocystis
- **R**ecurrent infection (e.g. meningitis, otitis media, bronchiectasis)
- Infants under 6 months with failure to thrive.

The most common PID is associated with antibody deficiency and patients tend to present with recurrent respiratory tract infection caused by pneumococcus and haemophilus influenzae. A check of Igs will be sufficient to exclude PID in many of these patients. In children take note of lymphocyte numbers. **However all patients in whom there is a clinical suspicion of immunodeficiency, should be discussed with the consultant immunologist.**

Latent TB testing

Active and latent TB should be excluded in patients undergoing immunosuppression e.g. anti-TNF therapy (BTS/NICE CG117). Interferon γ release assays (IGRA) and Mantoux tests may be used to test for latent TB, and there is no definitive data to suggest whether one is more sensitive than the other. However, IGRA tests are more specific for mycobacterium TB, especially in patients who have previously received BCG vaccination.
IGRA testing involves incubation with TB antigen and detection of subsequent IFN γ production by TB specific T cells. There is a negative control tube and a positive control tube (in which T cells have non-specific stimulation). If a patient is immunosuppressed, has few T cells or other non-specific T cell dysfunction, or there is a problem in sample collection/processing then the positive control can be negative and the result will be returned as indeterminate. Hence the test is not appropriate for annual monitoring on patients on active immunosuppression.
This is not a test for active TB as many patients with active TB have a negative IGRA test.

The protocol below has been agreed with the UHNM TB service and in collaboration with other relevant clinical groups.



* Lived in area >3 months, or other significant TB contact
http://www.hpa.org.uk/web/HPAweb&HPAwebStandard/HPAweb_C/1195733758290
** prednisolone >10mg, anti-TNFa and cyclosporin (or other organ Tx immunosuppression).

Allergy

The diagnosis of allergy is primarily clinical. The best supportive tests are skin prick tests, which are available through the respiratory medicine department (hospital users). Specific IgE testing can only be used to support clinical findings; it cannot exclude or definitely confirm a diagnosis. Testing of panels of allergens is not usually helpful, although aeroallergen panels may be useful in patients with rhinitis and asthma. A negative aeroallergen panel, would suggest that IgE mediated mechanisms are unlikely.

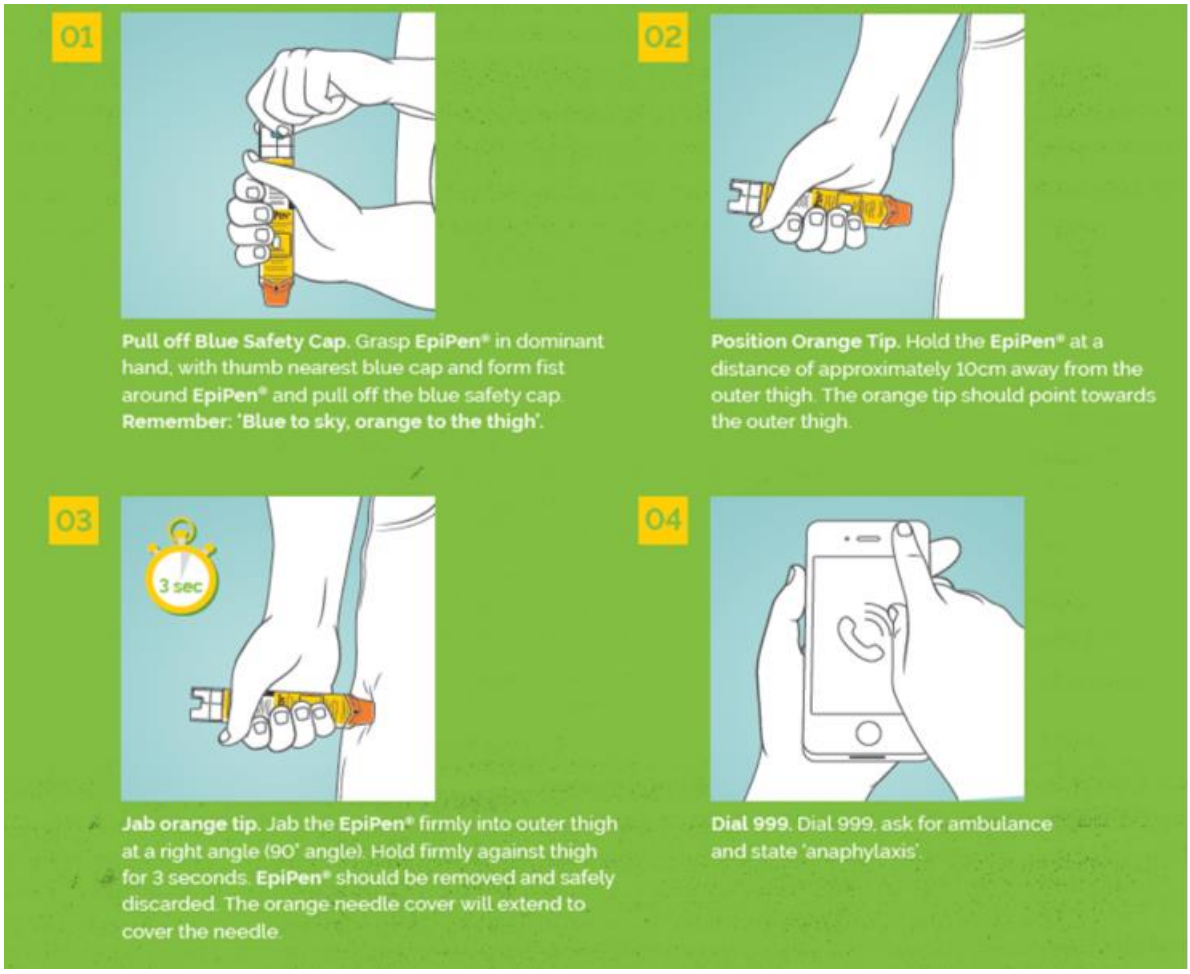
Patients diagnosed with allergy e.g. latex or nut, should be given a plan of management to include avoidance and management of accidental exposure. This may include use of an EpiPen. Patients must be taught how to use the EpiPen correctly.

To confirm a diagnosis of anaphylaxis it is extremely useful to have tryptase levels. Samples should be taken immediately, between 1-3 hours after onset of symptoms and a baseline level should be checked 12-24 hours after. These patients should be referred to the allergy clinic using the internal allergy referral form on the intranet or written referral. There are no investigations for food intolerance.

http://www.epipen.co.uk/patients/epipenr-user-guide/

There are a number of patient information sheets on the UHNM adult immunology and allergy website which can be accessed on

http://www.uhnm.nhs.uk/adultallergyandimmunology/Pages/More-information-about-allergy.aspx



USE AND INTERPRETATION OF TESTS

AUTOIMMUNITY	
Acetylcholine receptor antibodies	Specific test for myasthenia gravis 80-90% sensitive. Skeletal muscle (or tyrosine kinase) may be positive in AChR ab negative patients. Skeletal muscle abs are associated with thymoma.
Adrenal abs	Associated with Addison's Disease
Anti Nuclear abs **ANA**	A negative result can rule out connective tissue disease in most cases. A low titre may occur in inflammatory disease, infection and some normal people. High titre (> 1/1280) is suggestive of connective tissue disease. All ANAs >1/320 are automatically tested for ENA and dsDNA. No repeat testing within 6 months without prior discussion. ANA antibodies are detected by staining of cells and some patterns of staining are associated with disease. **Centromere** pattern is associated with the limited form of scleroderma, CREST. High titre **nucleolar** pattern is associated with scleroderma and related overlap disorders. **Speckled** pattern is often associated with Ro or La antibodies, and **homogenous** staining is seen in the presence of dsDNA antibodies.
Beta 2 Glycoprotein -1	Associated with anti-phospholipid syndrome. See information below for cardiolipin antibody. Performed by ELISA on Phadia 250
Cardiolipin abs	To satisfy the laboratory criteria there must be positive lupus anticoagulant (LA) or medium or high titre anti-phospholipid abs (or anti-cardiolipin abs) on two occasions at least 12 weeks apart. This is because transient non-specific antibodies are common, especially associated with infection. Performed by ELISA on Phadia 250

	P The LA test is less sensitive but more reliable as it is more specific. Lupus anticoagulant test cannot be carried out on anticoagulated patients. Also see advice in previous section.
Centromere abs	Associated with the limited variant of systemic sclerosis, CREST, and may also occur in some patients with primary biliary cirrhosis.
Cyclic citrullinated peptide abs	Present in about 70% of patients with rheumatoid arthritis, probably less at presentation. More specific than RF.
dsDNA abs	Associated with SLE, especially lupus nephritis. In some patients levels correlate with disease activity. Sometimes non-specific ssDNA abs are picked up, and for this reason new dsDNA abs are tested by crithidia staining, which is more specific. dsDNA is automatically tested on ANAs >1/320. dsDNA will not be tested on negative ANAs.
ENA **Extractable nuclear antigens**	• Sm — High specificity for SLE • Ro — Sjogren's S, also subacute cutaneous lupus, neonatal lupus and SLE • La — Sjogren's S and SLE • RNP (no Sm) — one of the criteria for mixed connective tissue disease • Scl70 — scleroderma • Jo-1 — myositis, often more aggressive, with lung involvement • Histone — drug-induced SLE • Ribosomal P — neuropsychiatric SLE ENA is automatically tested on ANAs >1/320.
Endomysial abs	Highly specific (>95%) for coeliac disease. Used to confirm new positive tissue transglutaminase abs (tTG), and for equivocal tTGs. Patients with undetectable IgA (deficient) will be automatically tested by IgG deamidated gliadin peptide antibody (high sensitivity in this group).
Ganglioside, GD1b, GM1, GQ1b **Myelin associated glycoprotein**	These antibodies are not strictly diagnostic, but may provide additional evidence. They are associated with motor and sensorimotor neuropathies. High titres of GM1 are typically associated with multifocal motor neuropathy. GQ1b is frequently associated with Miller-Fisher syndrome. MAG abs are often associated with a paraprotein. Titres of these antibodies vary with disease activity and may be used for monitoring.
Gastric parietal cell ab	Associated with pernicious anaemia, other autoimmune diseases and also occur in healthy older patients. Intrinsic factor is more specific for pernicious anaemia, but less sensitive. Intrinsic factor is automatically tested on all positive samples.
Glomerular basement membrane abs	Associated with Goodpasture's Syndrome
Glutamic acid decarboxylase abs	Associated with Stiff-man syndorme and in lower titres in type I diabetes.
68KDa inner ear protein abs	Previously called otoblot. Associated with autoimmune hearing loss.
Interferon gamma release assay (IGRA)	Quantiferon gold plus. Test for LATENT TB. Please see advice in earlier section.
Intrinsic factor abs	Supports a diagnosis of pernicious anaemia. A negative Intrinsic Factor does not exclude the diagnosis of pernicious anaemia since only 60% of patients with pernicious anaemia have this antibody The presence of anti-gastric parietal cell antibodies may or may not be concordant with that of Intrinsic factor antibodies and their measurement, in addition to, or in conjunction with measurement of Intrinsic Factor antibodies, may aid in the evaluation of patients with suspected pernicious anaemia.

Islet cell abs	Present in 75% of type I diabetics at diagnosis, may be used to screen at risk groups. Supports a diagnosis of autoimmune type I diabetes.
Liver-kidney microsomal abs	Associated with type II autoimmune hepatitis, but may occur in viral and drug-induced hepatitis.
Mitochondrial abs	M2 pattern mitochondrial abs are both specific and sensitive for primary biliary cirrhosis, although between 5-10% are mitochondrial ab negative.
Myositis ab screen	The following antibodies detected by Immunoblot assay Anti Ro-52 MI2 – Anti Mi-2 (Mi-2 alpha & Mi-2 beta) OJ – Anti OJ EJ – Anti EJ PL12 – Anti PL-12 PL7 – Anti PL-7 SRP – Anti SRP JO1 – Anti Jo-1 PS75 – Anti PM-SCL-75 PS10 – Anti PM-SCL-100 KU – Anti KU SAE – Anti SAE NXP2 – Anti NXP2 MDA5 – Anti MDA5 TIF – Anti TIF1 gamma Please request HMCGCR antibodies separately
Neuronal and Paraneoplastic abs	This is a rapidly evolving area of autoimmune testing. It is important to provide clinical detail in order to get the most appropriate investigations. There are a number of antibody tests available, if a large number of disparate antibodies are requested then the requesting consultant will be asked to confirm by email. Samples for paraneoplastic antibodies are screened by indirect immunofluorescence, and positive samples are then sent away for further identification. There is currently no clear evidence that either CSF or serum samples are preferable. Paired samples may be sent.
MPO and PR3 antibodies	Microscopic polyangiitis — usually MPO, may be PR3 Granulomatosis with polyangiitis (GPA). — PR3 Eosinophilic granulomatosis with polyangiitis (Churg-Strauss Syndrome) — usually MPO, may be PR3 <u>Other conditions associated with MPO/PR3 positivity</u> Subacute bacterial endocarditis — may have cANCA and PR3 Good pasture's (anti-GBM) — may also have ANCA, which is associated with better prognosis Ulcerative colitis — PR3 ; Associated with more extensive disease
Oligoclonal bands	Matched serum and CSF samples should be sent. Oligoclonal bands present in the CSF and not matched in the serum are associated with multiple sclerosis.
Rheumatoid factor	RF is positive in 70% of rheumatoid arthritis patients, but is also positive in other inflammatory conditions and in some healthy people (especially the elderly). High titres are associated with more severe disease and systemic complications. No repeat within one year.

Skin (intercellular and basement membrane)	Associated with pemphigus and pemphigoid.
Skeletal muscle (tyrosine kinase)	Associated with thymoma, and myaesthenia gravis.
Smooth muscle antibody	High titres are associated with autoimmune liver disease. Low or moderate titres are usually not significant and commonly associated with infection.
Systemic sclerosis screen	The following antibodies detected by Immunoblot Scl-70 CENP A CENP B RP11 RP155 Fibrillarin NOR90 Th/To PM-Scl 100 PM-Scl 75 Ku PDGFR Ro-52
Thyroid (TPO)	Associated with autoimmune thyroid disease: Hashimoto's thyroiditis and primary hypothyroidism, less sensitive for Grave's disease. In cases with a normal fT4 and raised TSH (compensated hypothyroidism), positive thyroid autoantibodies can sometimes be useful in deciding when to initiate treatment, though a normal value would not exclude early hypothyroidism. As a general rule, monitor those with TSH values between 5-10 unless symptomatic and consider treatment in those with TSH values >10.
TSH Receptor abs	This test is indicated in pregnant women with a history of Grave's disease, where there may be a risk of neonatal thyrotoxicosis.
Tissue transglutaminase (tTG)	Provided patient is not IgA deficient and is on gluten-containing diet, this is a highly sensitive and specific test for coeliac disease (>95% both). Diagnosis should be confirmed in a specialist setting. All new positive and equivocal results are confirmed by IgA endomysial antibody test. Patients with undetectable IgA (deficient) will be automatically tested by IgG deamidated gliadin peptide antibody (high sensitivity in this group). No repeat testing within 6 months without prior discussion.
IMMUNOCHEMISTRY	
C1 esterase inhibitor	Low levels are associated with hereditary and acquired angioedema. Normal C4 during an attack of angioedema excludes hereditary angioedema. Patients should be referred to Dr Goddard/ Dr Diwakar.
Functional C1 esterase inhibitor	Rarely protein levels are normal and there is a hereditary defect of function. Please discuss with clinical immunologist before requesting test.
Complement C3 and C4	C3 and C4 raised acute phase response Low C3, normal C4 post-streptococcal nephritis, gram negative sepsis, membranoproliferative GNitis, C3 nephritic factor (v low C3), rarely hereditary deficiencies of complement pathway control proteins. Low C4, normal C3 active SLE, C1 esterase inhibitor deficiency, cryoglobulinaemia, rarely hereditary deficiency. C3 and C4 low immune complex disease, e.g. SLE, sepsis, severe liver disease. Normal C4 during an attack of angioedema excludes hereditary angioedema.
Complement function	This tests the classical and terminal complement pathways and is really

CH50	indicated if a defect in a component of the pathway is suspected e.g. recurrent meningitis or recurrent bacterial infection. Rarely autoimmune diseases e.g. SLE and haemolytic uraemic syndrome are associated with early complement defects. See sample requirements in 'tests and tubes'.
Complement alternative pathway function AP50	This tests the alternative and terminal complement pathways and is again indicated in the investigation of complement defects. See sample requirements in 'tests and tubes'.
Functional Igs, pneumococcal, haemophilus influenzae B (HiB), tetanus	Defects in functional antibody responses to vaccination are associated with primary antibody deficiency e.g. common variable immunodeficiency. A defect of functional antibody responses, with normal Ig levels, is of debatable significance. Low levels should be tested by repeating 4 weeks after vaccination. May be used to assess risk of vaccination. Please phone for advice or consider referral to clinical immunology clinic. Pneumococcal ab requests are reported with serotype specific titres.
IgG subclasses	Rarely indicated. Raised IgG4 is associated with autoimmune pancreatitis
Leukocyte immunophenotyping and functional studies	Please phone for advice on patients with possible immunodeficiency.
Immunoglobulins (Biochemistry)	Measurement of immunoglobulins is indicated in patients suspected of a B cell malignancy, typically myeloma, or immunodeficiency. There are some other conditions e.g. PBC or HIV, which have characteristic Ig changes, but Igs are rarely key to making the diagnosis. Low Igs always require further investigation. Low Igs can occur due to loss from the renal or GI tract, tends to be mostly IgG lost. Low IgM can be due to chronic renal impairment, some drugs and occasionally lymphoproliferative disease. Low Igs in more than one class, especially if associated with features of immuno-deficiency should be referred to the clinical immunology clinic. Polyclonal increase is associated with some diseases, but is not specific and if the patient is clinicaly well, no further investigation is warranted.
Serum electrophoresis, urine BJP and serum free light chains	See biochemistry handbook
ALLERGY	
IgE	Associated with atopy, high levels associated with eczema, also associated with parasitic conditions, immunodeficiency, autoimmune disease, and rarely malignancy. Levels should be taken into account when interpreting specific IgE results.
Specific IgE	Specific IgE may be raised in association with specific allergy. There are frequently false positives, therefore screening is not recommended. Most useful in association with a clear history of an allergic reaction, to identify the specific cause e.g. which food in a meal, or which insect. Skin prick testing should be used in preference if available. Testing shortly after systemic reaction (within 6 wks) may be falsely low. Low positive specific IgE for drugs should be interpreted with caution in patients with total IgE> 500. Component resolved diagnostic tests have limited application in the diagnosis and management of allergies. Where allergen components are requested, full clinical details must be provided. Discussion with consultant immunologists before requesting components is encouraged.
Tryptase	Tryptase is released during mast cell degranulation, and during a systemic reaction (anaphylaxis), serum levels are increased. However as the half life is short, serial samples should be taken to observe a peak. Ideally immediately, 1-3 hours and 6-24 hours after reaction began.
Specific IgG (precipitins)	Aspergillus, This test is useful for the diagnosis of allergic broncho-pulmonary aspergillosis (ABPA) and aspergilloma. This test does not

	usually give a positive result for invasive aspergillosis (see Apergillus antigen test (galactomannan) Avian & *Micropolyspora Faeni* (Farmer's Lung)

Uncertainty Measurement estimates for quantitative assays can be made available as required to assist in the interpretation of results

APPENDIX I – Turnaround Times and Minimum retesting intervals

(taken from guidance published by the Royal College of Pathologists –March 2021)
https://www.rcpath.org/uploads/assets/253e8950-3721-4aa2-8ddd4bd94f73040e/g147_national-minimum_retesting_intervals_in_pathology.pdf

AUTOIMMUNITY	Turn around times (calendar days)	Reference ranges	Type of assay	Minimum retesting interval
Acetylcholine receptor antibodies	Referred 28	Positive >0.5 nmol/L		6 months
Adrenal abs	7 – Referred test	Pos/neg	Indirect immunofluorescence	repeat test of limited value; clinical context should dictate retesting frequency
Cardiolipin abs IgG	3	Positive >10	Fluorescence Enzyme linked immunoassay	12 weeks; Once diagnosis established repeat testing is not useful
Centromere	3	Pos/neg	Indirect immunofluorescence	Repeat testing not particularly useful.
Cyclic Citrullinated peptide (CCP abs)	3	Positive>7	Fluorescence Enzyme linked immunoassay	1 month Repeat testing once diagnosis is confirmed is of limited value
Anti nuclear antibody (ANA)	3	Pos/neg Titre >1/80 is reported as positive	Indirect immunofluorescence assay	Repeat testing is of limited value once diagnosis established
dsDNA	4	Positive >10 IU/ml	Fluorescence Enzyme linked immunoassay. Confirmation with crithidia by indirect immunofluorecence	3-6 months while on treatment
ENA Extractable nuclear antigens	Screen (neg) 4 Identification 8	Pos/neg	Fluorescence Enzyme linked immunoassay screen and immunoblot for confirmation and identification	repeat test of limited value; clinical context should dictate retesting frequency
Endomysial	7	Pos/neg	Indirect immunofluorescence	Not routinely indicated. Only useful for confirmation of tTg positives
Ganglioside, GD1b, GM1, GQ1b	Referred 28 days	Pos/neg Anti-Glycolipid Antibody		Not routinely required

		GM1 (IgG & IgM): <1:500 GM2 (IgG & IgM): <1:500 GD1a (IgG & IgM): <1:500 GD1b (IgG & IgM): <1:500 GQ1b (IgG & IgM): <1:500		
Gastric parietal cell	3	Pos/neg	Indirect immunofluorescence	1 month
Glomerular basement membrane	3	Positive >7	Fluorescence Enzyme linked immunoassay	Every 3-6 months while on treatment or more frequent if receiving plasma exchange therapy
Glutamic acid decarboxylase	Referred 21 Days	Positive >10 iu/mL		Not routinely recommended
Interferon gamma release assay IGRA	Referred 10	Pos/neg/indeterminate	ELISA	As advised by TB service
Intrinsic factor	7	Pos/neg	ELISA	not routinely required
Islet cell antibodies	7	Pos/neg	Indirect immunofluorescence	not routinely required
Liver-kidney microsomal	4	Pos/neg	Indirect immunofluorescence	1 month
Mitochondrial	4	Pos/neg	Indirect immunofluorescence	Repeat testing not routinely required
Neuronal paraneoplastic	7 (screen)	Pos/neg	Indirect immunofluorescence, confirmation of identity by medical school, Birmingham. See appendix II	1 month
MPO **PR3**		**Positive >3.5(equiv 3.5-5)** **Positive >2(equiv 2-3)**	Fluorescence Enzyme linked immunoassay	On treatment: six months or more frequent if receiving plasma exchange therapy Off treatment: Annually
Oligoclonal bands	Referred, 21 days			repeat test of limited value; clinical context should dictate retesting frequency
Rheumatoid factor	3	Positive >20	Nephelometry	Not routinely required
Skin (intercellular and basement membrane)	7	Pos/neg	Indirect immunofluorescence	On treatment: 6 months Off treatment: annually
Skeletal muscle (tyrosine kinase)	Referred 21 days	Pos/neg	Indirect immunofluorescence	repeat test of limited value; clinical context should dictate retesting

				frequency
Smooth muscle antibody	4	Pos/neg	Indirect immunofluorescence	1 month
Thyroid (TPO)	7	Positive /Negative	ELISA (Phadia)	3-6 months
Tissue transglutaminase (tTG)	5	Positive >20CU (equivocal 20-30CU)	Chemiluminescence	retesting at 6-12 months depending on pre-treatment value
IMMUNODEFICIENCY				
C1 esterase inhibitor (one serum sample for C1inh & C1 inh functional – see below)	Referred 28 days	0.15 – 0.35 g/l	Turbidimetry	Repeat testing not required for positives
Functional C1 esterase inhibitor (serum)	Referred 28 days	70 – 150%	Spectrophotometry	Repeat testing not required for positives
Complement C3 and C4	3	Normal range C3 0.75-1.65 g/l C4 0.14-0.54 g/l	Nephelometry	90 days; earlier frequency testing required in exceptional cases
Complement function CH50	Referred10 days	Normal range 23-49 u/ml	Functional assay	repeat once to confirm; Test only allowed with compatible clinical information
Complement alternative pathway function AP50	Referred 10 days	Normal range 75-125%		repeat once to confirm; Test only allowed with compatible clinical information
Functional Igs, pneumococcal, haemophilus influenzae B, tetanus	Referred 28 days	HIB <0.15 Tetanus <0.1 Pneumococcal <0.35	Multitiplex Immunoassay	6 weeks (post vaccination) Serial monitoring of limited value
IgG subclasses	Referred 14 days	Age related ranges	Turbidimetry	6 months
Leukocyte immunophenotyping and functional studies	TBNK 48 HOURS Further testing Referred 7 days	Varies with age: see Journal of Paediatrics; Mar 1997 p390	Flow Cytometry (Haematology)	As advised by Consultant
ALLERGY				
Total IgE	7	Levels vary with age. See report comments or contact laboratory.	Fluorescence Enzyme linked immunoassay	not routinely required
Specific IgE	7 (Referred allergens 14 days, may be more for rare allergens)	Positive >0.35 KU/L	Fluorescence Enzyme linked immunoassay	not routinely required
Tryptase	7 days	Normal range 2 – 14 ug/l	Fluorescence Enzyme linked immunoassay	As required following reaction

APPENDIX II – Referral Laboratories

• Cellular immunology • IGRA testing • Neutrophil Respiratory burst • S.Typhi	Regional Immunology and Clinical Chemistry Birmingham Heartlands Hospital Bordesley Green East Birmingham, B9 5SS Tel: 0121 424 1185
• IgG deamidated gliadin peptide • Anti-ganglioside ab • Anti-neuronal abs immunoblot • Oligoclonal bands • Anti-GAD • Autoimmune encephalitis screen	Clinical Immunology service (and neuroimmunology) The Medical School Vincent Drive Edgbaston Birmingham, B15 2TT Tel: 0121 414 3824
<u>Neuroimmune antibodies</u> • Anti MAG abs • Anti MOG abs • Anti voltage gated Ca channel abs • Anti Aquaporin 4 abs • MUSK (muscle specific kinase) • GABAR abs	Department of Immunology Churchill Hospital Old road Headington Oxford,OX3 7LJ Tel: 01865 225995
• Pneumococcal antibodies serotype specific • Tetanus antibodies • Haemophilus Influenzae B	2nd and 3rd Floors, Clinical Science Building Central Manchester and Manchester Children's University Hospital Trust Manchester Royal Infirmary Oxford Rd, Manchester, M13 9WL. Tel: 0161 276 4281
• Basal ganglia abs	Neuroimmunology and CSF Laboratory Room 917, Institute of Neurology Queens Square House, 33 Queen Square London. WC1N 3BG. Tel: 020 3448 3814
• Specific IgE (incl components) • TSH receptor abs • C3 nephritic factor • Pituitary gland abs • Ovarian abs • Adrenal abs • Salivary gland abs • PLA2R abs • Insulin abs • Striated muscle antibodies • IgG subclasses • C 1 inhibitor and function • CH50 • AP50	Department of Immunology PO Box 894 Northern General Hospital Herries Road Sheffield S5 7YT Tel 0114 271 5934 -Dr Wilde Tel 0114 271 5552 -lab
• HLA typing for renal transplant patients • HLA antibodies • HLA cross matches • HLA Typing for disease e.g Behcets, Coeliac	Tissue Typing Lab NBS, Birmingham Blood centre Vincent Drive Edgbaston Birmingham, B15 2SG
• Paediatric specialist immunology eg diGeorge S	Clinical Immunology, Level 4 Camelia Botnar Laboratories, Great Ormond St Hospital for Children, Great Ormond St, London. WC1N 3JH.

• Inner ear protein ab (Otoblot)	Assay has been withdrawn until provider can be sourced
• TH1 cytokine pathway testing	Department of Clinical Biochemistry and Immunology Level E4, Box 109 Addenbrooke's Hospital - Cambridge University Hospitals NHS Foundation Trust Hills Road; Cambridge CB2 0QQ; United Kingdom