University Hospitals of North Midlands NHS
NHS Trust

# IMMUNOLOGY LABORATORY HANDBOOK

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| 1 | Nov 2010 Rev No.2 | May 2010 Rev No. 1 | Inserted: Use of IgG Deamidated peptide test for coeliac disease | 5 | SG |
| 2 | | | Inserted: Advice for diagnosis and monitoring of myeloma & MGUS | 6 | SG |
| 3 | | | Inserted: Additional neuronal ab information | 8 & 9 | SG |
| 4 | | | Inserted: Indications for Thyroid ab testing | 10 | SG |
| 5 | | | Inserted: Pneumococcal ab testing in children | 10 | SG |
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| 7 | | | Changed: reference ranges and units for MPO/PR3 and dsDNA | 12 | SG |
| 8 | | | Inserted: Turn around times | 12 | SG |
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| 12 | | | Changed: send away test contact details | 14 | SG |
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| 14 | | | Changed CPA status | 4 | SG |
| 15 | | | Patient information weblinks | 5-6 | SG |
| 16 | | | ANA screening titre | 5 | SG |
| 17 | | | Changes to functional ab testing | 11& 15 | SG |
| 18 | | | Changes to inner ear protein ab | 9&15 | SG |
| 19 | | | Introduction of CCP | 5&9 | SG |
| 20 | | | Amend C3 and C4 reporting units to md/dl | 14 | KAS |
| 21 | | | Add TRT to Send away tests | 13 | KAS |
| 22 | May 2014 Rev No.5 | Feb 2013 Rev No.4 | IGRA testing information | 4, 7, 9 & 13 | SG |
| 23 | | | HLA testing in Coeliac disease | 6 | SG |
| 24 | | | CCP & RF assay information | 13 | SG |
| 25 | | | Send away information | 15 | SG |
| 26 | Aug 2016 Rev No.6 | May 2014 Rev No.5 | Add change from CPA to UKAS | 5 | SG |
| 27 | | | Sample requirements for immunodeficiency investigations | 5 | SG |
| 28 | | | Coeliac updated to NICE NG20 | 7 | SG |
| 29 | | | UHNS to UHNM | Throug hout | SG |
| 30 | | | New epipen guide | 10 | SG |

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| 31 | | | Removed protein chemistry information and referred to biochemistry handbook | 13 | SG |
| 32 | | | Specific IgE testing for drugs | 14 | SG |
| 33 | | | Reference policies for criteria for sample rejection, complaint procedure and protection of personal information. | 5 | SG |
| 34 | | | Change details of anti-TTG assay | 16 | SG |
| 35 | | | Change IGRA assay to quantiferon gold plus | 11 | SG |
| 36 | Nov 2016 Rev No 7 | Aug 2016 Rev No.6 | Coeliac ref to HLA testing/ESPGHAN | 8 & 13 | SG |
| | | | | | |

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## INTRODUCTION TO CLINICAL IMMUNOLOGY SERVICES

The clinical immunology service is 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 NEQAS. It has external accreditation with UKAS and is participating in training.
To provide the best quality service we rely on feedback and day-to-day communication with GPs and hospital users. Dr Sarah Goddard is available for clinical advice on use of the laboratory for diagnosis and management of autoimmunity, allergy and immunodeficiency. 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 (see intranet accident and emergency section: referral forms).

## HOW TO CONTACT THE LABORATORY

| | | | |
|---|---|---|---|
| Consultant Clinical Immunologist | Dr Sarah Goddard | 01782 (6) 74241 | sarah.goddard@uhns.nhs.uk |
| Lead Biomedical Scientist | Ms Karen Sneade | 01782 (6) 74240 | karen.sneade@uhns.nhs.uk |
| Laboratory | | 01782 (6) 74866 | |

Address : Immunology Laboratory, Pathology Department, University Hospital North Staffs, Newcastle Rd, Stoke-on-Trent, Staffs. ST4 6Q

The laboratory is open Monday-Friday 09.00-17.30. Dr Goddard is available at variable times throughout the week.
Rarely, urgent results are required and these should be discussed with the laboratory. New cANCA, MPO, PR3 and anti-GBM results will be phoned, and we will endeavour to phone any other results that appear to require particular attention.

If there is a problem and you are not happy with the service, in the first instance contact the department as above. Otherwise contact the Pathology Quality Manager: Mrs Katie Berger (01782) 674234. Complaints are responded to in accordance with Trust policies.

## 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 within specified time period (normally one month, see individual test details)

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

All tests require a clotted specimen (4ml red top tube), for several tests, please provide more than one tube. The minimum sample size required eg paediatric is 1ml, although for multiple tests please contact laboratory for advice. The following tests are exceptions:

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| CH50 | Clotted specimen to be received in the laboratory within 2 hours. |
| Functional C1 inhibitor | Citrated specimen (blue top) |

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| Oligoclonal bands | Serum and CSF sample 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 6-24 hours after reaction began. |
| Interferon gamma release assay | Specific 'quantiferon plus' IGRA assay tubes available in outpatients, Haywood, occupational health, the TB service at Cobridge and the laboratory at RSH. |
| Lymphocyte subsets (ICM code: TBNK) | EDTA (purple top). |
| 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 before collecting samples to ensure correct samples and transport arrangements. |

Samples will be stored for around three weeks and additional tests can be requested.

**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. IT07 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 you do not think there are real clinical grounds for suspecting the patient to have an autoimmune disease don't ask for autoantibodies. The result is unlikely to tell you anything 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 titred, 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, for this reason, positive ANAs are quantified. The higher the titre, the more likely will be the diagnosis of CTD. Further specificity can be gained by testing for antibodies to extractable nuclear antigens. ANA titres greater than 1/320 are automatically tested for dsDNA and ENA antibodies.

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|---|---|
| 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 |
| 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, 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. Typically IgG is raised.
A diagnosis of anti-phospholipid syndrome should be excluded in pregnancy and planned pregnancy, see below. A very small proportion of patients with Ro 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.
Investigations: ANA, complement C3 & C4, immunoglobulins
Disease monitoring: dsDNA and complement C3 and C4 may be used to monitor disease activity.

***Anti-phospholipid Syndrome:***
About half of patients have primary disease, and 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 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. 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: anti-cardiolipin antibodies on 2 occasions 12 weeks apart. Lupus anticoagulant (haematology)

***Vasculitis***

ANCA-associated vasculitis e.g. Wegener's granulomatosis, microscopic polyangiitis and Churg-Strauss S may present with rash, glomerulonephritis, pulmonary disease, and mononeuritis multiplex. Other causes of vasculitis include Henoch-Schonlein purpura, connective tissue disease, rheumatoid arthritis, drugs, and cryoglobulinaemia and ANCA testing is of no use in monitoring these conditions. The immunology laboratory has a number of tests available to aid diagnosis; it may be useful to discuss patients. ANCA are used as a screen and the more specific MPO(pANCA) and PR3 (cANCA) enzyme antibodies are automatically tested on positive samples. cANCA in the absence of PR3 is unlikely to be significant. pANCA in the absence of MPO is commonly associated with inflammatory bowel disease and primary sclerosing cholangitis. However it is not appropriate to test ANCA in the diagnosis of liver disease.

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| Microscopic polyangiitis | usually MPO, may be PR3 |
| Wegener's Granulomatosis | PR3 |
| Churg-Strauss Syndrome | usually MPO, may be PR3 |
| Subacute bacterial endocarditis | may have cANCA and PR3 |
| Good pasture's (anti-GBM) | may also have ANCA, which is associated with better prognosis. |

Cryoglobulinaemia may be associated with viral infection e.g. HepC and CTD. Great care must be taken in the collection of these samples, please contact biochemistry laboratory for advice.
Investigations: ANA, ANCA, RF, Immunoglobulins, complement C3 and C4, consider anti-GBM and cryoglobulins.
Disease monitoring: MPO and PR3 antibodies and complement 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 (not just a trace) of blood and, or protein. 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, cANCA or pANCA (with MPO), with the on call renal registrar.
Investigations: ANCA, anti-GBM, ANA, Immunoglobulins, complement C3 & C4, consider cryoglobulins.
See above for further details

***Coeliac Disease***

NICE guidance (CG86 & NG20). IgA tTG is the first line of investigation for adults and children. If the IgA tTG is negative in a patient on a gluten-containing diet* with normal/low IgA, then coeliac disease is unlikely. IgA endomysial ab test is done to confirm all new positives and equivocal results. 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)
Monitoring: IgA tTG
*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

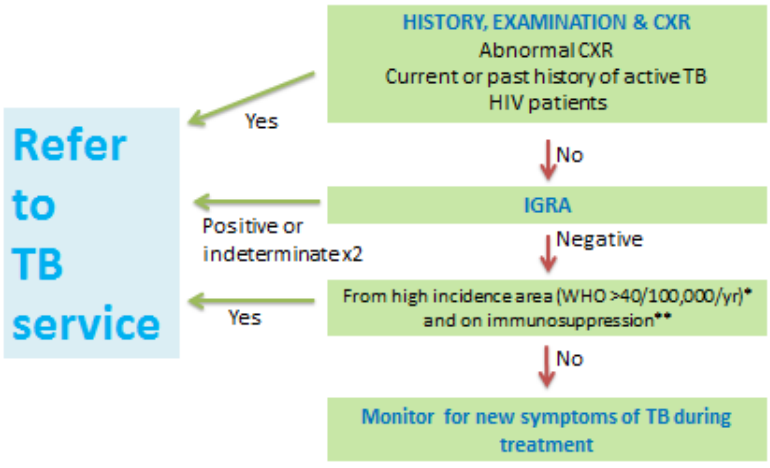
antibodies are very common, and often associated with infection. Another group have negative ANA and LKM antibodies. Sometimes pANCA and mitochondrial antibodies may be present.
Almost all patients with primary biliary cirrhosis have anti-mitochondrial antibodies. There are a number of patterns of mitochondrial 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.

***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:

- Serious infection e.g. severe chicken pox
- Prolonged i.e. difficult to treat infection
- Unusual infection and opportunistic infection e.g. staphylococcal liver abscess or pneumonia, atypical TB, pneumocystis
- Recurrent 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 which 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, for instance 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. **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




* 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).

groups.

***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 6-24 hours after. These patients should be referred to the allergy clinic using the internal allergy referral form on the intranet. There are no investigations for food intolerance.

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

**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 |
| **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. The LA test is less sensitive but more reliable as it is more specific. Lupus anticoagulant test cannot be carried out on anticoagulated patients. |
| **Centromere** | Associated with the limited variant of systemic sclerosis, CREST, and may also occur in some patients with primary biliary cirrhosis. |
| **Cyclic citrullinated peptide** | Present in about 70% of patients with rheumatoid arthritis, probably less at presentation. More specific than RF. |
| **dsDNA** | 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.<br>dsDNA is automatically tested on ANAs >1/320. dsDNA will not be tested on negative ANAs. |
| **ENA**<br>**Extractable nuclear antigens** | • Sm — High specificity for SLE<br>• Ro — Sjogren's S, also subacute cutaneous lupus, neonatal lupus and SLE<br>• La — Sjogren's S and SLE<br>• RNP (no Sm) — one of the criteria for mixed connective tissue disease<br>• Scl70 — scleroderma<br>• Jo-1 — myositis, often more aggressive, with lung involvement<br>• Histone — drug-induced SLE<br>• Ribosomal P — neuropsychiatric SLE<br>ENA is automatically tested on ANAs >1/320. |
| **endomysial** | Highly specific (>95%) for coeliac disease. Used to confirm new positive tissue transglutaminase abs (tTG), and for equivocal tTGs. IgG endomysial staining can be used in IgA deficiency. |
| **Ganglioside, GD1b, GM1, GQ1b**<br>**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** | 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** | Associated with Goodpasture's S |
| **Glutamic acid decarboxylase** | Associated with Stiff-man S 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** | Quantiferon gold plus. Test for LATENT TB. Please see advice in earlier section. |
| **Intrinsic factor** | Supports a diagnosis of pernicious anaemia. 50-70% sensitive. |
| **Islet cell antibodies** | Present in 75% of type I diabetics at diagnosis, may be used to screen at |

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| | risk groups. Supports a diagnosis of autoimmune type I diabetes. |
| **Liver-kidney microsomal** | Associated with type II autoimmune hepatitis, but may occur in viral and drug-induced hepatitis. |
| **Mitochondrial** | M2 pattern mitochondrial abs are both specific and sensitive for primary biliary cirrhosis, although between 5-10% are mitochondrial ab negative. |
| **Neuronal and paraneoplastic** | 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.<br><br>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. |
| **Neutrophil cytoplasmic ANCA** | Anti-neutrophil cytoplasmic antibodies (ANCA) are used as a screen and the more specific MPO (pANCA) and PR3 (cANCA) enzyme antibodies are automatically tested on positive samples. cANCA in the absence of PR3 is unlikely to be significant. pANCA in the absence of MPO is commonly associated with inflammatory bowel disease and primary sclerosing cholangitis.<br>• Microscopic polyangiitis — usually MPO, may be PR3<br>• Wegener's Granulomatosis — PR3<br>• Churg-Strauss Syndrome — usually MPO, may be PR3<br>• Subacute bacterial endocarditis — may have cANCA and PR3<br>• Good pasture's (anti-GBM) — may also have ANCA, which is associated with better prognosis. |
| **nuclear abs**<br>**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.<br>All ANAs >1/320 are automatically tested for ENA and dsDNA.<br>No repeat testing within 6 months without prior discussion.<br>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. |
| **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. |
| **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 |

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| | between 5-10 unless symptomatic and consider treatment in those with TSH values >10. |
| **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. |
| **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<br>• 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.<br>• Low C4, normal C3 active SLE, C1 esterase inhibitor deficiency, cryoglobulinaemia, rarely hereditary deficiency.<br>• C3 and C4 low immune complex disease, e.g. SLE, sepsis, severe liver disease.<br>Normal C4 during an attack of angioedema excludes hereditary angioedema. |
| **Complement function CH50** | This tests the classical and terminal complement pathways and is really 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.<br>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.<br>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.<br>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** | See biochemistry handbook |

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| **free light chains** | |
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| **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.<br>Testing shortly after systemic reaction (within 6 wks) may be falsely low.<br>Low positive specific IgE for drugs should be interpreted with caution in patients with total IgE> 500. |
| **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. |

**APPENDIX I**

| AUTOIMMUNITY | Turn around times (calendar days) | Reference ranges | Type of assay |
|---|---|---|---|
| **Acetylcholine receptor antibodies** | Send away, 28 | Positive >0.5 nmol/L | |
| **Adrenal abs** | 7 | pos/neg | Indirect immunofluorescence |
| **Cardiolipin abs** | 7 | Positive >10 | ELISA |
| **Centromere** | 3 | Pos/neg | Indirect immunofluorescence |
| **Cyclic Citrullinated peptide (CCP abs)** | 3 | Positive>7 | Fluorescence Enzyme linked immunoassay |
| **dsDNA** | 4 | Positive >10 IU/ml | Fluorescence Enzyme linked immunoassay. Confirmation with crithidia by indirect immunofluorecence |
| **ENA Extractable nuclear antigens** | Screen (neg) 4 Identification 8 | Pos/neg | Fluorescence Enzyme linked immunoassay screen and immunoblot for confirmation and identification |
| **endomysial** | 7 | Pos/neg | Indirect immunofluorescence |
| **Ganglioside, GD1b, GM1, GQ1b** | Send away 28 days | Pos/neg<br>Anti-Glycolipid Antibody<br>GM1 (IgG & IgM): <1:500<br>GM2 (IgG & IgM): <1:500<br>GD1a (IgG & IgM): <1:500<br>GD1b (IgG & IgM): <1:500<br>GQ1b (IgG & IgM): <1:500 | |
| **Gastric parietal cell** | 3 | Pos/neg | Indirect immunofluorescence |
| **Glomerular basement membrane** | 3 | Positive >7 | Fluorescence Enzyme linked immunoassay |
| **Glutamic acid decarboxylase** | Send away 21 Days | Positive >10 iu/mL | |
| **Interferon gamma release assay** | Send away 14 | Pos/neg/indeterminate | ELISA |
| **Intrinsic factor** | 7 | Pos/neg | ELISA |
| **Islet cell antibodies** | 7 | Pos/neg | Indirect immunofluorescence |
| **Liver-kidney microsomal** | 4 | Pos/neg | Indirect immunofluorescence |
| **Mitochondrial** | 4 | Pos/neg | Indirect immunofluorescence |
| **Neuronal paraneoplastic** | 7 (screen) | Pos/neg | Indirect immunofluorescence, confirmation of identity by medical school, Birmingham. See appendix II |
| **Neutrophil cytoplasmic ANCA**<br>**MPO**<br><br>**PR3** | 3 | **Pos/neg**<br><br>**Positive >3.5(equiv 3.5-5)**<br>**Positive >2(equiv 2-3)** | Indirect immunofluorescence<br><br>Fluorescence Enzyme linked immunoassay |
| **nuclear abs ANA** | 3 | **Pos/neg** | Indirect immunofluorescence |
| **Oligoclonal bands** | Send away, 21 days | | |
| **Rheumatoid factor** | 3 | Positive >20 | Nephelometry |

| | | | |
|---|---|---|---|
| **Skin (intercellular and basement membrane )** | 7 | Pos/neg | Indirect immunofluorescence |
| **Skeletal muscle (tyrosine kinase)** | Send away 21 days | Pos/neg | Indirect immunofluorescence |
| **Smooth muscle antibody** | 4 | Pos/neg | Indirect immunofluorescence |
| **Thyroid (TPO)** | 7 | Positive >75 | ELISA |
| **Tissue transglutaminase (tTG)** | 5 | Positive >20CU (equivocal 20-30CU) | Chemiluminescence |
| | | | |
| **IMMUNODEFICIENCY** | | | |
| **C1 esterase inhibitor** | Send away 28 days | | |
| **Functional C1 esterase inhibitor** | Send away 28 days | | |
| **Complement C3 and C4** | 3 | Normal range<br>C3 75-165 mg/dL<br>C4 14-54 mg/dL | Nephelometry |
| **Complement function CH50** | 10 | Normal range 23-49 u/ml | Functional assay |
| **Complement alternative pathway function AP50** | Send away 28 days | Normal range 75-125% | |
| **Functional Igs, pneumococcal, haemophilus influenzae B, tetanus** | Send away 28 days | | |
| **IgG subclasses** | Send away 28 days | | |
| **Leukocyte immunophenotyping and functional studies** | TBNK 1 day<br><br>Further testing Send away 7 days | Varies with age: see Journal of Paediatrics; Mar 1997 p390 | Flow Cytometry (Haematology) |
| | | | |
| **ALLERGY** | | | |
| **Total IgE** | 7 | Levels vary with age. See report comments or contact laboratory. | Fluorescence Enzyme linked immunoassay |
| **Specific IgE** | 7 (Referred allergens 14 days, may be more for rare allergens) | Positive >0.35 KU/L | Fluorescence Enzyme linked immunoassay |
| **Tryptase** | Send away 21 days | Normal range 2 – 14 ug/l | |

**APPENDIX II**

| | |
|---|---|
| • AcetylCholine receptor abs<br>• Cellular immunology<br>• IGRA testing<br>• Respiratory burst | Regional Immunology and Clinical Chemistry<br>Birmingham Heartlands Hospital<br>Bordesley Green East<br>Birmingham, B9 5SS<br>Tel: 0121 424 1185 |
| • IgG deamidated gliadin peptide<br>• Anti-ganglioside ab<br>• Anti-purkinje abs<br>• Oligoclonal bands<br>• Anti-GAD | Clinical Immunology service (and neuroimmunology)<br>The Medical School<br>Vincent Drive<br>Edgbaston<br>Birmingham, B15 2TT<br>Tel: 0121 414 3824 |
| • Neuroimmune antibodies<br>• Anti MAG abs<br>• Anti MOG abs<br>• Anti voltage gated K channel abs<br>• Anti voltage gated Ca channel abs<br>• Anti Aquaporin 4 abs<br>• NMDA receptor abs<br>• MUSK (muscle specific kinase) | Department of Immunology<br>Churchill Hospital<br>Old road<br>Headington<br>Oxford,OX3 7LJ<br>Tel: 01865 225995 |
| • Pneumococcal antibodies serotype specific<br>• Tetanus antibodies<br>• Haemophilus Influenzae B | 2nd and 3rd Floors, Clinical Science Building<br>Central Manchester and Manchester Children's University Hospital Trust<br>Manchester Royal Infirmary<br>Oxford Rd, Manchester, M13 9WL.<br>Tel: 0161 276 4281 |
| • Basal ganglia abs<br>• Beta interferon neutralising ab | Neuroimmunology and CSF Laboratory<br>Room 917, Institute of Neurology<br>Queens Square House, 33 Queen Square<br>London. WC1N 3BG.<br>Tel: 020 3448 3814 |
| • Serum tryptase<br>• Specific IgE<br>• TSH receptor abs<br>• C3 nephritic factor<br>• Pituitary gland abs<br>• Ovarian abs<br>• Salivary gland abs<br>• PLA2R abs<br>• Insulin abs<br>• Striated muscle antibodies<br>• Beta 2 glycoprotein antibodies<br>• IgG subclasses<br>• C 1 inhibitor and function | Department of Immunology<br>PO Box 894<br>Northern General Hospital<br>Herries Road<br>Sheffield<br>S5 7YT<br>Tel 0114 271 5934 -Dr Wilde<br>Tel 0114 271 5552 -lab |
| • HLA typing for renal transplant patients<br>• HLA antibodies<br>• HLA cross matches | Tissue Typing Lab<br>NBS, Birmingham Blood centre<br>Vincent Drive<br>Edgbaston<br>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) | Cambridge life sciences, 14 St Thomas' Place, Cambridgeshire Business park, Ely, Cambridgeshire.1 CB7 4EX |