Immunology Laboratory

Queen Elizabeth University Hospital, Glasgow

User Handbook

Written by- Dr Sai Murng
Approved by- Dr Moira Thomas
Document – MP_3 version 2
Revision – 17/04/2018
Review- 17/04/2019
IMMUNOLOGY LABORATORY HANDBOOK

Contents

INTRODUCTION .................................................................................................................. 3
CONTACT DETAILS .......................................................................................................... 3
  Consultant Immunologists ......................................................................................... 3
  Laboratory Manager ................................................................................................. 3
  Departmental Secretary ............................................................................................. 3
  Laboratory Enquiries .................................................................................................. 3
  Postal Address ........................................................................................................... 3
  Normal Laboratory Working Hours ........................................................................... 3
CLINICAL IMMUNOLOGY SERVICES ............................................................................ 4
  Immunodeficiency Clinics .......................................................................................... 4
  Allergy Clinics ............................................................................................................ 4
SAMPLES / REQUESTS / RESULTS .............................................................................. 5
  Sample Identification Requirements .......................................................................... 5
  High Risk Samples ..................................................................................................... 5
  Urgent Samples (must be discussed with immunology staff) ....................................... 5
  Electronic Requesting of Immunology Tests ............................................................. 5
  Reports and Results .................................................................................................... 5
  Repeat requests ......................................................................................................... 6
IMMUNOLOGY TESTS .................................................................................................... 7
  Allergy / Hypersensitivity Tests ................................................................................ 7
  Autoantibodies .......................................................................................................... 11
  Immunochemistry .................................................................................................... 27
  Cellular Studies ........................................................................................................ 33
GUIDE TO APPROPRIATE INVESTIGATIONS .............................................................. 35
INTRODUCTION

The Immunology Department comprises both Clinical and Laboratory Services. The Immunology Laboratory is based at the Queen Elizabeth University Hospital. During routine hours a member of the medical staff is available for consultation and provision of clinical advice. We are happy to answer enquiries about the use & interpretation of test results. A limited out of hours service is provided on weekend mornings to support the cardiac transplant service.

CONTACT DETAILS

Consultant Immunologists
Dr M J Thomas (Medical)
0141 232 7693 or ext 67693

Dr Sai Murng (Medical)
0141 232 7693 or ext 67693

Mrs Lauren Hennessy (Clinical Scientist)
0141 232 7693 or ext 67693

Laboratory Manager
Mrs Sylvia Arthur
0141 354 9103 or 89103

Departmental Secretary
Ms Yvonne Brown
Telephone 0141 232 7693 or ext 67693

Laboratory Enquiries
01413478872 Ext 68872

Postal Address
Department of Immunology
1st Floor, Laboratory Medicine & Facilities Management Building
Queen Elizabeth University Hospital
Govan Road
Glasgow
G51 4TF

Normal Laboratory Working Hours
0850 to 1650 Monday to Friday
CLINICAL IMMUNOLOGY SERVICES

Immunodeficiency Clinics
A comprehensive service is provided for the investigation and management of adults with suspected or confirmed primary immunodeficiency (including hereditary angioedema/ C1 inhibitor deficiency). Outpatient clinics are held at West Glasgow Ambulatory Care Hospital, Dalnair Street, G3 8SJ. Day ward facilities are available at Gartnavel General Hospital for patients requiring regular immunoglobulin replacement therapy and a home therapy training programme is taken place. Paediatric Immunodeficiency services are based at the Royal Hospital for Children.

Allergy Clinics
Allergy clinics are not provided directly by the Immunology department, although Consultant Immunologists contribute to the service. Adults with allergic problems may be referred either to the appropriate organ-based specialty or to the Anaphylaxis Service at the West Glasgow Ambulatory Care Hospital. Paediatric Allergy services are based at the Royal Hospital for Children.
SAMPLES / REQUESTS / RESULTS

Samples for immunology tests must provide patient identifiers, test(s) required and the return destination for the result. Inadequately labelled samples will be discarded. Samples accompanied by incomplete forms will not be processed. CHI number is essential for results to appear on SCI store and clinical portal.

Sample Identification Requirements

SAMPLES MUST HAVE
- patient’s full name (or proper coded identifier)
- date of birth and/or hospital or CHI number
- date and time of sample are essential for anaesthetic reactions and other serial samples.

REQUEST FORMS MUST HAVE
- patient’s full name (or proper coded identifier)
- date of birth and CHI number (if CHI unavailable, hospital number or patient’s address)
- destination for report
- name of patient’s consultant or GP
- tests required
- date and time of sample for anaesthetic reactions, cellular and complement tests

DESIRABLE
- relevant clinical information
- name and contact/bleep number of requesting clinician

High Risk Samples

Please contact laboratory to discuss patients with suspected viral haemorrhagic fever prior to sending any samples. Request forms and samples should also be labelled with yellow ‘Danger of Infection’ stickers and the nature of the hazard stated clearly on forms.

Urgent Samples (must be discussed with immunology staff)

There is a limited out-of-hours immunology service on weekend mornings for cardiac transplant samples. No other out-of-hours service is provided. Please contact the laboratory to discuss all urgent tests – writing ‘urgent’ on request forms is insufficient.

Electronic Requesting of Immunology Tests

Please use electronic requesting where available. Please contact IT for further advice if needed.

Reports and Results

95% of results are normally available within stated target turnaround times; samples requiring additional work such as titrations may take longer. Further details are provided in “Immunology Tests” below. Results are available on the Clinical Portal and Greater Glasgow & Clyde SCI store. Additionally results are sent out by internal or royal mail with the exception of sites which have opted for a paperless/electronic report only service. Please note that the laboratory computer system cannot generate extra ‘copy to’ reports.
Repeat requests
The laboratory uses request intervention software to minimise unnecessary repeat testing. The time interval is recorded under the individual tests below. All requests for repeat tests are checked by a member of staff and those with a valid reason for repeat testing are re-instated. Therefore if you require a repeat test, please ensure that the reason that the test needs to be repeated within this time interval is clearly stated on the request form or phone laboratory to discuss. Rejected tests are reported out through the normal channels.

The following tests should be sent directly to Biochemistry:

1. Bence-Jones Protein / Urinary Free Light chains
   Sample: 20mL urine in **plain preservative free container**

2. Immunoglobulins & electrophoresis
   Sample: 5 mL clotted, gel activated, blood (yellow top)

3. Cryoglobulin
   Sample: specific arrangement & flask is required - contact biochemistry before taking samples
IMMUNOLOGY TESTS

This section describes information, indications and interpretation of individual tests. Tests marked * are sent away.

**Allergy / Hypersensitivity Tests**

**TOTAL IgE**

<table>
<thead>
<tr>
<th>Sample:</th>
<th>serum 2 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method:</td>
<td>Fluorescence Enzyme Immunoassay (FEIA)</td>
</tr>
<tr>
<td>Turnaround:</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Normal range:</td>
<td>Adults &lt;120 kU/L</td>
</tr>
<tr>
<td>Repeat testing interval:</td>
<td>30 days</td>
</tr>
</tbody>
</table>

IgE binds to the high affinity receptors (FcɛRI) on mast cells, basophils, and eosinophil\(^1\). Allergen binding and cross-linking of these receptors may lead to degranulation and mediator release \(^2,3\). Serum concentration of IgE may be elevated in patients suffering from allergic asthma, allergic rhinitis or atopic eczema. The increase during childhood is slow, adult values are not reached until 15-20 years of age \(^1\). Raised total IgE levels can also be seen in patients with parasitic disease, Wiskott-Aldrich syndrome and Hyper-IgE syndrome. A normal IgE level does not exclude significant allergic disease. Monoclonal increase in IgE – see under paraproteins.

Reference:

**ALLERGEN SPECIFIC IgE (previously called ‘RAST’)**

<table>
<thead>
<tr>
<th>Sample:</th>
<th>serum 3 mL usually sufficient for 6-7 allergens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method:</td>
<td>Fluorescence Enzyme Immunoassay (FEIA)</td>
</tr>
<tr>
<td>Turnaround:</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Normal range:</td>
<td>&lt; 0.35 kU/L</td>
</tr>
<tr>
<td>Repeat testing interval:</td>
<td>30 days</td>
</tr>
</tbody>
</table>

These should be requested on the basis of a clinical history compatible with an IgE mediated allergic reaction. Typically this involves immediate allergy symptoms usually within an hour of exposure to the potential allergen. Testing is rarely of any value in the investigation of chronic urticaria or non-specific abdominal symptoms such as bloating. Test sensitivity and specificity varies between allergens. The presence of allergen specific IgE indicates sensitisation to the culprit allergen but does not necessarily imply clinical allergy. Negative results do not exclude allergy completely. Results should always be interpreted in the context of the clinical history.

Reference:
**ALLERGEN COMPONENT SPECIFIC IgE**

Sample: serum 3 mL usually sufficient for 6-7 allergens  
Method: Fluorescence Enzyme Immunoassay (FEIA)  
Turnaround: 3 weeks  
Normal range: < 0.35 kU/L  
Repeat testing interval: N/A

In conventional measurement of allergen specific IgE, the target allergen usually contains a mixture of allergenic proteins and peptides. In allergen component specific IgE testing the target allergens consist of single purified peptides. This can aid risk assessment of clinical allergy and can also help determine if sensitisation is primary or secondary to cross-reactive allergens. A limited range of component specific IgE tests are only available following formal assessment by an allergist or immunologist.

**IMMUNOCAP ISAC**

Sample: serum 5mL  
Method: Multiplexed immunoassay  
Turnaround: Sendaway  
Repeat testing interval: N/A

ImmunoCAP ISAC is a biochip based test using multiplexed component resolved diagnostic techniques to measure allergen specific IgE to a fixed panel of 112 components from 51 allergen sources in a semi-quantitative manner. This test can be useful in the investigation of idiopathic anaphylaxis. The test is only available following assessment by an allergist or immunologist and requires a formal cost approval from the service manager of the requesting clinician.

**TRYPTASE**

Sample: Serum 5mL  
Method: Fluorescence Enzyme Immunoassay (FEIA)  
Turnaround: 2 weeks  
Normal range: 0 – 13 µg/L  
Repeat testing interval: N/A

If samples will not reach the immunology laboratory within 3 days, they should be sent to the local Biochemistry lab to be separated, frozen and forwarded to Immunology lab the next working day.

Anaesthetic reactions / anaphylaxis – send 3 timed samples; proforma request form available

- Sample 1- at ~30mins (immediately after resuscitation)  
- Sample 2- at 1- 2 hrs (or as soon as possible after this)  
- Sample 3- at ~24hrs after onset of reaction.  
Post mortem samples – take as soon as possible after death

Note, resuscitation ALWAYS takes priority over collection of samples.  
State the time interval between reaction and blood sample on request form.  
Please provide information about nature of reaction and potential triggers.
The laboratory may add other tests depending upon the information provided – these may include C3 & C4, IgE to latex, chlorhexidine, ethylene oxide, suxamethonium, penicillins.

Tryptase typically peaks 1-2 hours post reaction returning to normal within 24 hours. However rises are not seen in all anaphylactic reactions especially those triggered by food. Reactions may be caused by a range of agents including anaesthetic drugs, other drugs (e.g. antibiotics, premedication), plasma expanders, chlorhexidine or latex. Results do not affect the immediate management. Persistently elevated tryptase levels may indicate an underlying systemic mast cell disorder. Close liaison with the laboratory is advised in the interpretation of results. West of Scotland patients may be referred to Anaphylaxis Service, West Glasgow Ambulatory Care Hospital. UK guidelines available at www.aagbi.org or www.bsaci.org.

**Post mortem samples**

Post mortem samples – blood from a peripheral vein (e.g. femoral veins) is preferred. Take the sample as soon as possible after death. Tryptase may be high in intra-cardiac samples after CPR/trauma. In addition tryptase levels tend to rise post mortem.

**Suspected mastocytosis / other mast cell disorders**

Please provide clinical details and state clearly that this is a random sample on request form. Normal tryptase levels do not completely exclude mast cell disorders.

**Reference:**


**ASPERGILLUS SEROLOGY (IgG and IgE antibodies to Aspergillus plus total IgE level)**

<table>
<thead>
<tr>
<th>Sample:</th>
<th>serum 2 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method:</td>
<td>Fluorescence Enzyme Immunoassay (FEIA)</td>
</tr>
<tr>
<td>Turnaround:</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Normal range:</td>
<td>IgG aspergillus – 0 – 40 mgA/L</td>
</tr>
<tr>
<td></td>
<td>IgE to aspergillus 0 – 0.35 kU/L</td>
</tr>
<tr>
<td></td>
<td>Total IgE (adults) 0 – 120 kU/L</td>
</tr>
<tr>
<td>Repeat testing interval:</td>
<td>30 days</td>
</tr>
</tbody>
</table>

Aspergillus IgG & IgE antibodies can be associated with aspergilloma, allergic bronchopulmonary aspergillosis (ABPA), extrinsic allergic alveolitis (EAA) and are a known complication of cystic fibrosis (CF). These antibodies indicate immune response to a prior or ongoing exposure to the antigen in question. A positive test should not
be, of itself, interpreted as representing a pathologic state. The absence of antibodies does not exclude the diagnosis since antibodies reduce when the disease is not in an acute state. Aspergillus IgG antibodies are sometimes termed Aspergillus precipitins.

Reference:

**AVIAN PRECIPITINS - IgG to Pigeon**

<table>
<thead>
<tr>
<th>Sample:</th>
<th>serum 2 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method:</td>
<td>Fluorescence Enzyme Immunoassay (FEIA)</td>
</tr>
<tr>
<td>Turnaround:</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Normal range:</td>
<td>0 – 10 mgA/L</td>
</tr>
<tr>
<td>Repeat testing interval:</td>
<td>30 days</td>
</tr>
</tbody>
</table>

Positive levels indicate exposure to pigeon antigens and may be associated with Pigeon Fancier’s Lung, a form of extrinsic allergic alveolitis. High levels may be found in severe acute disease. The presence of IgG precipitating antibodies is regarded as evidence of inhalational exposure to these antigens.

Reference:

**FARMER’S LUNG SEROLOGY - IgG to M. FAENI**

<table>
<thead>
<tr>
<th>Sample:</th>
<th>serum 2 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method:</td>
<td>Fluorescence Enzyme Immunoassay (FEIA).</td>
</tr>
<tr>
<td>Turnaround:</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Normal range:</td>
<td>0 – 10 mgA/L</td>
</tr>
<tr>
<td>Repeat testing interval:</td>
<td>30 days</td>
</tr>
</tbody>
</table>

Positive levels indicate exposure to the fungus *M. faeni* and may be associated with Farmer’s Lung. High levels may be found in severe acute disease.

Reference:
Autoantibodies

ANA - See under Nuclear Antibodies

ANCA - See under Neutrophil Cytoplasmic Antibodies

ADRENAL ANTIBODIES
Sample: serum 2 mL
Method: Indirect Immunofluorescence (IIF)
Turnaround: 4 weeks
Normal result: Negative
Repeat testing interval: 30 days

Adrenal antibodies are positive in up to 80% of Addison’s disease. Adrenal antibodies may also be detectable prior to development of adrenal failure. Positive adrenal antibodies in the context of autoimmune polyglandular autoimmune syndrome type 1 indicate 92% likelihood of developing of adrenal insufficiency. They may also be found in autoimmune ovarian failure.

Reference:

BETA 2–GLYCOPROTEIN 1 ANTIBODIES* (B2 GP1 antibodies)
Sample: serum 2 mL
Method: Fluorescence enzyme immunoassay (FEIA)
Turnaround: send away

The measurement of beta-2-glycoprotein 1 (B2 GP1) antibodies may be useful in patients suspected of having antiphospholipid syndrome who have negative results for lupus anticoagulant and cardiolipin antibodies (see under cardiolipin antibodies).

C1q ANTIBODIES*
Sample: serum 2 mL
Method: Enzyme Linked Immunosorbent assay (ELISA)
Turnaround: send away

C1q antibodies may be found in patients with Hypocomplementaemic Urticarial Vasculitis (HUV; C3 & C4 levels also very low). They are also found in patients with SLE and are a marker of renal involvement in SLE. Patients
without C1q abs have a low risk of developing lupus nephritis. In contrast, high titres of C1q abs indicate a high risk in developing lupus nephritis. Successful treatment of lupus nephritis typically decreases C1q ab titres.

Reference:

C3 NEPHRITIC FACTOR*
Sample: serum 2 mL
Method: Immunoelectrophoresis
Turnaround: send away

C3 nephritic factor is an IgG autoantibody which stabilises the alternate pathway C3 convertase (C3bBb), thereby permitting continual activation of the alternative complement pathway. Therefore most patients will have a low C3. Conversely, a normal C3 level makes C3 nephritic factor unlikely. The test should only be requested in patients with unexplained low C3, clinical features of partial lipodystrophy or unexplained glomerulonephritis. This test is not indicated in the routine investigation of chronic kidney disease.

Reference:

CARDIAC MUSCLE ANTIBODIES*
Sample: serum 2 mL
Method: Indirect Immunofluorescence (IIF).
Turnaround: send away

These antibodies are of limited clinical significance. Cardiac muscle antibodies are described in patients with Dressler’s syndrome after myocardial infarction, cardiomyopathy, myocarditis and in patients who have undergone cardiac surgery or have had rheumatic fever. The presence of these antibodies can occur without Dressler’s syndrome.

Reference:
CARDIOLIPIN ANTIBODIES (IgG & IgM)
Sample: serum 2 mL
Method: Fluorescence enzyme immunoassay (FEIA)
Turnaround: 4 weeks
Normal range: IgG cardiolipin ab 0 - 10 GPL-U/mL
IgM cardiolipin ab 0 - 10 MPL-U/mL
Repeat testing interval: 30 days

Cardiolipin antibodies are associated with Anti-Phospholipid Syndrome (APS), idiopathic spontaneous abortion and Systemic Lupus Erythematosus (SLE). Patients suspected of having APS should also be tested for lupus anticoagulant (send sample to Haematology; see handbook for details).

The International Society on Thrombosis and Haemostasis statement defined the diagnosis of APS based on the presence of at least one of the clinical criteria (vascular thrombosis or APS pregnancy morbidity) and one of the following laboratory criteria:

1. Lupus anticoagulant present in plasma, on two or more occasions at least 12 weeks apart.
2. Cardiolipin antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titre (i.e. >40GPL-U/mL), on two or more occasions, at least 12 weeks apart.
3. Beta 2–glycoprotein 1 antibody of IgG and/or IgM isotype in serum or plasma (in titre >the 99th centile), present on two or more occasions at least 12 weeks apart.

IgG cardiolipin antibodies are the most prevalent and demonstrate the greatest clinical correlation. The significance of isolated IgM cardiolipin antibodies is uncertain. Cardiolipin antibodies are frequently detected in syphilis, HIV infected patients and other viral, bacterial and parasitic infections but are not correlated with thrombosis risk or haematological manifestations of APS.

Reference:

CYCLIC CITRULLINATED (CCP) ANTIBODIES: only available for GGC rheumatology service
Sample: serum 2 mL
Method: Fluorescence enzyme immunoassay (FEIA)
Turnaround: 2 weeks
Normal range: 0 – 7 U/mL
Repeat testing interval: 30 days

This test is currently only funded for the GGC rheumatology service. NICE guidance recommends rheumatoid factor (RhF) as the initial investigation for rheumatoid arthritis (RA) in adults. CCP antibodies are more specific for RA and may appear early in the disease process. However CCP antibodies can be positive in other settings and negative CCP antibodies do not exclude RA.

Reference:

**CENTROMERE ANTIBODIES** (included in ANA screen)
Sample: serum 2 mL
Method: Indirect immunofluorescence (IIF) microscopy on Hep2000 cell line.
Turnaround: negative results available in 1 week; samples requiring confirmation take 4 weeks.
Normal result: Negative
Repeat testing interval: 1 year

Performed as part of the standard ANA screen (see under nuclear antibodies) i.e. ‘ANA negative’ means centromere antibodies are also negative.

Centromere antibodies are characteristic of the CREST syndrome, a variant of systemic sclerosis with limited skin involvement but associated with Calcinosis, Raynaud’s phenomenon oEsophageal immobility, Sclerodactyly and Telangectasia.

**dsDNA ANTIBODIES**
Sample: serum 2mL
Method: Fluorescence enzyme immunoassay (FEIA) used to screen samples
Crithidia Indirect Immunofluorescence (IIF) used for confirmation on new positives
Turnaround: 3 weeks for initial FEIA result; further 1 week for confirmatory IIF result
Normal range: FEIA immunoassay for dsDNA abs 0 - 10 IU/mL
Crithidia – normal result is negative
Repeat testing interval: 30 days

Antibodies to native double stranded DNA (dsDNA) are characteristic of SLE and titre may vary with disease activity. However they are only found in 40-60% of SLE patients. dsDNA abs may also be found in autoimmune hepatitis, rheumatoid arthritis and sometimes apparently healthy individuals. Confirmatory testing is carried out using indirect immunofluorescence on Crithidia – this test only detects high avidity antibodies to native dsDNA so is more specific but less sensitive than the FEIA method.
dsDNA abs are rarely found if ANA is negative. Therefore ANA remains the best screening test for connective tissue disorders. dsDNA abs will be added routinely to any new positive ANA with titre of 1/160 or above.

dsDNA abs should only be requested for monitoring patients known to have SLE.

Reference:

ENDOMYSIAL ANTIBODIES (IGA OR IGG)
Sample: serum 2 mL
Method: Indirect Immunofluorescence (IIF)
Turnaround: 4 weeks
Normal result: Negative
Repeat testing interval: 5 months

IgA TTG antibodies are the first line test for coeliac disease (see under TTG antibodies). IgA endomysial abs cannot be requested directly as they are now only used within the laboratory as a confirmatory follow on test for new positive or equivocal IgA TTG samples. IgG endomysial abs should only be requested in patients known to have IgA deficiency. The sensitivity and specificity of IgG EMA for coeliac disease is less than IgA based tests therefore a negative result does not exclude coeliac disease. There is no role for IgG endomysial abs in patients with normal IgA levels.

Reference:

EXTRACTABLE NUCLEAR ANTIGENS (ENA) ANTIBODIES
Sample: serum 2 mL
Method: Fluorescence enzyme immunoassay (FEIA)
Turnaround: 3 weeks (screening), further 1 week for identification of positives
Normal result: Negative
Repeat testing interval: 2 years

ENA antibodies are routinely performed on any new positive ANA or 1:160 or above.

Their presence is strongly associated with connective tissue diseases (CTD) although they are only positive in a subset of patients. Positive ENA antibodies are rarely found in the absence of a positive ANA. Therefore ANA is
recommended as the initial screening test and ENA should only be requested in selected patients with neonatal heart block or strong suspicion of CTD/dermatomyosistis. Direct requests for ENA abs will be tested ANA instead unless the clinical details provide a clear indication for ENA testing. Please contact laboratory to discuss testing if required.

ENA screen includes abs to Ro52, Ro60, La, Sm, RNP, Jo-1, Scl-70 and Centromere B (CENPB). ENA confirmation also includes ribosomal P abs. Jo-1 and Ribosomal P abs can be present without a positive ANA.

<table>
<thead>
<tr>
<th>ENA</th>
<th>Disease Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ro52</td>
<td>Isolated Ro52 antibodies are associated with SLE, rheumatoid arthritis, systemic sclerosis, Sjogren’s syndrome, myositis, interstitial lung disease and autoimmune liver disease</td>
</tr>
<tr>
<td>Ro60</td>
<td>SLE (particularly photosensitivity), cutaneous lupus, Sjogren’s syndrome neonatal lupus and congenital heart block</td>
</tr>
<tr>
<td>La</td>
<td>SLE, Sjogren’s syndrome</td>
</tr>
<tr>
<td>SmD</td>
<td>SLE.</td>
</tr>
<tr>
<td>U1-RNP</td>
<td>SLE, Mixed Connective Tissue Disease (MCTD)</td>
</tr>
<tr>
<td>Jo-1</td>
<td>Polymyositis or dermatomyositis especially with respiratory involvement</td>
</tr>
<tr>
<td>Scl-70</td>
<td>Systemic Sclerosis (generalised scleroderma)</td>
</tr>
<tr>
<td>CENPB</td>
<td>CREST syndrome (limited scleroderma)</td>
</tr>
<tr>
<td>Ribosomal P</td>
<td>SLE</td>
</tr>
</tbody>
</table>

Reference:

GLUTAMIC ACID DECARBOXYLASE (GAD) ANTIBODIES

Sample: serum 2 mL
Method: Enzyme Linked ImmunoSorbent Assay (ELISA)
Turnaround: 4 weeks
Normal result <5 IU/mL

GAD antibodies may be found in newly diagnosed type 1 diabetes. NICE guidelines (2015) recommend diabetes-specific autoantibodies should not be used routinely to confirm type 1 diabetes in adults or children. Carrying out tests for two different diabetes-specific autoantibodies (GAD, IA2, islet cell, insulin) reduces the false negative rate. The GAD ab test is semi-quantitative and has no role in monitoring disease.

GAD antibodies are also associated with stiff person syndrome. Samples from patients with suspected stiff person syndrome should be sent to Neuroimmunology, see Neuroimmunology handbook for details.

Reference:
1. NICE Guideline NG17. Type 1 diabetes in adults: diagnosis and management. 2015.
2. NICE Guideline NG18. Diabetes (type 1 and type 2) in children and young people: diagnosis and management. 2015.
GASTRIC PARIELTAL CELL ANTIBODIES
Sample: serum 2 mL
Method: Indirect Immunofluorescence (IIF) on mouse liver/stomach/kidney
Turnaround: 2 weeks
Normal result: Negative
Repeat testing interval: 1 year

Occur in 95% of patients with pernicious anaemia and may be detectable prior to the development of clinically apparent disease. They also occur in up to 15% of the normal population. Mitochondrial antibodies may mask gastric parietal cell antibody – in this case intrinsic factor antibodies should be requested if pernicious anaemia is suspected.

Reference:

GLOMERULAR BASEMENT MEMBRANE (GBM) ANTIBODIES
Sample: serum 2 mL
Method: Fluorescence enzyme immunoassay (FEIA)
Turnaround: 1 week
Normal range: 0 – 7 U/mL
Repeat testing interval: NA

GBM abs target the non-collagenous domains of type IV collagen. Positive GBM abs are strongly associated with anti-GBM disease (previously called Goodpasture’s syndrome). These antibodies are pathogenic, so GBM ab titres follow disease activity. Patients with GBM antibodies may also have a positive P-ANCA, usually due to myeloperoxidase antibodies although the significance of this is unclear. ANCA and GBM abs should both be requested in patients with glomerulonephritis and/or pulmonary haemorrhage.

Reference:

HISTONE ANTIBODIES*
Sample: serum 2 mL
Method: Enzyme Linked ImmunoSorbent Assay (ELISA)
Turnaroud: send away
Histone antibodies may be found in up to 95% of patients with drug-induced lupus. These patients are usually ANA positive but dsDNA antibody and ENA antibody negative. Histone antibodies may also be found in SLE.

Reference:

**IA2 ANTIBODIES (ANTI-TYROSINE PHOSPHOTASE ANTIBODIES)**

Sample: serum 2 mL
Method: Enzyme Linked ImmunoSorbent Assay (ELISA)
Turnaround: send away

IA2 antibodies may be found in newly diagnosed type 1 diabetes. NICE guidelines (2015) recommend diabetes-specific autoantibodies should not be used routinely to confirm type 1 diabetes in adults or children. Carrying out tests for two different diabetes-specific autoantibodies (GAD, IA2, islet cell, insulin) reduces the false negative rate.

Reference:
3. NICE Guideline NG17. Type 1 diabetes in adults: diagnosis and management. 2015.

**INSULIN ANTIBODIES**

Sample: serum 2 mL
Method: Fluorescence enzyme immunoassay (FEIA)
Turnaround: send away

Insulin antibodies may be found in newly diagnosed type 1 diabetes. NICE guidelines (2015) recommend diabetes-specific autoantibodies should not be used routinely to confirm type 1 diabetes in adults or children. Carrying out tests for two different diabetes-specific autoantibodies (GAD, IA2, islet cell, insulin) reduces the false negative rate.

Insulin antibodies may also be produced as a secondary phenomenon response to exogenous insulin.

Reference:
5. NICE Guideline NG17. Type 1 diabetes in adults: diagnosis and management. 2015.

**INTRINSIC FACTOR ANTIBODIES**

Sample: serum 2 mL
Method: Enzyme Linked ImmunoSorbent Assay (ELISA)
Turnaround: 2 weeks
Normal range: 0 – 20 U/mL
Repeat testing interval: 30 days
Positive in 50-70% of patients with Pernicious Anaemia. Intrinsic Factor ab s are more specific for pernicious anaemia than gastric parietal cell abs. Unlike older intrinsic factor ab assays this method is not affected by B12 treatment. The intrinsic factor ab test is semi-quantitative and has no role in monitoring disease.

Reference:

ISLET CELL ANTIBODIES*

Sample: serum 2 mL
Method: Indirect Immunofluorescence (IIF)
Turnaround: send away

Found in newly diagnosed type 1 diabetes. May also be present prior to the development of clinical symptoms. NICE guidelines (2015) recommend diabetes-specific autoantibodies should not be used routinely to confirm type 1 diabetes in adults or children. Carrying out tests for two different diabetes-specific autoantibodies (GAD, IA2, islet cell, insulin) reduces the false negative rate. Positive islet cell abs in patients with apparent NIDDM, gestational diabetes or in relatives of IDDM patients indicate increased risk of developing IDDM.

Reference:
7. NICE Guideline NG17. Type 1 diabetes in adults: diagnosis and management. 2015.

LIVER ANTIBODIES – comprises Smooth Muscle, Mitochondrial, LKM & LC1 antibodies

Sample: serum 2 mL
Method: Indirect Immunofluorescence (IIF) on mouse liver/stomach/kidney
Turnaround: 2 weeks
Normal result: Negative
Repeat testing interval: 1 year

Found in autoimmune liver disease. The different combinations of antibodies are associated with different types of autoimmune liver disease (see below). Unusual staining patterns may be sent to King’s College Hospital, London for confirmational testing, which may include immunoblot for anti-M2, anti-LKM, anti-soluble liver antigen (SLA) and anti-LC1 antibodies.

Liver cytosol 1 (LC1) antibodies
Found in a sub-group of patients with autoimmune hepatitis.

Liver kidney microsomal (LKM) antibodies
Found in a sub-group of patients with autoimmune hepatitis and is associated with a particularly aggressive form of the disease, especially in children.
Mitochondrial antibodies
Occur in 95% of patients with primary biliary cirrhosis and may be detectable prior to the development of abnormal liver function. Low titres may also be found in chronic active hepatitis. Samples with atypical mitochondrial antibody patterns will be referred for immunoblot analysis to King’s College Hospital, London.

Smooth muscle antibodies
Found in autoimmune hepatitis, often in association with positive ANA and occasionally mitochondrial abs. May also occur in other settings eg viral infections especially EBV and Hepatitis A. Only actin pattern smooth muscle antibodies are reported.

Reference:

MYELOPEROXIDASE (MPO) & PROTEINASE 3 (PR3) ANTIBODIES
Sample: serum 2 mL
Method: Fluorescence Enzyme Immunoassay (FEIA)
Turnaround: 1 week
Normal range: MPO <3.5 IU/mL; PR3 <5.0 IU/mL
Repeat testing interval: 21 days

See Neutrophil Cytoplasmic Antibodies (ANCA) section.
ANCA should be requested for new patients in whom systemic vasculitis is suspected
MPO/PR3 abs should be requested for monitoring patients with known ANCA positive vasculitis.

NUCLEAR ANTIBODIES (ANA)
Sample: serum 2 mL
Method: Indirect immunofluorescence (IIF) microscopy on Hep2000 cell line
Turnaround: 1 week plus additional week if titration required
Normal result: Negative
Repeat testing interval: 1 year

ANA is indicated in suspected connective tissue disease or autoimmune liver disease. Centromere autoantibodies are detectable on the ANA screen and do not need to be requested separately. ENA and dsDNA autoantibodies will be requested automatically on all new positive ANAs with titre of 1/160 or above.

Autoantibody-mediated inflammation and cell destruction may affect many organs of the body. The ANA test identifies autoantibodies that target substances contained inside cells. It can also be used to screen autoantibodies directed against nuclear components and cellular components that are contained within the cell cytoplasm, outside of the nucleus. By itself, a positive ANA test does not indicate the presence of an autoimmune disease or the need for therapy.

ANA can be positive in 37% of healthy people at 1:40 dilution and in 5% at 1:160 dilution. Positive ANAs are particularly common in the over 65s. However a negative ANA at 1:40 makes connective tissue disease very unlikely.

Positive ANA can be associated with the following conditions:
1. Systemic autoimmune diseases - SLE, Sjogren's, Scleroderma, drug-induced lupus, polymyositis, dermatomyositis, rheumatoid arthritis, pauciarticular juvenile chronic arthritis, polyarteritis nodosum, mixed connective tissue disease
2. Organ specific autoimmune diseases - thyroid (Hashimoto’s thyroiditis, Grave’s disease), gastrointestinal (autoimmune liver disease, inflammatory bowel disease), pulmonary fibrosis
3. Infection - tuberculosis, schistosomiasis, viral hepatitis, parvovirus and other infections.
4. Miscellaneous - neoplastic disease, relative of person with autoimmune disease

Reference:

NEUTROPHIL CYTOPLASMIC ANTIBODIES – ANCA

Sample:   serum 2 mL
Method:  Indirect Immunofluorescence (IIF) on ethanol fixed human neutrophil slides.
Turnaround: 1 week plus additional week if ANA is needed to confirm pattern
Normal result: Negative
Repeat testing interval: 30 days

Urgent request for ANCA must be discussed with the duty immunologist at the earliest opportunity – 0141 232 8872 or ext 68872.

ANCA should be requested for the investigation and diagnosis of suspected ANCA-associated vasculitis. If ANCA is positive MPO & PR3 antibodies will be added automatically. MPO & PR3 antibodies may be requested directly for the monitoring of patients with known ANCA positive vasculitis with previous positive MPO or PR3 antibodies.

There are three main patterns – C-ANCA, P-ANCA and atypical ANCA. These patterns relate to different antigenic specificities eg proteinase 3 (PR3), myeloperoxidase (MPO). All positive ANCAs are tested for specificities against PR3 and MPO. For normal ranges and turnaround times, see MPO/PR3 section. A strongly positive ANA may mask the presence of a P-ANCA but will not affect the detection of MPO or PR3 antibodies.

C-ANCA antibodies are principally directed against PR3. Other C-ANCA specificities include cationic protein S7 and cathepsin G. P-ANCA autoantibodies are principally directed against MPO. Other P-ANCA antigen specificities are elastase and lactoferrin. A positive C- or P-ANCA with strongly positive PR3 or MPO antibodies is suggestive but not diagnostic of an ANCA associated vasculitis (see table below). However all types of ANCA have been reported in a wide range of other conditions including infection, neoplasia, inflammatory disease, cocaine use as well as vasculitis. Conversely ANCA is typically negative in other forms of vasculitis.
Pattern Antigen Disease Association
C-ANCA PR3 Granulomatosis with polyangiitis (Wegener’s granulomatosis)
P-ANCA MPO Systemic vasculitis eg Microscopic Polyangiitis Eosinophilic granulomatosis with polyangiitis (Churg Strauss) Crescentic glomerulonephritis
Atypical ANCA various Wide range of inflammatory, infective & neoplastic diseases but the clinical utility of atypical ANCs has not yet been established.

Reference:

OVARIAN ANTIBODIES*
Sample: serum 2 mL
Method: Indirect Immunofluorescence (IIF)
Turnaround: send away

These may be found in premature ovarian failure.

PARATHYROID ANTIBODIES*
Sample: serum 2 mL
Method: Indirect Immunofluorescence (IIF)
Turnaround: send away
Parathyroid antibodies are associated with autoimmune hypoparathyroidism. PHOSPHOLIPID ANTIBODIES - see under Cardiolipin antibodies

PITUITARY ANTIBODIES*
Sample: serum 2 mL
Method: Indirect Immunofluorescence (IIF)
Turnaround: send away

Pituitary antibodies may be seen in 30% of patients with autoimmune hypopituitarism and 70% of patients with lymphocytic hypophysitis. They may also be seen in a variety of other autoimmune conditions and in some non-autoimmune pituitary conditions including pituitary tumours.

Reference:

PHOSPHOLIPASE A2 (PLA2) RECEPTOR ANTIBODIES*
Sample: serum 2 mL
Method: Enzyme Linked ImmunoSorbent Assay (ELISA)
Turnaround: send away

Indicated in the investigation of primary membranous nephropathy. Primary membranous nephropathy may have an autoimmune component, with 70% of cases positive for PLA2 receptor antibodies. IgG antibody binding to PLA2 receptors on kidney podocytes may result in complement deposition and renal damage. While PLA2 receptor antibody testing may be useful in distinguishing primary from secondary membranous nephropathy and in disease monitoring, it should not be viewed as a replacement for renal biopsy.

References:

RHEUMATOID FACTOR (RhF)
Sample: serum 2 mL
Method: Latex-enhanced turbidimetry
Turnaround: 3 days
Repeat testing interval: 1 year

Normal result: 0 – 29 IU/mL
Weak positive 30-90 IU/mL
Used in the investigation of inflammatory arthropathies to differentiate sero-negative from sero-positive arthritides. In rheumatoid arthritis, high titres may be associated with extra-articular manifestations e.g. vasculitis and nodules. RhF is not useful for monitoring disease activity. RFs may occur in other connective tissue/autoimmune diseases, cryoglobulinaemia (may be very high titre), infections and in some healthy individuals (often low titre). A negative RhF does NOT exclude rheumatoid arthritis.

Reference:

SKELETAL MUSCLE ANTIBODIES*
Sample: serum 2 mL
Method: Indirect immunofluorescence (IIF)
Turnaround: send away

Skeletal muscle antibodies are typically seen in patients with both thymoma and myasthenia gravis. They may also occur in some patients with hepatitis, acute viral infections and polymyositis. Acetyl choline receptor antibody testing should be performed in the initial investigation of myasthenia gravis – please contact Neuroimmunology for details.

SKIN REACTIVE ANTIBODIES
Sample: serum 2 mL
Method: Indirect Immunofluorescence (IIF)
Turnaround: 4 weeks
Normal result: Negative
Repeat testing interval: 30 days

Two varieties are recognised:
- Intercellular substance antibodies - found in Pemphigus
- Basement zone antibodies - found in Bullous Pemphigoid and Epidermolysis Bullosa Acquista.

Reference:

SOLUBLE LIVER ANTIGEN (SLA) ANTIBODIES*
Sample: serum 2 mL
Method: Immunoblot
Turnaround: send away
May be the only antibody found in some rare forms of autoimmune hepatitis. These may also be seen in hepatitis C. These antibodies are not detected by the conventional liver antibody indirect immunofluorescence screen.

Reference:

THYROID ANTIBODIES
Now measured in biochemistry

TISSUE TRANSGLUTAMINASE ANTIBODIES (IgA TTG)
Sample:  serum 2 mL
Method:  Fluorescence enzyme immunoassay (FEIA)
Turnaround:  2 weeks
Normal range:  0 – 7 U/mL
Repeat testing interval:  5 months (155 days)

Please ensure patients have been consuming sufficient gluten at time of testing to ensure reliable results. False negative results may be found if patients have been eating gluten less often than twice a day everyday for the previous 6 weeks. If patients have not been consuming sufficient gluten, advise delay testing.

IgA TTG abs are the first line test for coeliac disease (NICE guidance 2015) and have a reported specificity and sensitivity of >95% in untreated coeliac disease, provided patients are consuming sufficient gluten at time of testing. IgA TTG abs may also be found in dermatitis herpetiformis. IgA endomysial antibodies (EMA) will follow automatically in all samples with a new positive or equivocal IgA TTG result. Rarely, IgA TTG can be falsely positive in patients with high total IgA levels due to liver disease or IgA paraproteinaemia; these patients are usually negative for IgA endomysial abs.

There is no longer any need to measure immunoglobulins routinely as the IgA TTG ab assay is able to flag up samples with low IgA levels. In these patients, IgA will be measured and if confirmed as low IgG endomysial abs will follow.

Please note that all coeliac serology is likely to be less reliable in patients with panhypogammaglobulinaemia.

Reference:

**Immunology**

**ALTERNATE & CLASSICAL PATH HAEMOLYTIC COMPLEMENT (AP100/CH100)**

Sample: Fresh serum 5 mL clotted blood (red top) to reach laboratory on day of venepuncture or separated and frozen on day of venepuncture and transported frozen

Method: Radial immunodiffusion haemolytic assay

Turnaround: 8 weeks

Normal range:
- CH100: 392 - 1019 CH100U/mL
- AP100: 66 - 129%

Repeat testing interval: NA

Complement function tests are useful as a screen for rare inherited deficiencies in the complement pathway. CH100 measures integrity of the classical and terminal pathways and AP100 measures the integrity of the alternate and terminal pathway, therefore the two tests are always done together to identify the presence and location of any deficiency. Since this is a functional assay, attention to sample collection advice is important to avoid in vitro degradation of complement. The test is also best done in convalescence rather than at times of high in vivo complement activity e.g. sepsis, active SLE.

Rare inherited deficiencies in the classical pathway predispose to sepsis and immune complex disease and deficiencies in the alternate and common terminal pathways predispose to Neisserial infections. Therefore indications for the test are recurrent/atypical meningococcal infection, systemic gonococcal infection, atypical immune complex disorders e.g. early onset atypical SLE or a family history of these. Contact the lab to discuss abnormal results and coordinate further testing at a specialist centre.

Normal AP100/CH100 results may not exclude properdin deficiency or partial Factor H or I deficiency – contact the laboratory for further advice if these are suspected.

CH100/AP100 is also useful in monitoring the efficacy of Eculizumab suppression of in vivo complement activity. Very low levels in a correctly handled sample (see sample requirements) suggest effective suppression of complement activity by Eculizumab.

Reference:
1. PRU Handbook of Clinical Immunochemistry. 9th Ed. 2007.

**C1 INHIBITOR (FUNCTION)**

Sample: Fresh blood 5 mL EDTA (purple top) to reach lab on day of venepuncture.

Method: Spectrophotometry

Turnaround: 8 weeks

Normal range: 70 – 130%

Repeat testing interval: NA
See comments under C1 inhibitor (quantitative). The functional assay is only required in individuals with a personal or family history of angioedema plus low C4 level and normal C1 inhibitor (quantitative) level. Samples must be separated and frozen within 4 hours of venepuncture.

**C1 INHIBITOR (QUANTITATIVE)**

Sample: serum 2 mL sample; (also request C3 & C4)

Method: Immunoturbidimetry

Turnaround: 1 week

Normal range: 0.19 – 0.36 g/L

Repeat testing interval: NA

C1 inhibitor measurement is recommended in patients with a personal or family history of isolated angioedema (urticaria is not a typical feature of C1 inhibitor deficiency). C3 & C4 should also be checked as C4 is typically low in all forms of C1 inhibitor deficiency and a completely normal C4 level essentially excludes this diagnosis. Patients with angioedema, low or borderline C4 but normal C1 inhibitor (quantitative) levels should have C1 inhibitor function checked.

Reference:


**C1Q***

Sample: serum 2 mL

Method: Radial Immunodiffusion (RID)

Turnaround: send away

C1q measurement is only indicated for the differentiation of hereditary from acquired C1 inhibitor deficiency. Note this test measures C1q and NOT anti-C1q antibodies and is of NO value in SLE.

**C1Q ANTIBODIES***

See under Autoantibodies.

**C3 and C4**

Sample: 5mL sample of clotted, gel activated, blood (yellow top)

Method: Immunoturbidimetry

Turnaround: 3 days

Normal range: age/sex related ranges in g/L
**C3 C4**

<table>
<thead>
<tr>
<th></th>
<th>C3</th>
<th>C4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male &lt;14 yrs</td>
<td>0.80 – 1.70</td>
<td>0.14 – 0.44</td>
</tr>
<tr>
<td>Female &lt;14 yrs</td>
<td>0.82 – 1.73</td>
<td>0.13 – 0.46</td>
</tr>
<tr>
<td>Male &gt;14 yrs</td>
<td>0.82 – 1.85</td>
<td>0.15 – 0.53</td>
</tr>
<tr>
<td>Female &gt;14 yrs</td>
<td>0.83 – 1.93</td>
<td>0.15 – 0.57</td>
</tr>
</tbody>
</table>

Repeat testing interval: N/A

C3 and C4 levels measurement is useful in the investigation / monitoring of patients with connective tissue disease/other inflammatory disorders. Serial measurements are typically more useful than single levels.

<table>
<thead>
<tr>
<th>C3</th>
<th>C4</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>High</td>
<td>Acute phase response</td>
</tr>
<tr>
<td>Low</td>
<td>Low</td>
<td>SLE and other immune complex disorders</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sepsis (eg subacute bacterial endocarditis)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemodilution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypocomplementaemic urticarial vasculitis</td>
</tr>
<tr>
<td>Low</td>
<td>Normal</td>
<td>Sepsis (eg Gram negative septicaemia)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post-streptococcal nephritis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Membranoproliferative glomerulonephritis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C3 nephritic factor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inherited deficiency of C3, Factor H or I (rare)</td>
</tr>
<tr>
<td>Normal</td>
<td>Low</td>
<td>C1 inhibitor deficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cryoglobulinaemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inherited deficiency of C4 null alleles (common in SLE)</td>
</tr>
</tbody>
</table>

**BENCE-JONES PROTEIN / URINARY FREE LIGHT CHAINS** (sent via biochemistry)

A urine sample should accompany ALL serum samples in cases of suspected myeloma since up to 20% of myeloma patients have no detectable paraprotein in the serum.

**C3 NEPHRITIC FACTOR**

See under Autoantibodies

**CRYOGLOBULINS** (collection/screening by biochemistry, typing of positives by immunology)

Sample: 10-20mL clotted blood collected & transported at 37ºC (contact biochemistry)

Method Typing by immunofixation and latex-enhanced turbidimetry (rheumatoid factor)

Turnaround: 3 weeks

Normal range: Absent

Repeat testing interval: N/A

CONTROLLED DOCUMENT
Cryoglobulin studies are indicated in the investigation of patients with features of hyperviscosity, Raynaud’s or unexplained vasculitis. Detectable cryoglobulins are typed within immunology to determine composition, clonality and rheumatoid factor activity.

**FUNCTIONAL (SPECIFIC) ANTIBODIES**

Sample: serum 2 mL  
Method: Enzyme Linked ImmunoSorbent Assay (ELISA)  
Turnaround: 3 weeks  
Normal range: depends upon exposure and immunisation history  
  - Hib abs – minimum protective level 0.15 mg/L, optimal protective level 1mg/L  
  - Tetanus abs – minimum protective level 0.15 IU/mL  
  - Pneumococcal abs – minimum protective level not established  
Repeat testing interval: 20 days

Functional antibodies comprise antibodies to tetanus toxoid, pneumococci and Hib and are indicated as part of the investigation of suspected immunodeficiency. Levels of antibodies depend upon both exposure and immunisation. Interpretation of results should be in context of clinical picture, age and exposure/immunisation history. Where levels are low, test immunisation may be carried out to assess response. Post immunisation levels should be checked 4-6 weeks after administration. Please note that Hib refers to Haemophilus influenza b which causes systemic infection e.g. meningitis, epiglottitis and NOT the non-typeable Haemophilus influenzae commonly associated with respiratory infections.

**IgD**

Sample: serum 2 mL  
Method: Enzyme Linked ImmunoSorbent Assay (ELISA)  
Turnaround: send away

This is only of value in the assessment of rare periodic fever syndromes. Immunofixation vs IgD – see under Paraprotein.

**IgG SUBCLASSES**

Sample: serum 2 mL  
Method: Nephelometry  
Turnaround: send away

IgG subclasses may be requested in patients with suspected IgG4 disorders such as autoimmune pancreatitis.

**IMMUNOGLOBULINS - IgA, IgA, IgM & ELECTROPHORESIS** (now sent via biochemistry)

Immunoglobulins & electrophoresis are useful in the investigation of suspected immunodeficiency and lymphoproliferative diseases. A myeloma screen order set is available in the trakcare and GP order comms systems - search on ‘myeloma’.  
**Immune deficiency** - a wide range of immunoglobulin abnormalities can be seen in antibody deficiency and levels may be normal or even raised in other forms of immunodeficiency (e.g. T cell or neutrophil defects).
Therefore suggest discuss further investigation with an immunologist if there are clinical features of immune deficiency – eg unexplained serious, persistent, unusual or recurrent infections.

**Polyclonal elevations in immunoglobulins** occur in a variety of disorders including chronic infectious/inflammatory conditions and liver disease.

**Paraproteins** - if a paraprotein is detected, it will be typed and quantified. Immunofixation for IgD & IgE is available – referral labs requiring this test for further assessment of suspected light chain paraproteins should ensure that they request ‘immunofixation for IgD & IgE’ to avoid confusion with requests for quantitation of total IgD or IgE.

**Malignant Paraproteins** — are usually, but not always, of high concentration, associated with low levels of the non-paraprotein immunoglobulins (immunoparesis) and with the presence of free monoclonal light chains in the urine (Bence-Jones Protein). Most often occur in multiple myeloma but may also be seen in other lymphoproliferative diseases e.g. Waldenstrom’s Macroglobulinaemia, Chronic Lymphocytic Leukaemia and Non-Hodgkin’s Lymphoma.

**Monoclonal gammopathy of undetermined significance (MGUS)** – these are paraproteins found in patients without an identifiable underlying disease. The paraprotein is usually small and not accompanied by immunoparesis or free urinary light chains (BJP). MGUS may be caused by the same group of conditions which cause a polyclonal increase in immunoglobulins. MGUS may ultimately undergo malignant transformation (1-2% per annum).

Reference:

**SERUM FREE LIGHT CHAINS (sFLC)**
Sample: 5mL clotted blood
Method: Turbidimetry
Turnaround: 1 week
Normal range: serum free kappa 3.3 – 19.4 mg/L
serum free lambda 5.7 – 26.3 mg/L
K/L ratio 0.26 – 1.65. (up to 0.49 - 3.0 in renal impairment)
Repeat testing interval: 18 days

SFLC is indicated for monitoring of light chain or non-secretory myeloma, AL-amyloidosis, assessment of prognosis of MGUS. Serum free light chain test is not suitable for routine myeloma screening and a normal result does not exclude myeloma. If screening for myeloma send blood for immunoglobulins & electrophoresis PLUS urine for electrophoresis (BJP) – a myeloma screen order set is available in the trakcare and GP order comms systems (search on ‘myeloma’). Serum free light chains are also not indicated for the routine follow up of MGUS. In settings where there is immune stimulation (e.g. sepsis, inflammatory disorders etc) or renal
impairment causing reduced clearance of light chains, then both kappa and lambda light chains increase. In this setting the ratio remains similar.

Reference:
Cellular Studies

LYMPHOCYTE PHENOTYPING/SUBSETS

Lymphocyte subsets
Sample: 4ml EDTA blood to reach lab within 20 hours & before 3pm on Fridays. Do not refrigerate samples as this lowers the CD4 count.
Method: Flow cytometry
Turnaround: 1 week
Normal range: age specific normal ranges will be provided on the reports.
Repeat testing interval: N/A

- Indicated in the evaluation and monitoring of primary and secondary immunodeficiency disorders including HIV infections and therapies such as Rituximab and anti-thymocyte globulin.
- Please note that a CD4 count is an unreliable and unacceptable alternative to HIV testing.
- For suspected immunodeficiency patients, prior discussion with the laboratory is recommended to enable selection of the appropriate panel.

LYMPHOCYTE FUNCTION

Sample: 5-7ml lithium heparin blood from patient AND a healthy control (label this bottle ‘CONTROL’) to reach laboratory before 3.00pm (Monday, Tuesday and Friday) on the day of venepuncture. Prior arrangement with the laboratory is recommended. Samples cannot be processed on Wednesdays or Thursdays. Do not refrigerate samples. Samples without controls will not be analysed.
Method: mitogen driven proliferation assay with thymidine incorporation
Turnaround: 2 weeks
Repeat testing interval: N/A

Indication indicated in investigation of suspected cellular immunodeficiency- contact immunologists for advice on the interpretation of individual test results.

Reference:
NEUTROPHIL FUNCTION

Sample: 4ml EDTA blood from both patient AND a healthy control (label this bottle ‘CONTROL’). Prior arrangement with the laboratory is recommended. **Sample to reach laboratory before 3.00pm on day of venepuncture. Do not refrigerate samples. Samples without controls will not be analysed.**

Method: Dihydrorhodamine flow cytometry based assay

Turnaround: 1 week

Repeat testing interval: N/A

Neutrophil function test is indicated in suspected Chronic Granulomatous Disease (CGD). Assessment of neutrophil respiratory burst (replaces the NBT test). This assay checks the respiratory burst activity of neutrophils which is impaired in CGD due to a genetic defect in one of the components of the NADPH-oxidase complex that produces reactive oxygen intermediates. Note - neutrophil function cannot be reliably assessed if the neutrophil count is less than $1 \times 10^9$/L.

Reference:

1. Mauch L, et al. Chronic Granulomatous Disease (CGD) and complete myeloperoxidase deficiency both yield strongly reduced dihydrorhodamine 123 test signals but can be easily discerned in routine testing for CGD. Clin Chem. 2007. 53:890-896.

GUIDE TO APPROPRIATE INVESTIGATIONS

Allergy
Allergen specific IgE - must specify allergen(s)
Contact lab for list of available allergens if required

Anaesthetic reactions
3 samples ~30 mins, 1-3 hrs, 24 hrs after onset of reaction
If not requesting via trakcare, suggest use proforma request form

Angioedema (no urticaria)
C1 inhibitor level (quantitative), C3, C4

Arthritis
ANA, Rheumatoid factor

Autoimmune liver disease
Liver abs (mitochondrial, smooth muscle, LKM)
ANA, immunoglobulins

Coeliac Disease
Tissue transglutaminase IgA abs (TTG abs)
If known IgA deficiency – Endomysial IgG abs

Connective tissue disease
Initial screen – ANA, C3 & C4
Monitoring SLE - C3 & C4, dsDNA
Pregnancy –ANA, C3 & C4, ENA, cardiolipin antibodies

Glomerulonephritis (acute)
ANCA, ANA, GBM, C3 & C4
Consider cryoglobulins, myeloma screen

Immunodeficiency
Contact laboratory / medical staff for advice
Immunoglobulins and electrophoresis
Functional abs
Consider CH100/AP100, Lymphocyte subsets and other cellular assays

Myeloma screen
Serum for Immunoglobulins & electrophoresis
Urine for Bence Jones Protein

Urticaria
Allergen specific IgE rarely helpful unless intermittent
short episodes and possible trigger identifiable from history
Investigations are usually for checking the differential
diagnoses based on the clinical presentation (e.g. ANA
for urticarial vasculitis).
Patient leaflet & guidelines available at www.bad.org.uk
Guidelines for diagnosis and management

Vasculitis
ANCA, ANA, C3&C4 and consider cryoglobulins
If renal involvement -see also ‘glomerulonephritis tests’
If thrombosis is prominent, also consider cardiolipin antibodies.