Disclaimer

The information provided in this user guide is correct at the time of writing, however as clinical immunology is a rapidly developing speciality; techniques, reference ranges and details of investigations may change.

The information provided is a broad guideline to the use of more commonly used tests. However the Consultant Immunologist and staff of the Immunology Department are always happy to discuss the service & individual patients in more detail.

Feedback

We welcome feedback on all aspects of our service, both informal and formal. Feedback may be given to senior members of staff, or by using the departmental e-mail address.

Updates of User’s Handbook

This Immunology Department User’s Handbook is available on the Hospitals Internet and Intranet sites, and will be updated on a regular basis. We plan to produce a new print version every 2 years. If you have any suggestions for improvements please contact Dr. Keogan or Anne Clooney or use our Departmental E-mail immunologydepartment@beaumont.ie

Please note the most up to date version of this manual will be available online.
## Contents

1. Services Provided ...................................................... 5  
2. How to Contact Us .................................................... 6  
3. Routine & Urgent Service ............................................. 7  
4. How to Order Tests .................................................... 8  
5. Transportation of Specimens to the Laboratory ............... 9  
6. Obtaining Results of Laboratory Investigations ............... 10 
7. Test Profiles .......................................................... 11  
8. Frequency of Retesting Guidelines ................................. 14 
9. Repertoire of Tests Provided ........................................ 15 
10. Immunological Tests performed in other Laboratories in Beaumont Hospital 18 
11. Tests Referred to other Laboratories .............................. 19 
12. Uncertainty of Measurement ......................................... 21 
13. Information about commonly requested tests 
   13.1 Rheumatoid Factor ................................................ 22 
   13.2 Anti-CCP antibodies ............................................. 23 
   13.3 Anti-Nuclear Factor .............................................. 24 
   13.4 Anti-dsDNA antibodies ......................................... 27 
   13.5 Anti-ENA antibodies ............................................ 28 
   13.6 Anti-Nucleosome antibodies ................................... 30 
   13.7 Anti-Histone antibodies ........................................ 31 
   13.8 Anti-Ribosomal-P-protein antibodies ......................... 32 
   13.9 ANCA, Anti-MPO and Anti-PR3 ................................. 33 
   13.10 Anti-GBM antibodies .......................................... 39 
   13.11 Anti-Cardiolipin antibodies ................................. 40
13.12 Antibodies to Beta 2 Glycoprotein 1 42
13.13 Anti-Smooth muscle antibodies 44
13.14 Anti-LKM antibodies 45
13.15 Anti-Mitochondrial antibodies & M2 subtype 46
13.16 Anti-Gastric Parietal cell antibodies 47
13.17 Anti-Intrinsic factor antibodies 48
13.18 Anti-Thyroid Peroxidase antibodies (TPO) 49
13.19 Anti-Thyroid antibodies 50
13.20 Anti-Adrenal antibodies 51
13.21 Anti-Tissue Transglutaminase antibodies (tTG) 52
13.22 Anti-Endomysial antibodies 54
13.23 Anti-Neuronal antibodies 56
13.24 Anti-Skin antibodies 57
13.25 Total IgE and allergen-specific IgE 58
13.26 Complement C3 & C4 levels 60
13.27 Complement C2 62
13.28 Complement C1Q 63
13.29 Complement Function CH100 & AP100 64
13.30 Complement C1Esterase Inhibitor (C1 INH) 65
13.31 C1 Inhibitor Function 67
13.32 Anti-Streptolysin Titre (ASOT) 68
13.33 Mast Cell Tryptase 69
13.34 NMDA 71
13.35 Specific IgG: For Assessment of Immunodeficiency 72
13.36 Specific IgGs: To Assess for Immunological Reactivity 74
13.37 Mannose Binding Lectin (MBL) 76
13.38 Myositis Screen 77
13.39 IgG Subclasses 79

14. Query Test 80
15. Direct Immunofluorescence on Skin Biopsies 82
16. Immunology Test Turnaround Times 83
1. Services Provided
The Immunology Department provides both Clinical and Laboratory Services. Additionally we are keen to assist with the development of guidelines for investigations of potential immunological disorders, clinical audit and other educational activities.

Clinical Service
There is an immunology outpatient’s clinic held in Clinic A on Monday mornings. Additionally, a nurse-led clinic is held in the department on Thursday mornings. Referrals are accepted from hospital teams and GPs. Self-referrals from patients cannot be accepted. Appropriate referrals include known or suspected immunodeficiency, recurrent infections, serious allergy (anaphylaxis) or angioedema, as well as difficult autoimmune disease. A detailed referral letter including current medications, previous treatments and laboratory investigations with results should be sent to Dr. Keogan. Please ensure that the patients’ correct address and phone number is included. Appointments are allocated on the basis of clinical urgency. Due to the long waiting time, we do not routinely offer second appointments to patients who fail to attend without cancelling their appointment.

Laboratory Service
The Laboratory provides a large range of investigations for autoimmune and allergic disorders. The repertoire of investigations for possible immunodeficiency is being developed and expanded. Details of disease specific test profiles and test repertoire and disease specific test profiles are provided in Section 7 and 9. Some immunology tests are carried out in the Protein chemistry and Haematology laboratories. These are outlined in Section 10.

When we are unable to provide a clinically important assay, we will attempt to source a referral laboratory, to which specimens may be sent. We welcome input from interested clinicians in this process. The choice of laboratory is primarily based on quality grounds, with accredited laboratories being chosen preferentially. Other factors such as cost and turnaround times are also considered. A list of commonly requested referred investigations is included in Section 11.

Educational Activities
If you feel that Immunology input would be helpful in some of your training or audit activities, please contact Dr. Keogan.
2. How to Contact Us

The postal address of the laboratory is:

**Department of Immunology**
Beaumont Hospital
PO Box 1297
Beaumont Road
Dublin 9

Tel: 809 3026
Fax No (809) 3145 or (809) 3933
E-mail immunologydepartment@beaumont.ie

Visitors to the laboratory should go to the Pathology Reception Desk on the Lower Ground Floor. Staff at pathology reception will contact the Department and a member of staff will accompany them to the Laboratory.

**Telephone Numbers:**

**Departmental Secretary**  (809) 3026

Chief Medical Scientist
Anne Clooney  E-mail: an neclooney@beaumont.ie

Laboratory  (809) 2635
(809) 2619

Secretary to Dr. Keogan  (809) 2652
This number should be used for all queries in relation to appointments.
3. Routine & Urgent Service

3.1 Routine Hours of Opening

Laboratory hours are from **9.00 am to 5.00pm, on Monday to Friday.**
The laboratory is closed on Saturday, Sunday and on Bank Holidays. A reduced service is offered between Christmas & New Year and during the Easter holiday.

3.2 Out-of-Hours Service

There are no arrangements in place as yet to provide an out-of-hours service. On the rare occasions when there is genuine clinical urgency in performing an assay, every effort is made to perform the relevant test, however such a service cannot be guaranteed.

The Consultant Immunologist, Dr. Keogan can be contacted through the switch board for clinical advice out-of-hours. If immunological investigations would affect a patient’s management on an out-of hours or urgent basis, such requests should be discussed with Dr. Keogan by a senior member of the clinical team who is familiar with the patient’s history.

3.3 Urgent Requests

An urgent service is available for some assays. Immunology assays are generally time-consuming and as several controls and standards are required in each run, we generally process requests in batches. We will only consider performing urgent assays when the result obtained is likely to affect the patient’s management. It is helpful if requests for urgent samples can be made by telephone as early as possible in the day, before all members of staff embark on other assays.

Urgent requests must be discussed with a senior member of the scientific staff in the first instance, and may also require discussion with the consultant immunologist. Additionally when requesting an urgent test, please use the ‘STAT’ flag on the computer system. Please remember however, that a ‘STAT’ flag alone does not ensure that the specimen will be processed immediately.
4. How to Order Tests

Within the hospital, immunology tests or disease specific test profiles can be ordered using PIPE or BHIS systems. Some test profiles for common immunological disorders have been set up, to speed up this process (see P.11). The interpretation of many immunology results is critically dependent on the clinical situation. **It is essential to include clinical details and a contact number on all requests. Failure to include this information may result in considerable delay in processing a request.** Some tests including RASTs will not be processed unless such information is provided. A blue Immunology request form which travels with the specimen is available for requesting RASTs from OPD.

Immunology Request Forms can be supplied to sites outside the hospital, and are also available in Computer downtime boxes on the wards. It is essential that the forms are fully completed. **Specimens can only be processed if both specimen and form are identified with the patient’s name and a second unique identifier such as hospital number and/or date of birth.** These details must be legible on both form and bottle, and must correspond with each other. Inclusion of a contact name and number, as well as the address to which the report should be returned is mandatory. Clinical details, tests required and the date of specimen collection are also required.

If you are uncertain of how best to investigate the patient, you are welcome to contact Anne Clooney, Chief Medical Scientist, the Specialist Registrar (Bleep 797) or Dr. Keogan, Consultant Immunologist to discuss the individual case. We also run a system where a serum sample can be sent with **clinical details** and the senior staff will choose the appropriate tests for the clinical details given (see section 14).
5. Transportation of Specimens to the Laboratory

It is essential that all specimens are transported to the laboratory under conditions which

- Comply with the Hospital Safety Statement, as well as relevant National Postal and Health and Safety legislation and IATA regulations
- Protect postal workers, couriers, porters and laboratory staff
- Ensure the integrity of the analyte to be measured

Specimens where the external surface is contaminated with blood or other body fluids should not be submitted – another specimen should be collected. All specimens must be individually bagged in Biohazard Bags. **Within the hospital**, specimens for Immunology may be sent via the Chute system to the Immunology Terminal (2635).

**From outside the hospital** specimens should be sent in a kangaroo bag, with the form in a separate pocket to the specimen(s). Large batches of samples may be transported in groups of up to 10 specimens in a large biohazard bag, but the forms must be kept separate. It is the responsibility of referring hospitals to ensure that packaging complies with relevant legislation.

To comply with current regulations, specimens submitted from outside the hospital should be

- Inserted in a leak-proof secondary container with sufficient padding to absorb the contents of all specimens.
- The secondary container must be strong enough to withstand a 1.2 metre drop test
- The secondary container should be placed in outer packaging with cushioning
- Staples should never be used to close the outer packaging.
- Outer packaging should be marked as “Diagnostic Specimen” and have senders address

The laboratory uses First Direct Couriers for transport of specimens to/from Beaumont. It is also registered with the Hays DX Courier Service and the laboratory number is **DX:6007202**.

Specimens should be addressed to the laboratory, and never to an individual member of staff. If there have been prior discussions the form (not the envelope) should state which member of staff should be informed of the specimen’s arrival.

If a specimen arrives in a condition which places staff at risk, we regret that it cannot be processed. Where contact details are provided the requesting clinician will be informed, however we can take no responsibility for delays which occur due to the lack of contact details. It is our policy to report the receipt of unsafe samples to the hospital’s Risk Management Department.
6. Obtaining Results of Laboratory Investigations

All tests performed in the laboratory are reported on the BHIS system and can be accessed through PIPE. Additionally written reports are issued for all tests performed. These are green A5 immunology reports in the hospital.

Reports going outside the hospital to GPs or external agencies are included in pathology composite reports, which include all test results validated that day from all disciplines. Interpretative comments are routinely included. However if you have any queries in relation to a report, please contact us to discuss the result. Feedback from users about difficulty with reports helps us to improve the service.

Despite our best efforts, it is possible that an error can occur. If you have concerns about a report please draw it to our attention without delay, and we will investigate immediately. If an error is identified we issue an amended report, as well as identifying the cause of the error, and taking steps to minimise the risk of a recurrence. It is our policy to contact clinicians as soon as possible when a report is amended in any material way which is likely to affect patient management.

Copy reports may be issued if a report is missing. Please note that the format of copy reports is slightly different to original reports as a different computer programme is used to generate them. Copy reports may be faxed, however we require written confirmation that the fax number is secure and suitable for transmission of confidential information.

Reports of Immunology tests can also be received by e-mail in GP practices which have registered with the “HealthLink” service. This allows electronic transfer of results from most laboratories as well as discharge summaries and A & E attendance by patients registered to a particular GP. If you are interested in accessing this service please contact The National Healthlink Project. Tel. (01) 8825606. Email info@healthlink.doh.ie

Note: If a sample has been received in the lab, a result will follow. There is no need to send a repeat sample.
7. Test Profiles

To make test ordering more efficient we have set up a range of disease specific test profiles, for investigations of common potentially immunological disorders. Where screening tests are included in test batteries, positive screening tests lead to reflex ordering of appropriate follow-up tests, as detailed in Section 9.

### Acute Renal Failure Screen (ARF SCR)

<table>
<thead>
<tr>
<th>Tests Included</th>
<th>Indication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANF, ANCA, GBM, C3/C4, ASOT</td>
<td>Acute or acute-on-chronic renal failure.</td>
<td>Please discuss all pulmonary-renal syndrome or rapidly progressive GN, as urgent service available.</td>
</tr>
</tbody>
</table>

### Inflammatory Arthritis Antibodies (INFL ABS)

<table>
<thead>
<tr>
<th>Tests Included</th>
<th>Indication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF, CCP, ANF</td>
<td>Isolated inflammatory arthritis, in the absence of systemic features.</td>
<td>ANCA should be added if urinalysis is abnormal.</td>
</tr>
</tbody>
</table>

### Liver Autoantibodies (LIV ABS)

<table>
<thead>
<tr>
<th>Tests Included</th>
<th>Indication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANF, Anti-Smooth Muscle, Anti-Mitochondrial, Anti-LKM</td>
<td>Suspected chronic liver disease.</td>
<td>If MITO pos, M2 subtyping will be performed, on the first occasion only.</td>
</tr>
</tbody>
</table>

### Vasculitis Screen (VAS SCR)

<table>
<thead>
<tr>
<th>Tests Included</th>
<th>Indication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANF, ANCA, RF, C3/C4, DNA, ENA</td>
<td>Suspected vasculitis or connective tissue disease.</td>
<td>This battery is intended for diagnosis only. More selective tests should be used for monitoring once diagnosis established.</td>
</tr>
</tbody>
</table>
### Asthma RASTs

<table>
<thead>
<tr>
<th>Tests Included</th>
<th>Indication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAST - House dust mite</td>
<td>Allergic asthma.</td>
<td></td>
</tr>
<tr>
<td>RAST - Aspergillus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAST - Cat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAST - Dog</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Rhinitis RASTs

<table>
<thead>
<tr>
<th>Tests Included</th>
<th>Indication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAST - House dust mite</td>
<td>Perennial Rhinitis, thought to be allergic.</td>
<td></td>
</tr>
<tr>
<td>RAST - Cat</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Food RASTs

The Food Rast panel has been discontinued due to low yield. However all individual RASTs for milk, wheat, mixed nuts, sesame, soya, mixed fish and egg, are still available.

### Respiratory RASTs

<table>
<thead>
<tr>
<th>Tests Included</th>
<th>Indication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAST - Wheat</td>
<td>Severe asthma, when allergy history cannot be obtained.</td>
<td>More targeted testing is indicated when history suggests, or excludes, specific allergies.</td>
</tr>
<tr>
<td>RAST - Aspergillus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAST - Cat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAST - Dog</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAST - Egg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAST - Grass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAST - House dust mite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAST - Milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAST - Trees</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Eczema RASTs

<table>
<thead>
<tr>
<th>Tests Included</th>
<th>Indication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAST – House Dust Mite</td>
<td>Eczema, particularly when respiratory (upper or lower) allergy also present.</td>
<td>30% of patients with eczema, sensitive to HDM, improve when HDM reduction measures are followed.</td>
</tr>
<tr>
<td>RAST – Milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAST – Egg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Shellfish RASTs (RSTSHELL)

<table>
<thead>
<tr>
<th>Tests Included</th>
<th>Indication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAST - Lobster</td>
<td>Suspected allergy to shellfish.</td>
<td>Negative result does not rule out shellfish allergy. If this is suspected clinically referral to a Clinical Immunologist is advised.</td>
</tr>
<tr>
<td>RAST - Crab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAST - Shrimp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAST - Mussel</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Coeliac Screen (COEL SCR)

<table>
<thead>
<tr>
<th>Tests Included</th>
<th>Indication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-tTG</td>
<td>Suspected Coeliac Disease.</td>
<td>Anti-AGA has been discontinued.</td>
</tr>
<tr>
<td>Anti-EmA (if tTG is Equivocal or Positive)</td>
<td>Malabsorption.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anaemia.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gastrointestinal symptoms.</td>
<td></td>
</tr>
<tr>
<td>Anti-AGA has been discontinued.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
8. Frequency of Retesting Guidelines

<table>
<thead>
<tr>
<th>TEST</th>
<th>Routine minimum period between repeats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auto Antibody Screen</td>
<td>&gt;3 months (unless for LKM titre)</td>
</tr>
<tr>
<td>Anti-Intrinsic Factor</td>
<td>&gt;6 months</td>
</tr>
<tr>
<td>Anti-M2 antibodies</td>
<td>Performed once only</td>
</tr>
<tr>
<td>Anti-LKM</td>
<td>&gt;1 month</td>
</tr>
<tr>
<td>Anti TPO antibodies</td>
<td>&gt;6 months</td>
</tr>
<tr>
<td>Anti-Nuclear factor (Hep2)</td>
<td>No more than 3 monthly</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>&gt;3 weeks (unless plasmapheresis/discussion)</td>
</tr>
<tr>
<td>Anti-ENA</td>
<td>&gt;1 year unless pregnant</td>
</tr>
<tr>
<td>Anti-Cardiolipin (IgG &amp; IgM)</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Anti-Beta2GP 1</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>&gt;3 months</td>
</tr>
<tr>
<td>Anti-Histone</td>
<td>Once off (non-quantitative test)</td>
</tr>
<tr>
<td>Anti-Ribosomal P</td>
<td>Once off (non-quantitative test)</td>
</tr>
<tr>
<td>Anti-Nucleosome</td>
<td>Once off (non-quantitative test)</td>
</tr>
<tr>
<td>Anti-Endomysial (IgA)</td>
<td>&gt;3 months</td>
</tr>
<tr>
<td>Anti-tTG</td>
<td>&gt;3 months</td>
</tr>
<tr>
<td>Anti-GBM (Quantitative)</td>
<td>As requested &amp; discussed</td>
</tr>
<tr>
<td>ANCA</td>
<td>3 weeks, unless discussed</td>
</tr>
<tr>
<td>Anti-MPO</td>
<td>3 weeks, unless discussed</td>
</tr>
<tr>
<td>Anti-PR3</td>
<td>3 weeks, unless discussed</td>
</tr>
<tr>
<td>Anti-CCP</td>
<td>3 months</td>
</tr>
<tr>
<td>Anti-Skin antibodies</td>
<td>6 months. Pos. ICS as requested.</td>
</tr>
<tr>
<td>Anti-Adrenal antibodies</td>
<td>6 months</td>
</tr>
<tr>
<td>Anti-Neuronal antibodies (Hu &amp; Yo)</td>
<td>&gt;6 months</td>
</tr>
<tr>
<td>IgE (Total)</td>
<td>1 year</td>
</tr>
<tr>
<td>Specific IgE</td>
<td>1 year for same allergens</td>
</tr>
<tr>
<td>Mast Cell Tryptase</td>
<td>As requested/discussed</td>
</tr>
<tr>
<td>Complement levels (C3 &amp; C4)</td>
<td>As requested</td>
</tr>
<tr>
<td>C2</td>
<td>Once off</td>
</tr>
<tr>
<td>C1q</td>
<td>6 months, unless HAE on treatment</td>
</tr>
<tr>
<td>C1 inhibitor (immunochemical)</td>
<td>Once off if normal. As required if low.</td>
</tr>
</tbody>
</table>

Any of these guidelines may be overruled in a particular clinical situation, if the case is discussed with staff in the Immunology laboratory and/or the Consultant Immunologist.
9. Repertoire of Tests Provided
All tests are performed on serum samples. Up to 5 tests can be performed on a 10 mL sample. However separate samples are required for some tests to facilitate optimum handling.

9.1 Routine Autoantibody Tests

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Mnemonic</th>
<th>Ref Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Adrenal antibody</td>
<td>ADR</td>
<td>Negative</td>
</tr>
<tr>
<td>ANCA</td>
<td>ANCA</td>
<td>Negative</td>
</tr>
<tr>
<td>ANF (Anti-Nuclear factor)</td>
<td>ANF</td>
<td>Negative (1:80 of doubtful significance)</td>
</tr>
<tr>
<td>B2 Glycoprotein1 Antibody</td>
<td>Card Abs</td>
<td>Negative &lt; 7 U/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Equivocal 7-10 U/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elevated &gt;10 U/mL</td>
</tr>
<tr>
<td>Anti-Cardiolipin Abs</td>
<td>Card Abs</td>
<td>IgG &lt; 10 GPLU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgM &lt; 10 MPLU</td>
</tr>
<tr>
<td>Anti-CCP antibody</td>
<td>CCP</td>
<td>&lt;3 U/ml</td>
</tr>
<tr>
<td>Anti-dsDNA antibodies</td>
<td>DNA</td>
<td>&lt;10 U/mL</td>
</tr>
<tr>
<td>Anti-Endomysial (IgA) abs</td>
<td>EMA</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-Extractable Nuclear antibodies (includes anti-Ro, La, RNP, Sm, Jo-1 &amp; Scl-70)</td>
<td>ENA</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-Gastric parietal cell</td>
<td>GPC</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-GBM (glomerular basement membrane) Abs.</td>
<td>GBM</td>
<td>ELIA &lt; 10 U/mL</td>
</tr>
<tr>
<td>Anti-Histone antibodies</td>
<td>HIST</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-Neuronal antibodies (Anti-Hu, Yo)</td>
<td>HUYO</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-Intrinsic Factor Abs.</td>
<td>IF</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-LKM (liver-kidney microsomal) antibodies</td>
<td>LKM</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-Mitochondrial Abs (including M2 subtyping)</td>
<td>MITO</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-tissue transglutaminase antibody</td>
<td>tTG</td>
<td>&lt; 7 U/ml</td>
</tr>
<tr>
<td>Anti-Thyroid Peroxidase Abs</td>
<td>TPO</td>
<td>Negative &lt;50 IU/ml</td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>RF</td>
<td>Negative &lt;20 IU/mL</td>
</tr>
<tr>
<td>Anti-Pneumococcal antibodies</td>
<td>PNEU</td>
<td>Age-related. Mean adult titre</td>
</tr>
<tr>
<td>Skin Autoantibodies.</td>
<td>SKIN</td>
<td>Negative</td>
</tr>
<tr>
<td>Smooth muscle Abs</td>
<td>SMA</td>
<td>Negative</td>
</tr>
</tbody>
</table>
9.2 Autoantibodies Measured In Special Circumstances

<table>
<thead>
<tr>
<th>Test</th>
<th>Mnemonic</th>
<th>Ref Range</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Endomysial (IgG) antibody</td>
<td>EMAG</td>
<td>Negative</td>
<td>Only performed when IgA deficiency present.</td>
</tr>
<tr>
<td>Anti-MPO antibody</td>
<td>MPO</td>
<td>&lt;10 Units/mL</td>
<td>Follow-up of patients with known MPO-ANCA positive disease.</td>
</tr>
<tr>
<td>Anti-PR3 antibody</td>
<td>PR3</td>
<td>&lt;10 Units/mL</td>
<td>Follow-up of patients with known PR3-ANCA positive disease.</td>
</tr>
<tr>
<td>Anti-Ribosomal P-protein antibodies</td>
<td>RIBOP</td>
<td>Negative</td>
<td>Strong clinical suspicion of lupus with negative routine serology. Must discuss with Consultant Immunologist.</td>
</tr>
<tr>
<td>Anti-Nucleosome antibody</td>
<td>NUCSOME</td>
<td>Negative</td>
<td>Strong clinical suspicion of lupus with negative routine serology. Must discuss with Consultant Immunologist.</td>
</tr>
</tbody>
</table>

9.3 Other Immunology Tests

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Mnemonic</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASOT</td>
<td>ASOT</td>
<td>0 - 200 IU/mL</td>
</tr>
<tr>
<td>Complement, C3</td>
<td>C3, C4</td>
<td>0.75 - 1.65 g/L</td>
</tr>
<tr>
<td>Complement, C4</td>
<td>C1IN</td>
<td>0.14 - 0.54 g/L</td>
</tr>
<tr>
<td>C1 inhibitor</td>
<td></td>
<td>150 – 350 mg/L</td>
</tr>
<tr>
<td>Complement, C1q</td>
<td>C1Q</td>
<td>50– 250 mg/L</td>
</tr>
<tr>
<td>Complement, C2</td>
<td>C2</td>
<td>10 – 30 mg/L</td>
</tr>
<tr>
<td>Complement CH100 &amp; AP100</td>
<td>COMPFUNC</td>
<td>Normal</td>
</tr>
<tr>
<td>Mast Cell Tryptase</td>
<td>TRYP'TASE</td>
<td>2-14 ug/L</td>
</tr>
<tr>
<td>IgE</td>
<td>IgE</td>
<td>0 – 100 kU/L (Age Related)</td>
</tr>
<tr>
<td>Allergen specific IgE</td>
<td>See below</td>
<td>&lt; 0.0 kU/L = Grade 0</td>
</tr>
</tbody>
</table>
## 9.4 Current available Specific IgE Allergens

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSTALM</td>
<td>Almond specific IgE</td>
</tr>
<tr>
<td>RSTALTER</td>
<td>Altemaria alternate specific IgE</td>
</tr>
<tr>
<td>RAST_AMOX</td>
<td>Amoxicillan specific IgE</td>
</tr>
<tr>
<td>RAST_AMP</td>
<td>Ampicillin specific IgE</td>
</tr>
<tr>
<td>RSTASP</td>
<td>Aspergillus specific IgE</td>
</tr>
<tr>
<td>RSTBRL</td>
<td>Barley specific IgE</td>
</tr>
<tr>
<td>RSTBEE</td>
<td>Bee specific IgE</td>
</tr>
<tr>
<td>RSTBNUT</td>
<td>Brazil nut specific IgE</td>
</tr>
<tr>
<td>RSTCAND</td>
<td>Candida specific IgE</td>
</tr>
<tr>
<td>RSTCASH</td>
<td>Cashew nut specific IgE</td>
</tr>
<tr>
<td>RSTCAT</td>
<td>Cat specific IgE</td>
</tr>
<tr>
<td>RST_CEF</td>
<td>Cefaclor specific IgE</td>
</tr>
<tr>
<td>RSTCERL</td>
<td>Cereal mix</td>
</tr>
<tr>
<td>RSTCHS</td>
<td>Cheese specific IgE</td>
</tr>
<tr>
<td>RSTCITR</td>
<td>Citrus fruit mix</td>
</tr>
<tr>
<td>RSTCNU T</td>
<td>Coconut specific IgE</td>
</tr>
<tr>
<td>RSTCOD</td>
<td>Cod specific IgE</td>
</tr>
<tr>
<td>RSTCRAB</td>
<td>Crab specific IgE</td>
</tr>
<tr>
<td>RSTDOG</td>
<td>Dog specific IgE</td>
</tr>
<tr>
<td>RSTE GG</td>
<td>Egg specific IgE</td>
</tr>
<tr>
<td>RSTEGWHT</td>
<td>Egg White specific IgE</td>
</tr>
<tr>
<td>RSTFEATH</td>
<td>Feather mix</td>
</tr>
<tr>
<td>RSTFSH</td>
<td>Fish specific IgE</td>
</tr>
<tr>
<td>RSTGRS</td>
<td>Grass specific IgE</td>
</tr>
<tr>
<td>RSTHNUT</td>
<td>Hazelnut specific IgE</td>
</tr>
<tr>
<td>RSTHOR</td>
<td>Horse specific IgE</td>
</tr>
<tr>
<td>RSTHDM</td>
<td>House dust mite IgE</td>
</tr>
<tr>
<td>RSTKIWI</td>
<td>Kiwi specific IgE</td>
</tr>
<tr>
<td>RSTLTX</td>
<td>Latex specific IgE</td>
</tr>
<tr>
<td>RSTLOB</td>
<td>Lobster specific IgE</td>
</tr>
<tr>
<td>RSTMLK</td>
<td>Milk specific IgE</td>
</tr>
<tr>
<td>RSTMUSL</td>
<td>Mussel specific IgE</td>
</tr>
<tr>
<td>RSTNUT</td>
<td>Nut specific IgE</td>
</tr>
<tr>
<td>RSTPNUT</td>
<td>Peanut specific IgE</td>
</tr>
<tr>
<td>RAST_PEN</td>
<td>Penicillin specific IgE</td>
</tr>
<tr>
<td>RSTPOLL</td>
<td>Pollen specific IgE</td>
</tr>
<tr>
<td>RSTRYE</td>
<td>Rye specific IgE</td>
</tr>
<tr>
<td>RSTSLM</td>
<td>Salmon Specific IgE</td>
</tr>
</tbody>
</table>
RSTSES = Sesame specific IgE  
RSTSHP = Shrimp specific IgE  
RSTSOY = Soybean specific IgE  
RSTSTRAW = Strawberry specific IgE  
RSTTOMAT = Tomato specific IgE  
RSTTREES = Trees specific IgE  
RSTTUNA = Tuna specific IgE  
RSTWHT = Wheat specific IgE  
RSTWASP = Wasp specific IgE  
RSTWNUT = Walnut specific IgE  
RSTYEAST = Yeast specific IgE

Please request RSTOTHER, and state name of allergen, for anything not on the above list. If the history indicates an unusual allergen, the appropriate test will be sent to the UK.

10. Immunological Tests performed in other Laboratories in Beaumont Hospital

<table>
<thead>
<tr>
<th>Test</th>
<th>Mnemonic</th>
<th>Specimen</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoglobulins</td>
<td>IGGS</td>
<td>Clotted</td>
<td>Proteins (809) 2305</td>
</tr>
<tr>
<td>C Reactive Protein</td>
<td>CRP</td>
<td>Heparin</td>
<td>Clin Chem (809) 2668</td>
</tr>
<tr>
<td>Protein electrophoresis</td>
<td>SPEP</td>
<td>Clotted</td>
<td>Proteins (809) 2305</td>
</tr>
<tr>
<td>Urine electrophoresis</td>
<td>UPEP</td>
<td>24 hour urine collection</td>
<td>Proteins (809) 2305</td>
</tr>
<tr>
<td>(Bence Jones Protein)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2 Microglobulin</td>
<td>B2M</td>
<td>Clotted</td>
<td>Proteins (809) 2305</td>
</tr>
<tr>
<td>Cryoglobulins</td>
<td>CRYO</td>
<td>Special instructions on computer</td>
<td>Proteins (809) 2305</td>
</tr>
<tr>
<td>Lymphocyte subsets</td>
<td>LY-SUB</td>
<td>EDTA</td>
<td>Haematology (809)2763</td>
</tr>
</tbody>
</table>
### Tests Referred to External Laboratories

<table>
<thead>
<tr>
<th>Test</th>
<th>Mnemonic</th>
<th>Lab</th>
<th>Ref Range</th>
<th>Turn-Around times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine Receptor Antibodies</td>
<td>ACHR</td>
<td>2</td>
<td>0-5 (x10^{-10}M)</td>
<td>1 Month</td>
</tr>
<tr>
<td>Voltage gated calcium channel antibodies (Eaton Lambert Syndrome)</td>
<td>VGCC</td>
<td>1</td>
<td>0-45 PM</td>
<td>4-6 Weeks</td>
</tr>
<tr>
<td>Voltage gated potassium channel antibodies</td>
<td>VGKC</td>
<td>1</td>
<td>0-100 PM</td>
<td>4-6 Weeks</td>
</tr>
<tr>
<td>Glutamic Acid Decarboxylase Antibody</td>
<td>GAD</td>
<td>1</td>
<td>0-1 U/ml</td>
<td>2 Weeks</td>
</tr>
<tr>
<td>Ganglioside Antibodies</td>
<td>GANG AB</td>
<td>2</td>
<td>Negative</td>
<td>2 Weeks</td>
</tr>
<tr>
<td>Anti-MAG antibodies</td>
<td>MAG AB</td>
<td>1</td>
<td>0-1000BTU</td>
<td>3-4 Weeks</td>
</tr>
<tr>
<td>Cardiac Muscle antibodies</td>
<td>CMUS</td>
<td>3</td>
<td>Negative</td>
<td>2 Weeks</td>
</tr>
<tr>
<td>Striated Muscle antibodies</td>
<td>SMUS</td>
<td>3</td>
<td>Negative</td>
<td>2 weeks</td>
</tr>
<tr>
<td>Ovarian Antibodies</td>
<td>OVA</td>
<td>3</td>
<td>Negative</td>
<td>1 Month</td>
</tr>
<tr>
<td>Neutrophil Oxidative Burst</td>
<td>OXBURST</td>
<td>4</td>
<td>Normal</td>
<td>By Arrangement</td>
</tr>
<tr>
<td>Islet cell antibodies</td>
<td>ISLT</td>
<td>3</td>
<td>Negative</td>
<td>2 Weeks</td>
</tr>
<tr>
<td>Thyroxine Receptor Antibody</td>
<td>TSI</td>
<td>7</td>
<td>0-1.5 IU/L</td>
<td></td>
</tr>
<tr>
<td>Anti-HiB antibodies</td>
<td>HIB</td>
<td>5</td>
<td>For test immunisation expect post vaccination value to increase x 2 &amp; fall into protective range</td>
<td>Up to 2 Months</td>
</tr>
<tr>
<td>Anti-tetanus antibodies</td>
<td>TET</td>
<td>5</td>
<td></td>
<td>Up to 2 Months</td>
</tr>
<tr>
<td>Anti- diphtheria antibodies</td>
<td>DIPH</td>
<td>6</td>
<td></td>
<td>Up to 2 Months</td>
</tr>
<tr>
<td>Anti-NMDA</td>
<td>NMDA</td>
<td>1</td>
<td></td>
<td>3 weeks</td>
</tr>
</tbody>
</table>
Contact Details of External Laboratories

1. **Neurosciences Group, Institute of Molecular Medicine**
   John Radcliffe Hospital, Headington, Oxford OX3 9DF
   Tel: 00 44 1865 225 995  Fax: 00 44 1865 222 402

2. **Institute of Neurological Science**,  
   Southern General Hospital, Glasgow GS1 4TF  
   Tel: 00 44 141 201 2491  Fax: 00 44 141 201 2993

3. **Dept of Immunology**,  
   Northern General Hospital, Herries Road  
   Sheffield S5 7YT  
   Tel: 00 44 114 271 5552  Fax: 00 44 114 261 9893

4. **Dept. of Immunology**,  
   Central Pathology Laboratory, St James Hospital, Dublin 8  
   Tel: 01 416 2924/2925  Fax: 01 4113008

5. **Dept of Immunology**,  
   City Hospital NHS Trust,  
   Dudley Road, Birmingham B18 7QH  
   Tel: 00 44 121 507 4258  Fax: 00 44 121 507 4606

6. **Dept of Immunology**,  
   Heartlands Hospital, 51 Bordesley Green East,  
   Birmingham, B9 5ST  
   Tel: 00 44 121 424 1185  Fax: 00 44 121 424 0185

7. **Endocrinology Department**,  
   Central Pathology Laboratory, St James Hospital, Dublin 8  
   Tel: 01 416 2991  Fax: 01 4103446
12 Uncertainty of Measurement (UM)

Every measurement, including a laboratory result, is subject to a level of uncertainty. For example, blood pressure measured a few times within a single clinical visit may vary. This variation is made up of biological variation together with the uncertainty of measurement (and may be compounded further if any error is made). Systems in the laboratory are designed to minimise error – however if you are concerned that an error has occurred please contact us to let us investigate this. Even when error is eliminated, uncertainty of measurement affects all results.

When interpreting the results of a laboratory test the uncertainty of measurement (UM) of that result needs to be considered. UM is a numerical value & is an expression of the magnitude of uncertainty of a result. It characterizes the dispersion of values reasonably attributed to measurement. If not understood may lead to over interpretation of results.

 e.g. If the UM is 10% & the result is 100, then the true result probably lies between 90-110. Therefore is the result obtained due to clinical changes in the patient or imprecision of the test method itself?

Uncertainty is not error. Error tells us the difference between the true value & the measured value. Error can be corrected, uncertainty cannot. UM is the quantitative expression of doubt (uncertainty) & spread of a particular measurement. It is an estimate of the confidence in the result produced by the laboratory.

Uncertainty is a parameter associated with every result & is specific to each result. We are currently in the process of producing a document outlining the UM for the assays available & this will be posted on the internet/intranet.
**13.1 Rheumatoid Factor**

Specimen: **Serum, White tube**  
Minimum volume: **6 mls**

Method: **Nephelometry**  
Reference value: **<20 IU/mL**

Test turnaround time: **2-3 days**  
Frequency of retesting: **>3 Months**

**Indications:**
- Inflammatory arthritis
- Suspected vasculitis
- Interstitial lung disease
- Pleural/pericardial effusions

**INTERPRETATION OF RESULTS**

**Negative rheumatoid factor, <20 IU/mL**

A negative Rheumatoid factor makes diagnosis of Rheumatoid disease less likely, however as 10% of patients are RF negative, it does not exclude this diagnosis. Where there is a strong clinical suspicion of Rheumatoid Disease anti-CCP should be ordered.

**Weak positive, 20-70 IU/mL**

Some patients with Rheumatoid Disease will be only weakly positive and will fall into this range. However at this level Rheumatoid Factor is not specific for a diagnosis of Rheumatoid disease and a number of patients with weakly positive Rheumatoid Factor will have other inflammatory conditions. Anti-CCP will automatically be ordered and this will give more specific information. We generally suggest that you repeat the test in approximately 3-6 months time if clinical symptoms have persisted and only RF is positive. In Rheumatoid disease the assay should remain consistently positive, or may even be more strongly positive. However infection induced Rheumatoid Factor usually clears within weeks following successful treatment of the infection.

**Significantly positive rheumatoid factor, 71-250 IU/mL**

In an appropriate clinical setting a significantly positive Rheumatoid Factor is consistent with diagnosis of Rheumatoid disease. Anti-CCP will automatically be ordered.

**Strongly positive rheumatoid factor, >250 IU/mL**

Strongly positive Rheumatoid Factor is suggestive of Rheumatoid disease. The presence of a high level of Rheumatoid Factor at presentation is considered an adverse prognostic marker. Patients with Sjogren’s syndrome may have very high levels of RF despite only minor joint symptoms. Occasionally a similar level may be seen in patients with cryoglobulinaemia and if features suggestive of this disorder are present an appropriate sample should be sent to the Proteins Laboratory in Clinical Chemistry. Anti-CCP will automatically be ordered. Serial measurement of Rheumatoid factor is generally not useful in monitoring the response to therapy. Measurement of acute phase reactants (CRP & to a lesser extent ESR) are more useful.
13.2 Anti-Cyclic Citrullinated Peptide antibodies (CCP)

Specimen: Serum, White tube
Minimum volume: 6 mls
Method: IMMUNOCAP
Reference value: <3 U/ml
Test turnaround time: 1 week
Frequency of retesting: 3 Months

Indications:
- Inflammatory Arthritis
- Interstitial lung disease
- Suspected extra-articular rheumatoid disease

INTERPRETATION OF RESULTS

Anti-CCP antibody is a more sensitive (77%) and specific (97%) serological marker for Rheumatoid Arthritis (RA) than rheumatoid factor (RF: Sens= 74%; Spec= 65%). Studies show that the presence of Anti-CCP antibodies correlates with erosive RA disease progression. Anti-CCP antibodies are present earlier in disease than RF and therefore are useful in predicting the development of RA. The combination of RF and anti-CCP has a higher prognostic potential than either of them alone.

To date it is unclear whether monitoring changes in anti-CCP antibody levels is helpful. However, given that the half-life of IgG is 3 weeks, we do not recommend repeat testing more frequently than 3-monthly.

While CCP appears to be very helpful in diagnosing Rheumatoid Arthritis, it is less sensitive for diagnosis of extra-articular disease.

Positive > 7 U/ml
Relatively specific for Rheumatoid Disease. Suggest referral to Rheumatologist.

Note: Prior to June 2009 Anti-CCP antibodies were measured by ELISA. Reference value: <25 IU/mL
13.3  Anti-Nuclear Factor

Specimen: Serum, White tube  Minimum volume: 6 mls
Method: Indirect Immunofluorescence
Reference value: Negati ve.  
Weak positive (1:80 & 1:100) are commonly seen particularly in healthy older women
Test turnaround time: 2-3 days
Frequency of retesting: No more than 3 monthly

<table>
<thead>
<tr>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory arthritis</td>
</tr>
<tr>
<td>Suspected vasculitis/CTDs</td>
</tr>
<tr>
<td>Photosensitive/other typical skin rash</td>
</tr>
<tr>
<td>Pleural/pericardial effusions.</td>
</tr>
<tr>
<td>? autoimmune haemolytic anaemia, ITP, leucopenia</td>
</tr>
<tr>
<td>Renal impairment, proteinuria, haematuria</td>
</tr>
<tr>
<td>Unexplained CNS disease</td>
</tr>
</tbody>
</table>

The ANF method used in Beaumont is very sensitive, using Hep2 cells as substrate. ANF acts as a screen for Connective Tissue Disease. Once a diagnosis of connective tissue disease has been made, repeated measurement of ANF is rarely helpful in monitoring disease activity. In particular, for patients with SLE, we recommend that anti-dsDNA antibodies and complement levels (C3 & C4) be used for follow-up.

Because of the sensitivity of the technique in use, false positive results are common. A non-specific weak positive ANF is most common in women, and the prevalence increases with age. Approximately 20% of women aged 60 yrs or older will have a positive ANF unrelated to disease. Interpretation of ANFs is dependent on the clinical situation and age and sex of the patient. The information provided below is only a broad guide. Where there is a significant clinical suspicion of a connective tissue disease, it is reasonable to request anti-DNA antibodies and anti-ENA antibody testing, even if the ANF is only weakly positive.

INTERPRETATION OF RESULTS

Negative ANF
A negative result makes SLE or other connective tissue disease extremely unlikely.
Weak Positive 1:80
A weak positive ANF is of doubtful clinical significance, particularly in middle-aged or older females. However if connective tissue disease is strongly suspected clinically, please contact the laboratory to discuss further testing.

Positive 1:100
Weak ANF is only rarely associated with connective tissue disease. An ANF of this level is found in patients with a variety of inflammatory disorders, and may be seen in healthy women, particularly in the over-60s. However if connective tissue disease is suspected clinically, please contact the laboratory to arrange follow-on anti-dsDNA and anti-ENA antibody testing.

Positive 1:200
Approximately 10% of patients with an ANF of this level are subsequently found to have an anti-dsDNA or anti-ENA. We do not automatically add on these tests, however where the clinical features are consistent with a connective tissue disease, or no firm diagnosis is established, we are always happy to add on anti-dsDNA and anti-ENA if requested.

Positive 1:400 or greater
60% of patients with a strongly positive ANF are found to have anti-dsDNA or anti-ENA antibodies on follow on testing. Because of this high yield, we automatically add on an anti-dsDNA or anti-ENA test for requests originating within Beaumont Hospital. For external requests we are unable to do this unless the requesting hospital has agreed to this protocol.

We do not add on tests to strong anti-centromere antibodies, as this pattern of ANF is not associated with positivity in follow-on tests.

Patterns of ANF
Many textbooks suggest that the pattern of ANF is a reliable guide to the subsequent results of follow –on tests. Our experience with this approach has been disappointing, and that is why we do not limit anti-dsDNA testing to patients with a homogenous ANF and ENA testing to those with a speckled ANF. Anti-centromere and anti-nucleolar patterns may have particular clinical indications.

Anti-Centromere Antibody Pattern
This pattern is typically found in CREST syndrome (Calcinosis, Raynaud's phenomenon, Oesophageal dysmotility, Sclerodactyly & Telangiectasia). This is a limited form of scleroderma, generally not associated with visceral disease. However recent data has suggested an association with pulmonary hypertension, which is often clinically significant.
Anti-centromere antibodies may also be found in about 10% of patients with scleroderma, and in this setting this antibody is regarded as a good prognostic marker. 13% of patients with primary biliary cirrhosis (PBC) also have this autoantibody.

**Anti-Nucleolar Antibody Pattern**
This pattern is typically associated with scleroderma, but is by no means specific for this condition. When found in a patient with Raynaud's phenomenon and no other features of scleroderma, it may indicate future development of this condition.

A nucleolar pattern ANF is also frequently seen in autoimmune liver disease.
13.4 Anti-double-stranded-DNA Antibodies

<table>
<thead>
<tr>
<th>Specimen:</th>
<th>Serum, White tube.</th>
<th>Minimum volume: 6 mls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method:</td>
<td>IMMUNOCAP</td>
<td>Reference value: &lt;10 IU/mL</td>
</tr>
<tr>
<td>Test turnaround time:</td>
<td>2-3 days</td>
<td>Frequency of retesting: &gt;3 Weeks</td>
</tr>
</tbody>
</table>

**Indications:**
- Strong clinical suspicion of SLE
- ANF positive 1:400 or greater
- Clinical suspicion of SLE with ANF of 1:100 or 1:200
- Follow-up of known SLE patients

Strongly positive anti-dsDNA is suggestive of SLE, but may also be found in autoimmune hepatitis. Weakly positive anti-dsDNA antibodies may also be found in patients with other connective tissue diseases, and occasionally in non-autoimmune inflammatory disorders. Anti-dsDNA is useful in monitoring activity of SLE. However as the half-life of IgG is 3 weeks, it is seldom helpful to measure more frequently than monthly. However, when patients are undergoing plasmapheresis we are happy to receive daily samples to monitor therapy.

**Negative Result ( <10 IU/mL)**
SLE unlikely, however a small number of SLE patients may be negative when first tested. Therefore if clinical suspicion is high, serology should be repeated in 3-6 months.

**Borderline Result (10-15 IU/mL)**
Most patients with non-inflammatory disorders have values less than 15 IU/mL. At this level we suggest repeating serology in 3-6 months to exclude an evolving connective tissue disease, unless an alternative diagnosis is established in the meantime.

**Weak Positive Result (16-30 IU/mL)**
A number of patients with SLE will have antibody levels in this range, particularly in mild disease. However the specificity of weak antibody levels is less than 50% for SLE. Similar antibody levels may be seen in patients with other connective tissue diseases, including RA. Occasionally similar levels will be seen in patients who do not have autoimmune disorders.

**Significant Positive (>30 IU/mL)**
This result is suggestive of SLE, but may also be seen occasionally in autoimmune chronic hepatitis.
13.5 Anti-ENA (Extractable Nuclear Antigen) Antibodies

This test includes: Anti-Ro, Anti-La
                                Anti-RNP, Anti-Sm
                                Anti-Jo-1, Anti-Scl-70

Specimen: Serum, White tube  Minimum volume: 6 mls
Method: ELISA with confirmation by Immunoblot
Reference value: Negative for all 6 components
Test turnaround time: 1-2 weeks
Frequency of retesting: >1 year unless patient is pregnant

<table>
<thead>
<tr>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANF positive 1:400 or greater</td>
</tr>
<tr>
<td>Clinical suspicion of SLE/CTDs with ANF of 1:100/1:200</td>
</tr>
<tr>
<td>Clinical &amp; Biochemical evidence Polymyositis</td>
</tr>
<tr>
<td>Suspected Sjogren’s syndrome</td>
</tr>
<tr>
<td>DLE/Subacute cutaneous lupus</td>
</tr>
<tr>
<td>Congenital heart block – test mother &amp; child</td>
</tr>
</tbody>
</table>

Antibodies to extractable nuclear antigens (ENA) refer to antibodies to a group of antigens found within the nucleus (+/- cytoplasm), which are associated with connective tissue diseases. While approximately 70 such antigens have been described, only antibodies to 6 are routinely available, and play a well-validated role in patient management.

The majority of patients who are anti-ENA positive will also have a positive ANF. However as both Ro and Jo-1 are primarily located in the cytoplasm occasionally patients with these antibodies may have a negative ANF.

Anti-ENA antibodies are useful in diagnosis, but not follow-up of patients. There is little indication for repeated measurement of these antibodies as the assays are qualitative, and antibody levels have never been shown to reflect disease activity. The only exception is when a patient is seen with a short history, and serology is negative – on repeat some months later, the picture may be more helpful. Additionally in view of the obstetric implications, it is reasonable to repeat an ENA when a patient with SLE or other connective tissue disease becomes pregnant.
Anti-Sm
This antibody is found in 30% of patients with SLE, and is regarded as specific for this diagnosis.

Anti-RNP
This antibody is typically seen in mixed connective tissue disease. This is an overlap syndrome with features of SLE, polymyositis and scleroderma, in varying proportions. Anti-RNP may also be significantly positive in patients with SLE, however in this group anti-dsDNA will also be elevated. Weakly positive anti-RNP may be found in other connective tissue diseases.

Anti-Ro
Anti-Ro antibodies are found in 70% of patients with Sjogren's syndrome and 30% of patients with SLE. This antibody is also often found in subacute cutaneous lupus erythematosus (SCLE). Anti-Ro is often present in lupus patients with photosensitivity.

Antibodies cross the placenta from early in the second trimester, and anti-Ro cross-reacts with the fetal cardiac conducting system. A minority of babies born to anti-Ro-positive mothers may develop congenital heart block. The birth of a baby with congenital heart block may be the presenting feature of SLE, and both mother and baby should be screened. Congenital heart block may cause a late intrauterine death or a stillbirth.

Anti-La
Anti-La antibodies are usually found in association with anti-Ro, and are rarely found alone. It is found in approximately 30% of Sjogren's patients and 10% of lupus patients.

Anti-Jo-1
Anti-Jo-1 is found in 30% of patients with polymyositis (anti-synthetase syndrome). Typically anti-Jo-1 positive patients have or will develop interstitial lung disease, Raynaud's phenomenon, and thickened, sausage shaped fingers.

Anti-Scl-70
Anti-Scl-70 is found in 30% of patients with scleroderma, and when significantly positive is regarded as specific for this condition. The antibody may predate clinical signs of disease. The presence of anti-Scl-70 is regarded as a poor prognostic marker.
13.6 Anti-Nucleosome Antibodies

Specimen: Serum, White tube Minimun volume: 6 mls
Method: Immunoblot Reference value: Negative
Test turnaround time: 4-6 weeks Frequency of retesting: Once off

<table>
<thead>
<tr>
<th>Indications:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strong clinical suspicion of SLE</td>
</tr>
<tr>
<td></td>
<td>Suspected Sjogrens syndrome</td>
</tr>
<tr>
<td></td>
<td>Suspected Systemic Sclerosis</td>
</tr>
</tbody>
</table>

INTERPRETATION OF RESULTS.

Negative
Normal value. This does not exclude systemic Lupus as the sensitivity of this assay is 97%. Results should be considered in conjunction with anti-dsDNA, anti-ENA and complement C3 and C4 levels. If all of these are negative/normal, systemic lupus is highly unlikely. If clinical suspicion of lupus remains high, particularly with recent onset symptoms, serology should be repeated in 6 months.

Positive
Positive anti-nucleosome antibodies is strongly suggestive of Lupus, even in the absence of anti-dsDNA antibody. The blot we use is 2nd generation with a specificity of > 95% for Lupus, which is considerably higher than early reports with 1st generation assays. Very occasionally false positives have been described in Sjogrens syndrome and Systemic Sclerosis, even when using 2nd generation assays. Anti-nucleosome antibodies can be ordered following discussion with the Immunology team.
13.7 Anti-Histone Antibodies

Specimen: Serum, White tube  Minimum volume: 6 mls
Method: Immunoblot  Reference value: Negative
Test turnaround time: 4-6 weeks  Frequency of retesting: Once off

<table>
<thead>
<tr>
<th>Indications:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspected drug-induced SLE (90% Positive)</td>
</tr>
<tr>
<td>Felty's Syndrome (70% Positive)</td>
</tr>
<tr>
<td>Juvenile Chronic Arthritis</td>
</tr>
</tbody>
</table>

Anti-histone antibodies were originally thought to be markers for drug-induced lupus. However following more intensive investigation it was found that although present in 90% of patients with drug-induced lupus, they are also found in 40% of idiopathic lupus patients. Hence they are not specific for drug-induced disease.

Anti-histone antibodies are positive in a high proportion of patients with Felty’s syndrome and ANF-positive juvenile chronic arthritis. If these conditions enter the differential diagnosis for a patient, the poor specificity of anti-histone antibodies should be considered.
13.8 Anti-Ribosomal-P-Protein antibodies

Specimen: Serum, White tube
Method: Immunoblot
Test turnaround time: 4-6 weeks
Minimum volume: 6 ml
Reference value: Negative
Frequency of retesting: Once off

Indications: High index of suspicion of SLE and routine serology negative (i.e. dsDNA, ENA)

This test is performed infrequently, and is only available after detailed discussion, and when the results of routine serology are known.

This antibody was initially thought to be relatively specific for cerebral lupus. This has not been confirmed. Anti-Ribosomal-P-Protein antibodies are found in 20-40% of patients with definite SLE. Anti-ribosomal-P-Protein appears to be relatively specific for SLE, although it does NOT appear specific for any particular clinical manifestation. We have retained this test in our repertoire because of a small number of reports of positivity in patients with lupus when the anti-dsDNA and anti-ENA are negative.

Interpretation

Negative
Negative result does not exclude SLE, as this antibody is only present in a minority of patients.

Positive
Anti-ribosomal-P-Protein is thought to be specific for SLE. Previously reported associations with cerebral lupus have NOT been confirmed.
13.9  Anti-Neutrophil Cytoplasm Antibodies (ANCA)

Anti-Myeloperoxidase Antibodies (Anti-MPO)
Anti-Proteinase 3 Antibodies (Anti-PR3)

Specimen: Serum, White tube  Minimum volume: 6 mls

ANCA IIF
Method: Indirect Immunofluorescence
Normal value: Negative
Test turnaround time: Next day if negative, 2-3 days if positive.
Frequency of retesting: 3 Weeks, unless discussed.

Anti-MPO
Method: IMMUNOCAP
Normal value: <3.5 Units/mL
Test turnaround time: 2-3 days, or as required
Frequency of retesting: 3 weeks unless discussed

Anti-PR3
Method: IMMUNOCAP
Normal value: <2 Units/mL
Test turnaround time: 2-3 days, or as required
Frequency of retesting: 3 weeks unless discussed

Urgent service and plasmapherisis monitoring available.

Indications:
- Suspected vasculitis
- Renal impairment, haematuria
- Haemoptysis, pulmonary nodules
- Chronic upper respiratory tract inflammation
- Unexplained CNS disease, painful neuropathy

All samples from new patients are screened by indirect immunofluorescence to detect ANCA antibodies. When this is negative, no further testing is undertaken and the sample is reported as Negative.

When any positivity is identified on IIF screening, further testing is undertaken by ELIA, to identify antibodies to MPO and/or PR3, which are the major clinically significant subtypes of ANCA.

In patients who are known to be ANCA positive, in whom their autoantibody specificity has previously been documented as MPO-ANCA or PR3-ANCA, follow-up samples for the purpose of disease monitoring will be tested by ELIA for the relevant antibody only.
Note: Since 03/10/11 the assay for MPO & PR3 antibodies has changed to a new sensitive assay. Unfortunately, evaluation of the new assays in this & other laboratories have shown that whilst the new assay might be an improvement, there are considerable differences in the values that are obtained with the new assays compared with the old. Since these quantitative assays are used in part to monitor treatment & to detect relapse of disease, these changes in values need careful management in the short term to avoid the wrong impression being given. The problem should only affect results in the “changeover” period & once the new assays have been used for a few months then values will again be directly comparable from test to test.

The situation has not been helped by the fact that cut off values & the overall range of the tests have also been changed. The new PR3sensitive & MPOsensitive are calibrated against CDC PR3 ANCA & CDC MPO ANCA Reference Sera respectively & thus the results will be reported in International Units (IU/ml).

To clarify the situation, the test mnemonics will stay the same for ordering requests for MPO & PR3 however the test name on reports will change from anti-PR3 to PR3sensitive & from anti-MPO to MPOsensitive.

Changes to basic parameters of the assays:

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Negative result</th>
<th>Equivocal Result</th>
<th>Positive Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLD ASSAY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-MPO Units/mL</td>
<td>MPO</td>
<td>&lt;7</td>
<td>7-10</td>
</tr>
<tr>
<td>NEW ASSAY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-MPO IU/mL</td>
<td>MPOS</td>
<td>&lt;3.5</td>
<td>3.5-5.0</td>
</tr>
<tr>
<td>OLD ASSAY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-PR3 Units/mL</td>
<td>PR3</td>
<td>&lt;7</td>
<td>7-10</td>
</tr>
<tr>
<td>NEW ASSAY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-PR3 IU/mL</td>
<td>PR3S</td>
<td>&lt;2</td>
<td>2-3</td>
</tr>
</tbody>
</table>

As with all assays that change calibration & units, it is necessary to re-baseline patients who are being monitored for treatment. We have been running the two methods in parallel since April 2011 & have compiled data on a large cohort of patients. Some patients may not have been reviewed in this time period & if we are informed we can hold some of the old reagents to run the samples on both assays for comparison (until the expiry date of the reagents).
A strongly positive ANCA particularly with specificity for PR3 is highly suggestive of vasculitis. However because of the implications of this diagnosis, it is preferable where possible to obtain biopsy confirmation of the diagnosis. Occasionally biopsy may not be possible, due to the rapidity of disease progression or in the case of neurological disease. In such cases it is particularly important to consider and eliminate possible causes of a false positive ANCA. False positivity is less common with PR3-ANCA than with MPO-ANCA. ANCA positivity in the absence of vasculitis is most frequently seen in:

- Chronic and granulomatous infection (including TB)
- Inflammatory Bowel Disease
- Autoimmune hepatitis
- Connective tissue diseases

False positive results are fare less common with the new assay than was previously seen.

We are frequently asked about the relationship of ELISA results to ANCA patterns. When ANCA were first described in the late 1980s, a number of patterns which could be distinguished subjectively when looking at IIF on ethanol-fixed neutrophil slides were described. These included cytoplasmic or C-ANCA, perinuclear or P-ANCA and atypical ANCAs. Initial studies were based on these appearances as the precise antigens had not been identified. However, it should be remembered that the patterns are artefacts due to redistribution of charged proteins within the neutrophil following fixation. The same sera can produce a different pattern on different preparations of neutrophils, and even on different batches of neutrophils prepared in a similar way, as subtle changes in fixation may affect results. It is therefore much more reliable to classify patients according to the ELISA results rather than IIF pattern. However, C-ANCA patterns are most commonly seen in patients with antibodies directed against PR3, with only about 10% of C-ANCA patterns subsequently identified as an MPO-ANCA or occasionally a minor specificity. P-ANCA patterns are due to antibodies to MPO in approximately 50% of cases with 20-30% being due to antibodies to PR3. Other P-ANCAs are due to antibodies to a variety of minor antigens including elastase, lysozyme, Cathepsin G and occasionally BPI or lactoferrin.

Atypical ANCAs produce a variety of patterns of positivity on immunofluorescence and are negative for antibodies to MPO and PR3. These patterns may be seen with antibodies to BPI, elastase, Cathepsin G, lysozyme and lactoferrin, as well as other neutrophil proteins. The clinical relevance of these antibodies is uncertain. Anti-BPI have frequently been reported in patients with cystic fibrosis and non-CF bronchiectasis, but there is no evidence to suggest that measurement of these antibodies provides
useful clinical or prognostic information. **Atypical ANCAAs are NOT specific for vasculitis.**

When a positive antinuclear factor is present it is impossible to exclude the presence of an additional perinuclear ANCA by immunofluorescence. In these cases we report the **ANCA (immunofluorescence) as OBSCURED.** Sera are tested by ELISA to exclude the presence of a PR3-ANCA or MPO-ANCA.

**INTERPRETATION OF RESULTS**

**Negative ANCA**
Active, systemic Wegener’s granulomatosis or microscopic polyarteritis is unlikely, as over 90% of patients with these conditions are positive. However this result does not completely exclude a diagnosis of vasculitis. ANCA is only positive in about 30% of patients with medium vessel vasculitis (Churg-Strauss syndrome and Polyarteritis Nodosa) and is rarely positive in large vessel vasculitis (Giant cell arteritis and Takayasu’s arteritis) or hypersensitivity vasculitis.

ANCA may also be negative in patients with localised small vessel vasculitis, or in patients with treated or inactive disease.

As detailed above the assay for anti PR3 and anti MPO antibodies was changed in October 2011. In order to differentiate the results by the old and new methods, the results by the new method are known as anti -PR3S and anti – MPOS, where the S stands for sensitive. The results by the old method remain anti – PR3 and anti – MPO.

Further audits will be done after introduction of the new assay in order to determine the clinical significance of high, moderate and low positive values.

**ANCA Positive, Anti-PR3S Positive**
Consistent with vasculitis. Patients should be assessed for manifestations of vasculitis. Haematuria and renal function should be assessed without delay, even if vasculitis was not originally considered. Biopsy confirmation should be obtained where possible.

If there is no evidence of vasculitis the patient should be followed up until a diagnosis is established or serology normalises.

**ANCA Positive, Anti-MPOS Positive**
Consistent with vasculitis, or pauci immune glomerulonephritis. Patients should be assessed for manifestations of vasculitis. Haematuria and renal function should be
assessed without delay, even if vasculitis was not originally considered. Biopsy confirmation should be obtained where possible.

If there is no evidence of vasculitis the patient should be followed up until a diagnosis is established or serology normalises. Positive anti-MPOS may be seen in patients with connective tissue diseases, inflammatory bowel diseases and chronic active hepatitis.

**Atypical ANCA**
Clinical significance is uncertain. These antibodies are not suggestive of vasculitis.

**Monitoring disease activity – Serial Measurement of Anti-MPO or Anti-PR3**
In patients who have ANCA associated vasculitis, monthly measurement of anti-MPO or PR3 is helpful in monitoring disease activity. As the half life of IgG is 3 weeks, the test is slow to respond, unless the patient is undergoing plasmapheresis. In the early stages of treatment, frequent measurement of CRP is often helpful in monitoring disease control.

The majority of patients will become antibody negative on treatment. However a proportion of patients in remission, with no clinical or biochemical evidence of inflammation, may continue to be positive, usually at a much lower plateau antibody level than when disease was diagnosed.

A rise in antibody level is followed by relapse in about two thirds of patients, and therefore is an indication for close monitoring and assessment. However ANCA levels alone should not be used to adjust therapy.

Please see below for interpretation of results for anti MPO and anti PR3 by the previous method, for samples prior to October 2011.

**ANCA Positive, PR3 Positive**

<table>
<thead>
<tr>
<th>Value</th>
<th>Interpretation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR3 &gt; 40 Units/mL</td>
<td>Strongly Positive</td>
<td>Suggestive of vasculitis, particularly Wegener’s Granulomatosis</td>
</tr>
<tr>
<td>PR3 21 – 40 Units/mL</td>
<td>Moderately Positive</td>
<td>Consistent with vasculitis. Patients should be carefully assessed for manifestations of vasculitis</td>
</tr>
<tr>
<td>PR3 8 – 20 Units/mL</td>
<td>Weakly positive</td>
<td>Of uncertain clinical significance.</td>
</tr>
</tbody>
</table>
## ANCA Positive, MPO Positive

<table>
<thead>
<tr>
<th>Value</th>
<th>Interpretation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO &gt;50 Units/mL</td>
<td>Strongly Positive</td>
<td>Suggestive of vasculitis or pauci immune glomerulonephritis.</td>
</tr>
<tr>
<td>MPO 21 – 50 Units/mL</td>
<td>Moderately Positive</td>
<td>Consistent with vasculitis or pauci immune glomerulonephritis. Can be seen in connective tissue diseases, inflammatory bowel disease and chronic active hepatitis.</td>
</tr>
<tr>
<td>MPO 6 – 20 Units / mL</td>
<td>Weakly positive</td>
<td>Of uncertain clinical significant. False positives frequently seen at this level.</td>
</tr>
</tbody>
</table>
13.10 Anti-Glomerular Basement Membrane Antibodies (Anti-GBM)

Specimen: Serum, White tube.  
Minimum volume: 6 mls
Method: IMMUNOCAP
Reference value:  
<10 U/mL, Negative  
10-15 U/mL Equivocal  
>15 U/mL Positive.

Test turnaround time: As required
Frequency of retesting: As requested & discussed
Urgent service and Plasmapheresis monitoring available.

<table>
<thead>
<tr>
<th>Indications:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary Haemorrhage</td>
</tr>
<tr>
<td>Acute Renal Failure</td>
</tr>
<tr>
<td>Haematuria of renal origin</td>
</tr>
</tbody>
</table>

INTERPRETATION OF RESULTS

Negative anti-GBM
Active anti-GBM disease extremely unlikely. Even without treatment patients with anti-GBM disease usually become antibody negative within 6-24 months of onset of disease.

Positive (>15 U/mL)
Suggestive of anti-GBM disease. Urgent renal consultation should be arranged, and renal biopsy is usually indicated.

Equivocal (10-15 U/mL)
Active anti-GBM disease is usually associated with substantially higher levels of antibodies. False positive results may be seen in this range, but are unusual. Urgent assessment of renal function and urinalysis is indicated, together with nephrology consult.

Treatment of anti-GBM disease usually involves rapid removal of pre-formed antibodies by plasmapheresis, as well as steroids and cyclophosphamide to minimise further production of antibody. Monitoring antibody levels is useful to determine the duration of plasmapheresis. This can be arranged by discussion with the Consultant Immunologist.

A minority of patients with anti-GBM disease are also positive for ANCA (usually MPO). These patients appear to have a vasculitic component to their disease, and some studies suggest that these patients may respond better than patients with anti-GBM alone to aggressive immunosuppression.
### 13.11 Anti-Cardiolipin Antibodies (IgG and IgM)

**Specimen:** Serum, White tube  
**Minimum volume:** 6 mls  
**Method:** IMMUNOCAP  
**Reference value:**  
- IgG: 0-10 GPLU/mL  
- IgM: 0-10 MPLU/mL  
**Test turnaround time:** 1 week  
**Frequency of retesting:** 12 weeks

<table>
<thead>
<tr>
<th>Indications:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arterial or venous thrombosis</em></td>
<td></td>
</tr>
<tr>
<td><em>Pregnancy associated Morbidity:</em></td>
<td></td>
</tr>
<tr>
<td>Recurrent miscarriage (x 3)</td>
<td></td>
</tr>
<tr>
<td>Mid or third trimester fetal loss</td>
<td></td>
</tr>
<tr>
<td>Severe pre-eclampsia or intrauterine growth retardation requiring delivery before 36 weeks</td>
<td></td>
</tr>
<tr>
<td>Known SLE</td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td></td>
</tr>
<tr>
<td>Ischaemic stroke &lt;50 years</td>
<td></td>
</tr>
<tr>
<td>Transverse myelopathy</td>
<td></td>
</tr>
<tr>
<td>Mesenteric infarction</td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction in the absence of risk factors</td>
<td></td>
</tr>
</tbody>
</table>

**Diagnosis of Anti-Phospholipid Syndrome (APS)**

Establishing a diagnosis of the anti-phospholipid syndrome requires demonstration of a diagnostic clinical manifestation, together with a diagnostic laboratory abnormality, which must be demonstrated on at least two occasions, 12 weeks apart.

**Diagnostic clinical manifestations are:**
- Arterial or venous thrombosis
- Pregnancy associated morbidity (outlined above)

Other clinical features, mentioned above, are associated with the APS, but are not considered specific enough to establish the diagnosis.

**Laboratory diagnostic criteria are:**
- Moderately positive (>40) IgG or IgM anti-cardiolipin
- Lupus anticoagulant
- Anti- Beta 2 glycoprotein 1 antibody

While many patients with APS will have abnormal results in both tests, approximately 10% of patients are positive for lupus anticoagulant only with normal anti-cardiolipin antibodies. Therefore when APS is suspected both anti-cardiolipin and lupus...
anticoagulant should be routinely requested. When clinical suspicion of APS is high, β2Glycoprotein 1 should also be requested.

**INTERPRETATION OF RESULTS**

**Negative IgG and IgM anti-cardiolipin antibodies**

Anti-phospholipid syndrome is unlikely. However the lupus anticoagulant test should also be performed to exclude this diagnosis. Occasionally during an episode of thrombosis, anti-cardiolipin antibody levels fall and so false negative results are obtained. If a diagnosis of APS is strongly suspected, measurement should be repeated 1 to 2 months later.

**Weak positive (IgG and/or IgM anti-cardiolipin <40 GPLU/mL or MPLU/mL)**

Weakly positive values do not fulfil the diagnostic criteria for APS, and are usually non-specific findings. Polyclonal activation of B cells due to infection or inflammation for any cause may produce this result. It is advisable to repeat the test at least 3 months after the patient has recovered from the acute illness, to ensure that levels have reduced or normalised.

**Moderate Positive (IgG and/or IgM anti-cardiolipin 40-79 GPLU/mL or MPLU/mL)**

Consistent with the antiphospholipid syndrome if a diagnostic clinical criterion is present. To establish the diagnosis a follow-up sample must be submitted at least 12 weeks later. Please ensure that the request for this sample indicates that it is a follow-up, and if ordered under a different episode number, please quote the episode or specimen number of the previous sample.

**Strongly Positive (IgG and/or IgM anti-cardiolipin >80 GPLU/mL or MPLU/mL)**

Highly suggestive of the anti-phospholipid syndrome. To establish the diagnosis, a follow-up sample must be submitted for testing at least 12 weeks later. Additionally the diagnosis requires documentation of a clinical manifestation. Please ensure that the request for a follow-up sample indicates that it is a follow-up, and if ordered under a different episode number, please quote the episode or specimen number of the previous sample.

**Note:** Prior to March 2011 Anti-cardiolipin antibodies were measured by ELISA. Reference value: IgG = 0-10 GPLU/mL; IgM = 0-7 MPLU/mL
13.12 Antibodies to Beta 2 Glycoprotein 1

Specimen: Serum, White tube  Minimum volume: 6 mls
Method: IMMUNOCAP  Reference value: <7 U/mL
Test turn around time: 1 week  Frequency of retesting: 12 weeks

<table>
<thead>
<tr>
<th>Indications:</th>
<th>Suspected Antiphospholipid syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>See Section 13.11</td>
</tr>
</tbody>
</table>

The antiphospholipid syndrome (APS) is defined by two major components. Firstly, the presence of at least one type of antiphospholipid antibody (aPL) which are antibodies directed against phospholipid-binding plasma proteins. Secondly, the occurrence of at least one clinical feature:

- **Clinical** — One or more episodes of venous, arterial, or small vessel thrombosis and/or morbidity with pregnancy.
- **Thrombosis** — Unequivocal imaging or histologic evidence of thrombosis in any tissue or organ, OR
- **Pregnancy morbidity** — Otherwise unexplained death at ≥10 weeks gestation of a morphologically normal fetus, OR
- One or more premature births before 34 weeks of gestation because of eclampsia, preeclampsia, or placental insufficiency, OR
- Three or more embryonic (<10 week gestation) pregnancy losses unexplained by maternal or paternal chromosomal abnormalities or maternal anatomic or hormonal causes.
- **Laboratory** — The presence of antiphospholipid antibodies (aPL), on two or more occasions at least 12 weeks apart and no more than five years prior to clinical manifestations.

Although the clinical manifestations of APS occur in other disease populations, in the APS they occur by definition in the context of aPL. APL may be detected by:

- Lupus anticoagulant tests
- Anticardiolipin antibody
- Anti-ß2 glycoprotein antibodies

**Interpretation**

**Negative**
Normal
Positive
A positive B2 glycoprotein 1 antibody may be indicative of anti phospholipid syndrome. A diagnosis can only be made if the above criteria are met. Anti cardiolipin antibody (both IgM and IgG) and lupus anticoagulant should be measured. A second sample should be sent 12 weeks after the first for repeat B2 glycoprotein 1 antibody, anti cardiolipin antibody and lupus anticoagulant. The patient should be carefully examined for clinical manifestations such as livedo reticularis and a history of venous thromboembolism or miscarriage should be sought.
13.13 Anti-Smooth Muscle Antibodies

Specimen: Serum, White tube
Method: Indirect Immunofluorescence
Reference value: Negative
Test turnaround time: 3-5 days
Frequency of retesting: >3 months

<table>
<thead>
<tr>
<th>Indications:</th>
<th>Persistently abnormal Liver Function Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Other signs of chronic liver disease</td>
</tr>
<tr>
<td></td>
<td>Investigation of hypergammaglobulinaemia</td>
</tr>
</tbody>
</table>

INTERPRETATION OF RESULTS

Negative
Normal result

Weak Positive, 1/40
Weak positive anti-smooth muscle antibody is of doubtful clinical significance. Common in the elderly or in patients with infection/inflammation of any cause.

Positive, 1/80
Weak positive value, not specific for autoimmune hepatitis.

Positive 1/160
Moderate positive value is consistent with but not specific for autoimmune hepatitis. Other causes of liver disease should be excluded.

Strong Positive 1/320 or greater.
Strongly positive value is suggestive of autoimmune hepatitis.
13.14 Anti-Liver-Kidney Microsomal (LKM) Antibodies

Specimen: Serum, White tube Minimum volume: 6 mls
Method: Indirect Immunofluorescence + Immunoblot if IIF positive
Reference value: Negative
Test turnaround time: 3-5 days Frequency of retesting: >1 month

Note: When IIF results demonstrate an anti-LKM antibody, the specificity of this result is confirmed by an immunoblotting system using the specific antigen cytochrome P450.

Indications: Persistently abnormal Liver Function Tests
Other signs of chronic liver disease
Investigation of Hypergammaglobulinaemia

Type II autoimmune hepatitis (associated with LKM antibodies) can progress rapidly. The history is often considerably shorter than with Type I autoimmune hepatitis, which is much more common and associated with the presence of anti-smooth muscle antibodies.

INTERPRETATION OF RESULTS

Negative
No serological evidence of type II autoimmune hepatitis.

Positive IIF, Positive Immunoblot
The presence of anti-LKM antibodies is associated with type II autoimmune hepatitis or hepatitis C. The titre of the antibody is not helpful in distinguishing these disorders, and hepatitis serology should be performed.

Positive IIF, Negative Immunoblot
There are a small number of antibodies which generate a pattern (positivity) on IIF which is indistinguishable from LKM antibodies, but the staining is due to binding to antigens other than cytochrome P450. Such antibodies include anti-endoplasmic reticulin antibodies. The clinical significance, if any, of such antibodies is uncertain.

Serial measurement of anti-LKM titre can be useful in monitoring a patients response to therapy.

Because of the rapidity with which Type II autoimmune hepatitis progresses, it is departmental policy to telephone clinicians when a new positive result is detected and contact details are available.
13.15 Anti-Mitochondrial Antibody & M2 subtyping

Specimen: Serum, White tube  Minimum volume: 6 mLs
Method: Indirect Immunofluorescence + ELISA if positive
Reference value: Negative
M2 ELISA <10 IU/ml
Test turnaround time: 3-5 days (2 weeks if ELISA positive)
Frequency of retesting: >3 months M2 performed only once

All newly detected anti-mitochondrial antibodies are tested for reactivity to pyruvate dehydrogenase (M2 subtype) using an ELISA system. M2 type anti-mitochondrial antibodies are highly specific for primary biliary cirrhosis (PBC). M2 testing by ELISA was introduced in January 2005. Prior to this, immunoblot testing was used, which is less sensitive. If a patient had positive IIF with a negative immunoblot, prior to January 2005, we recommend testing for M2 positivity by ELISA. This is particularly important if PBC remains a diagnostic possibility.

<table>
<thead>
<tr>
<th>Indications:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistently abnormal Liver Function Tests</td>
</tr>
<tr>
<td>Other signs of chronic liver disease</td>
</tr>
<tr>
<td>Investigation of hypergammaglobulinaemia</td>
</tr>
<tr>
<td>Pruritis</td>
</tr>
</tbody>
</table>

INTERPRETATION OF RESULTS.

Negative
Normal value

Positive IIF, Positive M2 ELISA.
Suggestive of PBC. Occasionally may be seen in undifferentiated connective tissue disease. The titre of the anti-mitochondrial antibody is usually high (1/320 or greater). However even when the antimitochondrial antibody titre is lower, detection of the M2 subtype is suggestive of PBC. Occasionally M2 positive anti-Mitochondria can be seen in undifferentiated Connective Tissue Disease

Positive IIF, Negative M2 ELISA.
The IIF pattern of staining is frequently atypical (less granular than an M2 type, and with different staining of tissues). This combination of results is not specific for PBC, and may be seen in a wide variety of conditions including undifferentiated connective tissue disease, anti-phospholipid syndrome, infections and other inflammatory conditions.

Note: When an anti-mitochondrial antibody is present granular staining of mitochondria in the liver, kidney tubules and gastric parietal cells is seen. In the presence of a strong anti-mitochondrial antibody, it is not possible to exclude the presence of an anti-gastric-parietal cell antibody, which is obscured.
13.16 Anti-Gastric-Parietal Cell Antibodies (Anti-GPC)

Specimen: Serum, White tube
Minimum volume: 6 mls

Method: Indirect Immunofluorescence

Reference value: Negative

Test turnaround time: 3-5 days
Frequency of retesting: >3 months

| Indications: | Low B12 | Macrocytic anaemia | Suspected subacute combined degeneration of the spinal cord |

INTERPRETATION OF RESULTS.

Negative
Normal value

Positive
Anti-GPC antibodies are present in about 90% of people with atrophic gastritis or pernicious anaemia, however these antibodies are relatively non-specific. Anti-GPC antibodies are present in 20% of relatives of patients with pernicious anaemia, 20% of patients with other autoimmune endocrine disease, as well as 25% of patients with iron deficiency anaemia. They are also present in 16% of females over the age of 60 years. It is recommended that vitamin B12 levels be checked. Sera in which anti-GPC antibodies are found are automatically tested for antibodies to intrinsic factor.

Obscured
When an anti-mitochondrial antibody is present granular staining of mitochondria in the liver, kidney tubules and gastric parietal cells is seen. In the presence of a strong anti-mitochondrial antibody, it is not possible to exclude the presence of an anti-gastric-parietal cell antibody, which is obscured. If pernicious anaemia is suspected, an anti-intrinsic factor antibody should be requested.
13.17 Anti-Intrinsic Factor Antibodies

Specimen: Serum, White tube
Minimum volume: 6 mls
Method: ELISA
Reference value: <6 U/ml Negative
>= 6 U/ml Positive

Test turnaround time: 1 week
Frequency of retesting: >6 months

Indications:
- Low B12
- Macrocytic anaemia
- Suspected subacute combined degeneration of the spinal cord

INTERPRETATION OF RESULTS

Negative
Negative anti-Intrinsic Factor antibody does not exclude a diagnosis of pernicious anaemia, as this antibody is only found in approximately 60% of subjects with pernicious anaemia.

Positive
Positive result is suggestive of pernicious anaemia, and measurement of vitamin B12 is recommended. Patients with a normal vitamin B12 may have latent pernicious anaemia, and follow-up with at least annual measurement of Vitamin B12 level is recommended.
13.18 Anti Thyroid Peroxidase Antibodies (anti-TPO)

Specimen: Serum, White tube  
Minimum volume: 6 mls

Method: ELISA

Reference value:  

<table>
<thead>
<tr>
<th>Value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50 IU/ml</td>
<td>Negative</td>
</tr>
<tr>
<td>50-75</td>
<td>Equivocal</td>
</tr>
<tr>
<td>&gt;75</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Test turnaround time: 1 week  
Frequency of retesting: >6 months. If Equivocal >3 months.

### Indications:

- Hypothyroidism
- Hyperthyroidism
- Goitre
- Other autoimmune endocrinopathy

TPO is the specific antigen causing reactivity in the anti-thyroid microsomal assays. In line with current recommendations we now use this more sensitive and specific assay for all requests.

**INTERPRETATION of RESULTS**

**Negative (Anti-TPO < 50 IU/ml)**  
Autoimmune thyroid disease unlikely.

**Equivocal (Anti-TPO 50-75 IU/ml)**  
Indicates thyroid autoreactivity, however autoimmune thyroid disease is usually associated with higher titres of antibodies. Please repeat in 3-6 months.

**Positive (Anti-TPO > 75 IU/ml)**  
Positive anti-TPO antibodies indicate current or future risk of autoimmune thyroid disease. Thyroid function should be checked now and at 1-2 year intervals.
13.19 Anti-Thyroid Antibodies

Anti-Thyroid Microsomal Antibodies (Anti-TM)
(Please note: In May 2006 we changed the method used to measure anti-thyroid antibodies to an ELISA based anti-thyroid peroxidase (TPO) assay. Anti-TM can no longer be ordered. Information on the anti-TM assay is still included here as results from this assay are still listed in patients' records.)

Method: Particle Agglutination
Reference value: Anti-TM Negative <1/100

INTERPRETATION OF RESULTS

Negative
Autoimmune thyroid disease unlikely

Weak Positive (Anti-TM <= 1/1600)
Indicates thyroid autoreactivity, however autoimmune thyroid disease is usually associated with higher antibody levels. It is suggested that thyroid function be checked, and TFTs and anti-thyroid antibody levels be repeated in 2-3 years.

Care should be taken in patients with goitre and thyroid nodules. Weakly positive anti-thyroid antibodies are not uncommon in people with non-immunological thyroid disease (up to 30% of patients with thyroid cancer).

Positive (Anti-TM >1/1600)
Indicates current or future risk of autoimmune thyroid disease. Thyroid function should be checked, and repeated annually to facilitate early diagnosis of hypothyroidism. Occasionally, significantly positive levels of antibodies are seen in patients with thyroid cancer and therefore thyroid nodules should be fully assessed, even when autoantibodies are detected.

Note: Anti-thyroid antibodies may be identified transiently in De Quervain's thyroiditis. During pregnancy detection of anti-thyroid antibodies indicates significant risk for post-partum thyroiditis, which is associated with an increased incidence of post-natal depression.

Measurement of anti-thyroglobulin (TG) antibodies add little to assessment of autoimmune thyroid disease. However anti-TG may be of value in patients with thyroid cancer, where use of thyroglobulin measurements is planned to monitor response to therapy and/or recurrence. Measurement of thyroglobulin is unreliable in the presence of anti-thyroglobulin antibodies.
13.20 Anti-Adrenal Antibodies

Specimen: Serum, White tube Minimum volume: 6 mls
Method: Indirect immunofluorescence
Reference value: Negative
Test turnaround time: 2 weeks Frequency of retesting: 6 months

<table>
<thead>
<tr>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypocortisolaemia</td>
</tr>
<tr>
<td>Other autoimmune endocrinopathy</td>
</tr>
<tr>
<td>Hyperpigmentation</td>
</tr>
</tbody>
</table>

**INTERPRETATION OF RESULTS**

**Negative**
Negative result does not exclude autoimmune adrenalitis, as antibodies are detected in approximately 70% - 80% of these patients.

**Positive**
Suggestive of autoimmune adrenalitis. However anti-adrenal antibodies are found in about 5% of patients with adrenal destruction due to non-immunological disease. Anti-adrenal antibodies may indicate future risk of developing autoimmune adrenalitis.

Patients with autoimmune Adrenal Disease should be screened for other autoimmune endocrinopathies (thyroid, ovarian, testis and islet cell antibodies). There may also be an association with other non-endocrine organ specific disorders including Pernicious Anaemia and rarely Myasthenia Gravis. Testing for rare associations is only indicated when symptoms are present.
13.21 Anti- Tissue Transglutaminase Antibodies (anti-tTG)

Specimen: Serum, White tube  
Minimum volume: 6 ml

Method: IMMUNOCAP

Reference value:  
- <7 U/ml = Negative
- 7-10 U/ml = Equivocal
- >10 U/ml = Positive

Please note that anti-tTG is the appropriate screening test for coeliac disease. Equivocal or positive sera will be automatically tested for anti-endomysial antibodies. Our assay and reference ranges have been extensively validated internally, to ensure that an appropriately low threshold for triggering anti-endomysial antibody testing is in place.

Test turnaround time: 1 week  
Frequency of retesting: >3 months

<table>
<thead>
<tr>
<th>Indications:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspected coeliac disease</td>
</tr>
<tr>
<td>Malabsorption (including low iron, Vit B12 or albumin)</td>
</tr>
<tr>
<td>Anaemia</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
</tr>
<tr>
<td>Down's syndrome (increased risk of coeliac disease)</td>
</tr>
<tr>
<td>IDDM (increased risk of coeliac disease)</td>
</tr>
<tr>
<td>Dermatitis Herpetiformis</td>
</tr>
<tr>
<td>Osteoporosis &amp; Osteomalacia</td>
</tr>
<tr>
<td>Peripheral Neuropathy</td>
</tr>
<tr>
<td>Unexplained Infertility</td>
</tr>
<tr>
<td>Unexplained weight loss</td>
</tr>
</tbody>
</table>

In addition to classical presentations with GI symptoms and malabsorption, coeliac disease is found in about 3.4% of those with osteoporosis, 12% of those with Type I diabetes mellitus and up to 1% of the general population.

tTG has been identified as the target antigen against which anti-EMA is directed. The anti-tTG ELIA is used as an initial screening test and all equivocal/positive sera will be further tested for EMA antibodies. IgA levels will be measured on all negative anti-tTGS to exclude IgA deficiency. Anti-tTG has a high sensitivity for untreated coeliac disease, while the anti-endomysial antibody is more specific. Sequential testing offers optimal diagnostic utility.
INTERPRETATION OF RESULTS

**Negative (<7 U/ml)**
Coeliac disease unlikely if the patient is on a normal diet. If clinical suspicion is high, should be repeated in 3-6 months, ensuring that the patient is on a diet with a normal gluten content.

**Equivocal 7-10 U/ml**
All equivocal results will be further tested for IgA anti-EMA.

**Positive >10 U/ml**
Suggestive of Coeliac Disease. However false positives may occur therefore all samples with positive anti-tTG by ELISA will be further tested for EMA antibodies by indirect immunofluorescence.
### 13.22 IgA Anti-Endomysial Antibodies (EMA)

**Specimen:** Serum, White tube  
**Minimum volume:** 6 mls  
**Method:** Indirect Immunofluorescence  
**Reference value:** Negative  
**Test turnaround time:** 1 week  
**Frequency of retesting:** >3 months

| Indications: | Positive anti-tTG (automatically added as reflex test)  
Biopsy suggestive of coeliac disease, despite negative tTG**  
Strong clinical suspicion of coeliac disease, despite negative tTG** |

**Discussion with clinical team essential to have test performed for these indications.**

In patients with normal levels of IgA, IgA anti-endomysial antibodies are more than 90% sensitive (up to 98% sensitive in some studies) and relatively specific (>95%) for coeliac disease. When an anti-endomysial antibody request is received in this laboratory, we also measure IgA levels to exclude IgA deficiency. If IgA deficiency is identified serum is sent to the Proteins Laboratory in Clinical Chemistry for further assessment of immunoglobulins.

IgA deficiency is present in about 1:30 patients with coeliac disease (and about 1:600 of the general population). When IgA deficiency is present serology is less helpful in assessing the likelihood of coeliac disease. However in patients with IgA deficiency we perform an IgG anti-endomysial antibody which if strongly positive is suggestive of coeliac disease.

**INTERPRETATION OF RESULTS**

**Negative IgA anti-endomysial antibodies**

Coeliac disease is unlikely if patient is on a normal diet. However false negative results may be seen in IgA deficiency, and also in patients on a gluten free diet. The clinical significance of a negative EMA in a patient with a positive anti-tTG is uncertain, however an expert GI opinion should be sought in this situation, as biopsy may still be indicated.
Positive IgA anti-endomysial antibodies
Suggestive of coeliac disease

Negative IgA anti-endomysial antibodies, Low IgA
In this setting, negative anti-endomysial antibody does not exclude coeliac disease. If there is a high clinical suspicion of coeliac disease, or if the IgG anti-endomysial antibody is strongly positive, biopsy is indicated.

Negative IgA and IgG anti-endomysial antibodies, Low serum IgA
The negative predictive value of serology in this setting is not well established, and if there is a strong clinical suspicion of coeliac disease, biopsy is necessary to exclude coeliac disease.

If a low IgA is detected, serum is sent to the Proteins Laboratory in Clinical Chemistry for immunoglobulins and SPEP. This is to exclude a more extensive hypogammaglobulinaemia. However patients with isolated IgA deficiency are at risk of infections, allergy, autoimmune disease and serious transfusion reactions. You may wish to arrange for a Clinical Immunology appointment for further assessment.
13.23 Anti-Neuronal Antibodies – Anti-Hu & anti-Yo

Specimen: Serum, White tube
Minimum volume: 6 mls

Method: Indirect Immunofluorescence with confirmation by Immunoblot

Reference value: Negative
Test turnaround time: 1 week
Frequency of retesting: >6 months

<table>
<thead>
<tr>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspected paraneoplastic neurological syndromes</td>
</tr>
<tr>
<td>Esp – acute or subacute cerebellar syndromes</td>
</tr>
<tr>
<td>Encephalomyelitis</td>
</tr>
<tr>
<td>Sensory &amp; autonomic neuropathy</td>
</tr>
<tr>
<td>Axial ataxia</td>
</tr>
<tr>
<td>Opsoclonus-myoclonus</td>
</tr>
</tbody>
</table>

A screening indirect immunofluorescence assay is performed in-house. All positive results are confirmed using an Immunoblot. The presence of an ANF renders the IIF test difficult to interpret. ANF positive specimens are also run on the Immunoblot. If you are concerned about some of the more recently described antibodies please discuss the case with Senior Laboratory Staff or Dr. Keogan.

Negative anti-Hu and anti-Yo
Negative results do not exclude a paraneoplastic syndrome. Novel autoantibodies are being described associated with these syndromes, and the existing panel is relatively insensitive.

Positive anti-Hu
This antibody is typically associated with encephalomyelitis (including limbic encephalomyelitis, brainstem encephalitis and cerebellar degeneration) and also sensory-autonomic neuropathy. The antibody is usually associated with small cell lung tumours and rarely neuroblastoma, prostate, rhabdosphcoma and seminoma.

Positive anti-Yo is associated with cerebellar degeneration and mild non-cerebellar involvement. It is commonly associated with ovarian and breast tumours; rarely also with tumours of the Fallopian tube and lung.

While the above paragraphs outline the classical associations, recent data suggest that the neurological associations are less clear-cut, and this should be considered when ordering tests.
13.24 Anti-Skin Antibodies

Specimen: Serum, White tube  
Minimum volume: 6 mL

Method: Indirect Immunofluorescence

Reference value: Negative

Test turnaround time: 1 week

Frequency of retesting: 6 months, but pos ICS as requested

| Indications: | Blistering skin disorders – pemphigus & pemphigoid |

**Pemphigus** is associated with antibodies to the epidermal intercellular substance (ICS). Anti epidermal ICS is thought to be pathogenic in this condition, and serial measurement of antibody titre is of value in monitoring the disease and response to therapy.

**Pemphigoid** is associated with antibodies to basement membrane zone (BMZ). Although antibodies of some IgG subclasses are thought to be pathogenic, the total IgG antibody titre does not reflect disease activity. We therefore do not offer titration of this antibody.

**Interpretation**

**Negative**

Negative result does not exclude these conditions as the sensitivity of antibodies is only about 80% in systemic disease. It is considerably lower in patients with localised forms of pemphigoid.

**Positive anti-epidermal ICS**

Suggestive of pemphigus, particularly when strongly positive. Occasionally weak positive results may be found as a non-specific feature, particularly in burns and SLE.

**Positive anti-BMZ**

Suggestive of bullous pemphigoid, or rarely epidermolysis bullosa acquisita or herpes gestationis.
### 13.25 Total IgE and allergen specific IgE

**Specimen:** Serum, White tube  
**Method:** IMMUNOCAP  
**Minimum volume:** 6 mls.

**Reference value:** Total IgE  
**Range is age related.**  
**Adult reference range 0-100 kU/L**

**Specific IgE**

<table>
<thead>
<tr>
<th>Class</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 0</td>
<td>&lt;0.35 Units</td>
</tr>
<tr>
<td>Class 1</td>
<td>0.35-0.7</td>
</tr>
<tr>
<td>Class 2</td>
<td>0.7-3.5</td>
</tr>
<tr>
<td>Class 3</td>
<td>3.5-17.5</td>
</tr>
<tr>
<td>Class 4</td>
<td>17.5-52.5</td>
</tr>
<tr>
<td>Class 5</td>
<td>52.5-100</td>
</tr>
<tr>
<td>Class 6</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

(Source: Protein Reference Unit Handbook)

**Test turnaround time:** 1 week  
**Frequency of retesting:** 1 year

### Indications: - Total IgE:

- Suspected allergic disease - (angiodema, rhinitis, asthma, anaphylaxis, acute urticaria,)
- Aids interpretation of allergen specific IgE results
- Suspected Churg-Strauss Syndrome
- Possible hyper-IgE Syndrome – (immunodeficiency with eczema, recurrent Staph Aureus infections, boils & abscesses coarse facial features)
- Suspected parasitic infection

### Indications – Allergen-Specific IgE

- Known allergic disease, to identify allergens
- Suspected allergic bronchopulmonary aspergillosis (ABPA)
- **Allergens**

Allergen specific IgE should be requested for limited number of allergens suggested by history. Disease specific profiles of suggested allergens are listed on Page 12. If history is vague, skin testing is more useful to test for large number of allergens. When skin tests cannot be performed due to extensive skin disease/dermographism/patient unable to stop antihistamines/unacceptable risk of anaphylaxis, a more extensive range of RAST testing may be ordered after discussion with Senior laboratory or Medical staff.
Interpretation

Interpretation of allergen-specific IgE is linked with the level of total IgE, as well as the class of allergen specific IgE. Interpretation of both types of tests are considered below.

**Normal Total IgE**
Excludes atopy. However, a normal IgE does not exclude sensitisation to individual allergens. As a general rule even weakly positive allergen-specific IgE may be clinically relevant in patients with a low normal IgE. However the relevance of allergen specific IgE must be carefully assessed in the context of the clinical history.

**Raised Total IgE**
Consistent with atopy. Atopy denotes a genetic susceptibility to make IgE responses. This does not imply that atopic disease is present. The possible role of atopy in the patients clinical presentation should be carefully assessed. False positive results for allergen-specific IgE, particularly of class 1 & 2 become more common the higher the total IgE. In patients with a raised IgE >1000kUA/L, even class 3 allergen-specific IgEs may be false positives. The clinical relevance of allergen-specific IgE measurements must be considered in the clinical context. If uncertain, you may consider referring the patient to the immunology clinic.

Raised IgE may also be due to parasitic infection (eosinophilia usually also present) and Churg-Strauss syndrome.

**Total IgE > 5000kUA/L**
If patient has infections consider the Hyper-IgE syndrome. If this is a diagnostic possibility, please contact the Immunology Department to arrange accurate quantification of level (and clinical consultation if required).
Values of IgE > 5000kUA/L are not uncommon in patients with atopic eczema alone. In such patients allergen-specific IgE results must be assessed with extreme caution.
13.26 Complement - C3 and C4

Specimen: Serum, White tube  
Minimum volume: 6 mLs
Method: Nephelometry
Reference value: C3 0.75 – 1.65g/L  
C4 0.14 – 0.54g/L
Test turnaround time: Next day  
Frequency of retesting: As requested

<table>
<thead>
<tr>
<th>Indications:</th>
<th>Diagnosis of suspected immune complex disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monitoring immune complex disease including</td>
</tr>
<tr>
<td></td>
<td>cryoglobulinaemia and SLE</td>
</tr>
<tr>
<td></td>
<td>Angioedema (without urticaria)</td>
</tr>
<tr>
<td></td>
<td>Glomerulonephritis</td>
</tr>
<tr>
<td></td>
<td>Suspected anaphylactoid reaction eg to IVIg, colloid infusions</td>
</tr>
</tbody>
</table>

Complement components act as acute phase reactants, and thus inflammation causes a rise in levels. Activation of the complement cascade causes depletion of C3 and C4 (classical and lectin pathways) or C3 alone (alternative pathway). However in most circumstances when complement is consumed, inflammation also occurs and so the opposing acute phase response may mask complement consumption. In difficult cases we can send serum to the UK for measurement of complement activation products. Please discuss any difficult cases with Dr. Keogan.

Complement levels are normally increased in pregnancy, and this may also mask a fall in complement levels due to disease. Complement is activated during dialysis and plasmapheresis and therefore samples should be collected before these procedures are undertaken.

Measurement of C3 and C4 is not the investigation of choice when complement deficiency is suspected (because of recurrent infections, repeated neisserial infections, immune complex disease at a young age, personal or family history of combinations of these features). The appropriate test is the CH100, which tests the functional integrity of the entire classical pathway. However if the functional CH100 assay is abnormal, measurement of individual components is advised. It is important to remember that complement deficiency results both from protein deficiency as well as production of normal amounts of dysfunctional protein. The standard C3 and C4 assays do not distinguish between normal functional and abnormal dysfunctional protein.

The reference range for C4 levels in particular is broad. This is because C4 is encoded by 4 different genes. Null genes are present quite commonly, and the normal population includes people with one, two or three null genes. If you are a person with 4 functional
genes, your “normal” C4 level will be in the higher quartile of the reference range. Even with significant complement consumption the C4 level may remain within the reference range for the population. Therefore a fall in C4 levels within the reference range may be clinically very significant.

**Interpretation**

**Raised C3, raised C4 or raised C3 and C4.**
These are common findings during an acute phase response. However measurement of complement is not recommended to assess the acute phase response – CRP is the most valuable marker.

**Reduced C3 but Normal C4**
Suggestive of complement activation usually via the alternative pathway. This is typical of post-streptococcal glomerulonephritis and Type II membranoproliferative glomerulonephritis (associated with the presence of nephritic factor). However this pattern may be due to complement consumption via the classical pathway in a patient who usually runs a high normal C4 level (see above).

**Reduced C3 and C4**
Indicates complement consumption via the classical pathway, usually associated with immune complex disease. Occasionally low levels may be seen in the absence of complement consumption when hepatic synthetic function is seriously impaired.

**Reduced C4, Normal C3**
Typically this pattern is seen with activation of the early classical pathway (usually due to fluid phase activation of the classical pathway). If the patient has angioedema or abdominal pain, C1-Inhibitor deficiency should be considered. Cryoglobulinaemia may also be associated with similar findings. This pattern may reflect conventional activation of the classical pathway in patients who normally run a high normal C3, particularly when the C3 is in the lower quartile of the reference range.
13.27 Complement C2

Specimen: Serum, White tube Minimum volume: 6 mls
Method: RID
Reference value: 10 – 30mg/L
Test turnaround time: 2-4 weeks Frequency of retesting: Once off

Indications:
- Diagnosis of immune complex disease
- Monitoring of immune complex disease
- Glomerulonephritis
- Infections

C2 is low in conditions in which classical complement pathway activation has occurred, usually as a consequence of immune complex mediated activation. It is often a more sensitive indicator of complement activation than either C3 or C4. C2 null alleles are probably the most common genetic abnormality of the complement system (estimated gene frequency 1:100 to 1:500) and low concentrations may be due to heterozygous deficiency. Complete deficiency should result in an absent CH100.

Interpretation

C2 Reduced
Suggests C2 consumption via activation of the classical pathway, usually associated with immune complex disease. CH100 should be done to assess complement function.

C2 Absent
May be due to genetic abnormality, complement functional integrity should be assessed.
13.28 Complement C1Q

Specimen: Serum, White tube  
Minimum volume: 6 mls
Method: RID
Reference value: 50 – 250mg/L
Test turnaround time: 4-6 weeks
Frequency of retesting: 6 months unless HAE on treatment

<table>
<thead>
<tr>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angioedema without Urticaria</td>
</tr>
<tr>
<td>SLE</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
</tr>
<tr>
<td>Recurrent bacterial infections</td>
</tr>
</tbody>
</table>

There are few clinical situations which require measurement of C1Q levels, except as part of the assessment of an absent CH100 in a patient with increased susceptibility to infection. The one exception to this is the identification of the consumptive defect in acquired C1 Inhibitor deficiency, where levels are always subnormal.

Interpretation

**C1Q Reduced or Absent**
If patient has angioedema C1 INH testing should be done.
13.29 Complement Function CH100 and AP100

Specimen: Serum, white tube Minimum volume: 6 mls
(It is essential that serum is separated and frozen within 3 hours maximum after venepuncture)
Method: Gel diffusion haemolysis
Test turnaround time: 4-6 weeks Frequency of retesting: 3 weeks

<table>
<thead>
<tr>
<th>Indications:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune complex disease such as SLE</td>
</tr>
<tr>
<td>Recurrent infections</td>
</tr>
<tr>
<td>Immune complex disease with recurrent infections</td>
</tr>
<tr>
<td>Family history of complement deficiency or any of the above</td>
</tr>
</tbody>
</table>

CH100 tests the functional integrity of the Classical pathway and AP100 tests the Alternative pathway. If abnormal results are obtained the assay will be repeated. If the repeat test is abnormal we will request a repeat sample to ensure the abnormal result was not an artefact of inappropriate sample handling or storage. A time period of 3-4 weeks post acute infection should be allowed before testing Complement function.

Interpretation

CH100 Normal
Classical pathway functioning normally

CH100 Reduced or Absent
Decreased complement activity may be caused by deficiencies of any of the individual components of the Classical pathway, hereditary or acquired, glomerulonephritis, SLE or vasculitis.

AP100 Normal
Alternate pathway functioning normally

AP100 Reduced or Absent
Decreased complement activity may be caused by deficiencies of any of the individual components of the Alternate pathway, hereditary or acquired,
13.30 Complement C1 Esterase Inhibitor (C1INH)

Specimen: Serum, White tube  Minimum volume: 6 mls
Method: RID
Reference value: 150 – 350mg/L
Test turnaround time: 4-6 weeks
Frequency of retesting: Once off if normal, as required if low

| Indications: | Oedema of skin, gastrointestinal or respiratory tract |

**Hereditary angioedema (HAE):** deficiency of C1 esterase inhibitor is the most frequent of the inherited complement component deficiencies. The condition is inherited as an autosomal dominant trait and several members of a family are usually affected. The commonest symptoms are episodes of painless swellings on the limbs or trunk which subside in 24-48 hours. Recurrent abdominal pain or respiratory obstruction, which can be fatal, may also form part of the clinical picture.

In view of the autosomal dominant inheritance of this condition full family studies are essential in all cases where the diagnosis is proven. The investigation can initially be restricted to quantitation of C3 and C4 levels. Antigenic and functional assay of C1INH can be reserved for those family members who have been shown to have subnormal C4 concentrations with normal concentrations of C3.

Two forms of the inherited deficiency exist. In the classic **Type 1**, low concentrations of C1 INH are found by both antigenic and functional assay. **Type 2** is characterised by normal or elevated concentrations of C1 INH by the antigenic assay but absent functional activity. The assay of functional C1 INH is essential for this diagnosis.

**Acquired C1 inhibitor deficiency:** There is a rare form of C1 INH deficiency which presents for the first time in adult life. Most reported cases have been secondary to lymphoma or myeloma. This is a consumptive rather than a synthetic defect and is associated with low concentrations of C1Q.

**Interpretation**

**C1 INH Low (**\( \leq 50 \text{ mg/L} \)**)
Profound reduction in C1 INH is consistent with hereditary or acquired C1 INH deficiency.

**C1 INH Reduced (50 – 99 mg/L)**
Significant reduction in C1 INH may be due to consumption but deficiency cannot be excluded.
C1 INH Borderline (100 - 149 mg/L)
Borderline C1 INH is commonly seen with activation of complement via the classical pathway, or in patients on treatment for hereditary angioedema. Profound reduction in C1 INH is usually seen in untreated C1 INH deficiency, but this should be considered if patient has angioedema.

C1 INH Normal (150-350 mg/L)
Normal levels of C1 INH. However a small number of cases of C1 INH deficiency are due to a dysfunctional protein with normal or high C1 INH levels. If a patient has angioedema in the absence of urticaria further testing of functional C1 INH may be indicated. C1 INH testing is not indicated in patients with urticaria or without angioedema.

C1 INH raised (> 350 mg/L)
A small number of cases of C1 INH deficiency are due to a dysfunctional protein with normal or high C1 INH levels. If a patient has angioedema in the absence of urticaria further testing of functional C1 INH may be indicated. C1 INH testing is not indicated in patients with urticaria or without angioedema.
13.31 C1 Inhibitor Function

Specimen: Serum, White tube  Minimum volume: 6 mls
Method: ELISA  Reference value: >68%
Test turn around time: 4 months
Frequency of retesting: Discuss with Consultant Immunologist

<table>
<thead>
<tr>
<th>Indications:</th>
<th>Suspected hereditary angioedema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Angioedema without urticaria AND low C4 during an attack</td>
</tr>
</tbody>
</table>

A deficiency of functionally active C1-INH may lead to angioedema. There are 2 major forms of C1-INH deficiency: the congenital form, termed hereditary angioedema (HAE), and the acquired form that is usually secondary to lymphomas or myeloma.

Two forms of the inherited deficiency exist. In the classic Type 1, low concentrations of C1 inhibitor are found by both antigenic and functional assay. Type 2 is characterised by low levels of functional activity but normal or elevated concentrations of C1 inhibitor that is dysfunctional.

The acquired form of C1 inhibitor deficiency is a consumptive rather than a synthetic defect and is associated with low concentrations of C1Q.

Interpretation

Normal
Normal C1 inhibitor function excludes hereditary angioedema Types 1 and 2 and acquired C1 inhibitor deficiency.

C1 Inhibitor Function Equivocal
Equivocal C1 inhibitor function may be due to C1 inhibitor deficiency or may indicate incorrect specimen handling, so a repeat sample should be performed. Please discuss with the immunology team at bleep 797.

C1 Inhibitor Function Low
Low values are often caused by incorrect specimen transport and handling, so a repeat sample should be performed. Low C1 inhibitor function on repeat testing is suggestive of hereditary angioedema or acquired C1 inhibitor deficiency. Suggest urgent referral to Clinical Immunologist.
13.32 Anti-Streptolysin-O Titre (ASOT)

Specimen: Serum, White tube
Method: Nephelometry
Minimum volume: 6 mls
Reference value: <200 IU/mL
Test turnaround time: 2-3 days
Frequency of retesting: >3 weeks

Indications:
- Suspected current or recent streptococcal infection
- Possible rheumatic fever
- Glomerulonephritis & acute renal failure
- Reactive arthritis

Anti-streptolysin-O antibodies may be produced following infection with Group A Streptococci. Only a proportion of the subtypes of group A Strep can cause rheumatic fever or glomerulonephritis in genetically susceptible individuals, usually with an onset 2-4 weeks after the infection. The ASOT does not distinguish between nephritogenic and non-nephritogenic strains – a positive result merely indicates current or recent infection with streptococcus.

If rheumatic fever is suspected, evidence of recent streptococcal infection is required for diagnosis. If cultures and ASOT are negative, it may be of value to measure anti-DNAase, an additional antibody which may be produced following a Streptococcal infection.

Interpretation

Negative ASOT (<200 IU/mL)
Negative result does not exclude Group A Streptococcal infection as this antibody is present in only 80-85% of patients with Streptococcal pharygitis. A smaller proportion of patients with skin infection are antibody positive.

Positive ASOT (>200 IU/mL)
Indicates current or recent infection with Group A Streptococci.
13.33 Mast Cell Tryptase

Specimen: Serum, White tube
Minimum volume: 6 mls
Method: IMMUNOCAP
Reference value: <14 ug/L (Ante-mortem specimens only)
Test turnaround time: 1 month
Frequency of retesting: As requested/discussed

Indications:
- Assessment of possible anaphylaxis (Requires serial samples: following resuscitation, 4-6 hours and >24 hours after the event)
- Systemic mastocytosis – diagnosis & monitoring
- Hypereosinophilic syndromes
- Post-Mortem assessment of sudden death, if anaphylaxis considered likely/possible

Tryptase is released following mast cell degranulation, and while elevated levels indicate that mast cell degranulation occur, this test provides no information about the cause of mast cell degranulation. Following an anaphylactic reactions levels typically peak within an hour, remain elevated for about 6 hours and return to baseline by 24 hours.

In systemic mastocytosis, levels are typically raised, and levels may be useful to monitor disease burden. In localised or cutaneous limited mastocytosis, tryptase levels may be within the normal range. Hence persistent elevation of tryptase supports a diagnosis of mastocytosis, however normal levels do not exclude this diagnosis.

In the hypereosinophilic syndromes, there is some data to suggest that an elevated tryptase may be a poor prognostic factor.

Post-mortem levels of tryptase are affected by factors such as time between death and blood sampling, trauma, use and duration of CPR. Hence the interpretation of post-mortem samples is undertaken by the Consultant immunologist, in consultation with the Consultant pathologist who undertook the post mortem.
INTERPRETATION

Serial samples, Post-resuscitation or 2nd sample elevated, normal levels at 24 hours: Indicates mast cell degranulation has occurred. While this is usually due to a severe IgE mediated allergic reaction, similar results may be seen following administration of drugs which cause direct mast cell degranulation such as contrast media.

Serial samples: all normal
No evidence to support anaphylaxis, however results do not exclude this diagnosis. Tryptase is not a sensitive marker of anaphylaxis due to food allergy. Elevations are more likely to be seen following reactions to parenteral administration of drugs and venom allergy.

Persistently elevated levels
Mastocytosis or hypereosinophilic syndrome should be considered. If no evidence of disease at present patient should be monitored, with repeat bone marrow and other appropriate biopsies in the future.

In the setting of documented hypereosinophilic syndrome, persistently elevated tryptase appears to be a poor prognostic marker.

Normal single level
Systemic mastocytosis unlikely, however limited disease cannot be excluded. Tryptase is not useful in the diagnosis of hypereosinophilic syndrome, hence normal level does not exclude this condition.
13.34 NMDA

Specimen: Serum, White tube  Minimum volume: 6 mls
Method: Indirect Immunofluorescence
Reference value: Negative
Test turn around time: 3 weeks
Frequency of retesting: Discuss with Consultant Immunologist

<table>
<thead>
<tr>
<th>Indications</th>
<th>Suspected NMDA Encephalitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients who develop rapid changes in behaviour, psychosis, abnormal postures or movements including catatonia, seizures and signs of autonomic instability</td>
</tr>
</tbody>
</table>

NMDA Receptor antibody was first found in young female patients with ovarian tumours and prominent psychiatric symptoms, amnesia, seizures, dyskinesias, autonomic dysfunction and decreased level of consciousness. The antibodies are now also found in males and females with no known tumour and in children.

Interpretation

Negative
Normal Value

Positive
Positive result is suggestive of anti-NMDA receptor encephalitis. Their presence suggests an immunotherapy responsive condition.

This assay is currently being validated, however it is technically demanding. During the validation period, specimens will be sent to Oxford as well as processed in-house. A preliminary report will be issued on any clear positive or negative results from the in-house screening assay. Equivocal results will await definitive assessment from Oxford.
13.35 Specific IgG: For Assessment of Immunodeficiency

Specimen: Serum, White tube
Method: ELISA
Minimum volume: 6 mls
Test turn around time: 2-4 weeks
Frequency of retesting: Pre-vaccine, 1 month post, 3-6 months & then annually for Pneumococcal. Pre & Post vaccine for others.

Indications: Suspected humoral immunodeficiency

Specific IgG used for assessment of Immunodeficiency include Anti – pneumococcal antibody, anti – Hib antibody, anti – Tetanus antibody and anti – Diptheria antibody.

Selective antibody deficiency may be identified as part of a host of distinct primary or secondary immunodeficiency disorders or it may exist in isolation.

Anti-Pneumococcal Antibodies
The polysaccharide pneumococcal vaccine is widely used to assess immune function and identify immunodeficiency in patients with recurrent and/or severe infections. Pneumococcal antibodies are measured before vaccination with a polysaccharide pneumococcal vaccine and 4 weeks after.

Interpretation

Normal Response
A normal response to vaccination is a four fold increase in the level of titres. These may not indicate protection to all serotypes and does not exclude humoral immunodeficiency. If there is clinical concern regarding immunodeficiency, please contact the clinical immunology team at bleep 797.

Suboptimal Response
Pre and post vaccination results with 2-3 fold increase in levels is a suboptimal rise in antibody levels. If the patient has a significant history of recurrent bacterial infections, please discuss clinical details with Immunology clinical team.
Poor Response
Pre and post vaccination results with no significant rise in antibody levels is a poor vaccine response. In patients with significant clinical history of recurrent bacterial infections, poor vaccine response is suggestive of specific antibody deficiency. Please discuss with clinical Immunology team.

Anti-HIB antibodies
Are measured to assess level of protective antibodies (IgG) to HIB

Interpretation
Minimal Protective level of Anti HIB antibodies: 0.15mg/l
Optimum Protective level: 1.0mg/l

Anti-Tetanus antibodies
Measured to assess level of protective antibodies (IgG) to Tetanus

Interpretation

<0.01 IU/ml - indicates susceptible individual.
0.01–0.09 IU/ml - some degree (basic) protection.
0.1–0.9 IU/ml - indicates full protection level.
>1.0 IU/ml - provides long term protection.

Anti-Diphtheria Antibodies
Measured to assess level of protective antibodies (IgG) to Diptheria

Interpretation

<0.01 IU/ml - indicates susceptible individual.
0.01–0.09 IU/ml - some degree (basic) protection.
0.1–0.9 IU/ml - indicates full protection level.
>1.0 IU/ml - provides long term protection.
**13.36 Specific IgGs: To Assess for Immunological Reactivity**

**Specimen:** Serum, White tube  
**Minimum volume:** 6 mls

**Method:** IMMUNOCAP

**Reference value:** <40 mgA/l

**Test turn around time:** 1 week  
**Frequency of retesting:** >6 months

<table>
<thead>
<tr>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspected APBA</td>
</tr>
<tr>
<td>Suspected extrinsic allergic alveolitis eg</td>
</tr>
<tr>
<td>Farmer’s Lung or Bird Fancier’s Lung</td>
</tr>
</tbody>
</table>

**Specific IgG to Aspergillus**  
Measured to assess immunological reactivity to aspergillus in the assessment of allergic bronchopulmonary aspergillosis, especially in patients with asthma or cystic fibrosis

**Interpretation**

**Normal Value (<40 mgA/l)**  
Negative

**Weakly positive 40-90 mgA/L**  
IgG Apergillus at this level may be clinically significant in non - Cystic Fibrosis patients. However, in patients with CF this level may not be significant. Suggest clinical correlation with clinical, microbiological and serological factors.

**Strongly positive > 90mgA/L**  
Raised level of specific IgG to aspergillus suggests an immunological reactivity to aspergillus. Possibility of allergic bronchopulmonary aspergillosis should be considered.

**Specific IgG to Micropolyspora faeni**  
Measured to assess immunological reactivity to micropolyspora faeni in the assessment of possible extrinsic allergic alveolitis.

**Interpretation**

**Normal Result <40 mgA/l**  
Negative

**High > 22mgA/l**
Raised level of specific IgG to micropolyspora faeni suggests an immunological reactivity to micropolyspora faeni. The possibility of Farmer’s Lung should be considered.

**Specific IgG to Budgie or Pigeon**
Measured to assess immunological reactivity to avian antigens in the assessment of possible extrinsic allergic alveolitis.

**Interpretation**

**Normal Result < 40 mgA/l**
Negative

**High Specific IgG to Budgie > 30 mgA/l**
Raised levels suggest an immunological reactivity to avian antigens. Possibility of Bird Fancier’s Lung should be considered.

**High Specific IgG to Pigeon > 38 mgA/l**
Raised levels suggest an immunological reactivity to avian antigens. Possibility of Bird Fancier’s Lung should be considered.
13.37 Mannose Binding Lectin (MBL)

Specimen: Serum, White tube  Minimum volume: 6 mls
Method: ELISA
Reference value: 0.55 – 4.00 mg/L.
Test turn around time: 1 month  Frequency of retesting: Once off

**Indications:**

| Suspected Immunodeficiency (Recurrent Bacterial Infections) |

MBL deficiency was originally described as a cause of recurrent bacterial infections in early childhood, which abated with maturation of the antibody system.

More recently, MBL deficiency has been recognised as a significant cofactor when patients have other minor immunodeficiencies such as IgA deficiency or selective antibody deficiency. MBL deficient patients are more likely to experience severe infections when undergoing chemotherapy.

**Interpretation**

**Normal**
Normal Value is 0.55 – 4.0 mg/L

**Low MBL < 0.55 mg/L**
MBL deficiency is not thought to be significant in the context of normal immune function. However, it may increase the risk of infection in children, patients with other subtle immune defects or post chemotherapy. Please discuss with clinical immunologist if patient has recurrent infections.
13.38 Myositis Screen

Specimen: Serum, White tube  Minimum volume: 6 mls
Method: Immunoblot, correlated with ANF appearance
Reference value: Negative
Test turn around time: 4-6 weeks  Frequency of retesting: Once off

Indications: Suspected dermatomyositis or polymyositis
Suspected idiopathic myositis

The myositis screen includes antibodies to Mi-2, Ku, PM-Scl 100, PM-Scl 75, SRP, Ro-52 and the anti – synthetase antibodies; Jo-1, PL-7, PL-12, EJ, and OJ.

Interpretation

Normal Value
Negative

Positive Anti-Mi-2 Antibody
This antibody is highly specific for dermatomyositis. It can be found in 15% – 20% of dermatomyositis patients and in 8%- 12% idiopathic myositis.

Positive Anti-Ku Antibody
This antibody can be associated with myositis, scleroderma, SLE or overlap syndromes.

Positive Anti – PM-Scl 100 Antibody
This antibody is associated with an overlap syndrome with a combination of symptoms associated with polymyositis/ dermatosynovitis and systemic sclerosis.

Positive Anti PM-Scl 75
This antibody is associated with diffuse systemic sclerosis. It can also be associated with an overlap syndrome with a combination of symptoms associated with polymyositis/ dermatosynovitis and systemic sclerosis.

Positive Anti SRP Antibody
Antibodies against the Signal Recognition Particle (SRP) occur in 4% - 5% of myositis patients.
Positive Anti Ro-52 Antibody
This antibody is found in 5 – 81% of autoimmune and infectious diseases and is not associated with a specific disease.

Positive Anti Synthetase Antibodies:
These antibodies occur in anti-synthetase syndrome, a subset of myositis patients characterized by interstitial lung disease, systemic polyarthritis, Raynaud's Phenomena, fever and Mechanic's Hand. They occur with differing prevalence and are often associated with other, simultaneously occurring autoimmune diseases.

Positive Anti Jo – 1 Antibody
This antibody occurs in 25 – 55% of patients with anti-synthetase syndrome

Positive Anti – PL-7 Antibody
This antibody occurs in 3 - 6% of patients with anti-synthetase syndrome

Positive PL-12 Antibody
This antibody occurs in up to 3% of patients with anti-synthetase syndrome

Positive anti – EJ Antibody
This antibody occurs in 1% of patients with anti-synthetase syndrome

Positive anti OJ Antibody
This antibody occurs in 1% of patients with anti-synthetase syndrome
13.39 IgG Subclasses

Specimen: Serum, White tube
Minimum volume: 6 mls

Method: Nephelometry

Reference value:
- IgG 6.0-16.1 g/L
- IgG1 3.2-10.2 g/L
- IgG2 1.2-6.6 g/L
- IgG3 0.2-1.9 g/L

Test turn around time: 6 weeks
Frequency of retesting: >Annually

Indications:
Suspected Humoral Immunodeficiency
i.e. Recurrent bacterial infections

A patient with recurrent infections or severe infections and a low total IgG or IgG subclass may have a humoral immunodeficiency. Suggest discussion with or referral to a Clinical Immunologist.

Interpretation:

Normal Total IgG, IgG1, IgG2, IgG3
This does not exclude humoral immunodeficiency. If there is clinical concern regarding recurrent infection, suggest referral to clinical immunology as further investigations may be indicated.

Low Total IgG
A low total IgG requires further investigation with serum electrophoresis and quantification of IgG, IgA and IgM. This sample will be sent to the proteins laboratory for further evaluation.

Low IgG1
IgG1 deficiency can be associated with recurrent infection.

Low IgG2
IgG2 deficiency can be associated with recurrent sinopulmonary infection, particularly when it occurs with IgA deficiency or other immune defects.

Low IgG3
The clinical significance of low IgG3 is controversial. While this is occasionally seen in healthy adults, it may be clinically relevant, particularly if other immune defects are present.
14. **Query Test**

**Specimen:** Serum, White tube

**Minimum volume:** 6mLs

**Full clinical details**

**Contact Bleep Number**

**Method:** Consultant, SPR or Chief Medical Scientist

will select appropriate tests

**Reference value:** As appropriate

**Indications:** When uncertain about the most helpful investigations and/or unable to contact us

We are always happy to discuss patients however it may not always be convenient to interrupt a busy clinic.

For convenience we have included the “Query Test” which facilitates sending serum together with clinical details, and ensures that the most helpful investigations are chosen for your patient.

In our pilot scheme many users found it useful.
15. Direct Immunofluorescence (DIF) on Skin Biopsies

Specimen: Fresh skin biopsy, transported on damp gauze to the laboratory. Unless special arrangements have been agreed specimen MUST reach the immunology laboratory by 4pm

Method: Direct Immunofluorescence

Test turnaround time: 1 week (Urgent service available)

<table>
<thead>
<tr>
<th>Indications: DIF should be considered when a skin biopsy is being taken for the following conditions:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Blistering skin disorders – such as pemphigus &amp; pemphigoid</td>
</tr>
<tr>
<td>• Dermatitis Herpetiformis</td>
</tr>
<tr>
<td>• Lupus Erythematosus</td>
</tr>
<tr>
<td>• Vasculitis</td>
</tr>
</tbody>
</table>

Direct immunofluorescence (DIF) is a technique for assessing deposition of immunoglobulins and complement in tissues. This technique is part of the routine investigation of selected skin biopsies.

Normal fixation techniques degrade complement and some epitopes on immunoglobulins, therefore fresh tissue samples must be submitted to the laboratory. The tissue is rapidly frozen and thin sections cut. Sections are incubated with FITC-conjugated antibodies (to C3, C4, IgA, IgG, IgM, Fibrin, Kappa & Lambda.) washed and any staining assessed by microscopy. Slides are interpreted by a trained pathologist and the immunofluorescence pattern must be interpreted in the context of the morphology in the biopsy.

Some immunoreactants are relatively rapidly degraded. Biopsies must be taken directly to the laboratory for processing. Classical findings in many skin diseases are dependent on a biopsy taken from the correct site and at the correct time. Optimum biopsy sites for some common conditions are outlined in the table below.

False negative results may be seen in many skin conditions and it is usually advisable to request appropriate serology at the time of biopsy, as this may be sufficient to confirm a diagnosis in the presence of typical histology, even if DIF is negative.

A biopsy for DIF should always be accompanied by a sample for routine histology as DIF must be assessed by an experienced pathologist in the context of the histological appearances. False positive findings may be seen, particularly in the presence of dermal inflammation.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Typical Finding</th>
<th>Site to Biopsy</th>
<th>Age of lesion</th>
<th>Accompanying Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pemphigus</td>
<td>Linear IgG positivity in a chicken-wire pattern in the epidermis</td>
<td>Perilesional skin</td>
<td>Close to new lesion</td>
<td>Antibodies to epidermal intercellular substance</td>
</tr>
<tr>
<td>Pemphigoid</td>
<td>Linear IgG (+/- C3) along the dermoepidermal junction.</td>
<td>Perilesional skin</td>
<td>Close to new lesion</td>
<td>Antibodies to epithelial basement membrane</td>
</tr>
<tr>
<td>Dermatitis Herpetiformis</td>
<td>IgA (+/- C3 &amp; fibrin) in granular or fibrillary pattern in the papillary dermis</td>
<td>Peri-lesional, non-erythematous skin</td>
<td>Close to new lesion</td>
<td>Anti-endomysial antibody.</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>Granular deposition of C3 (+/- C4) with at least one isotype of immunoglobulin in dermal vessels</td>
<td>Lesion</td>
<td>Fresh, preferably &lt;24 hours</td>
<td>C3, C4. Cryoglobulins ANF + follow ANCA RF</td>
</tr>
<tr>
<td>DLE</td>
<td>Granular deposition of one or more immunoreactants along the dermoepidermal junction (lupus band)</td>
<td>Lesion</td>
<td>&gt;3 months</td>
<td>ANF, Anti-DNA Anti-ENA</td>
</tr>
</tbody>
</table>
### 16. Immunology Test Turnaround Time Guidelines

<table>
<thead>
<tr>
<th>INTERNAL TESTS</th>
<th>Turnaround time</th>
<th>Urgent Service</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Adrenal antibody</td>
<td>2 WEEKS</td>
<td></td>
</tr>
<tr>
<td>ANCA</td>
<td>Next day if Negative</td>
<td>2-3 days if Positive*</td>
</tr>
<tr>
<td>ANF (Anti-Nuclear factor)</td>
<td>Next day if Negative</td>
<td>2-3 days if Positive*</td>
</tr>
<tr>
<td>Anti-Cardiolipin Abs</td>
<td>1 WEEK</td>
<td></td>
</tr>
<tr>
<td>Anti-Beta 2 Glycoprotein 1</td>
<td>1 WEEK</td>
<td></td>
</tr>
<tr>
<td>Anti-CCP antibody</td>
<td>1 WEEK</td>
<td></td>
</tr>
<tr>
<td>Anti-dsDNA antibodies</td>
<td>2-3 DAYS</td>
<td>On Request</td>
</tr>
<tr>
<td>Anti-Endomysial (IgA) abs</td>
<td>1 WEEK</td>
<td></td>
</tr>
<tr>
<td>Anti-Extractable Nuclear antibodies (includes anti-Ro, La, RNP, Sm, Jo-1 &amp; Scl-70)</td>
<td>1-2 WEEKS</td>
<td></td>
</tr>
<tr>
<td>Anti-Gastric Parietal Cell</td>
<td>3-5 DAYS</td>
<td></td>
</tr>
<tr>
<td>Anti-GBM (glomerular basement membrane) Abs.</td>
<td>AS REQUIRED</td>
<td>On Request</td>
</tr>
<tr>
<td>Anti-Histone antibodies</td>
<td>4-6 WEEKS</td>
<td></td>
</tr>
<tr>
<td>Anti-Neuronal antibodies (Anti-Hu,Yo)</td>
<td>1 WEEK</td>
<td></td>
</tr>
<tr>
<td>Anti-Intrinsic Factor Abs.</td>
<td>1 WEEK</td>
<td></td>
</tr>
<tr>
<td>Anti-LKM (liver-kidney microsomal) antibodies</td>
<td>3-5 DAYS</td>
<td></td>
</tr>
<tr>
<td>Anti-Mitochondrial Abs (including M2 subtyping)</td>
<td>3-5 DAYS (2 WEEKS)</td>
<td></td>
</tr>
<tr>
<td>Anti-Tissue Transglutaminase antibody</td>
<td>1 WEEK</td>
<td></td>
</tr>
<tr>
<td>Anti-Thyroid Peroxidase Abs</td>
<td>1 WEEK</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>2-3 DAYS</td>
<td></td>
</tr>
<tr>
<td>Skin Autoantibodies.</td>
<td>1 WEEK</td>
<td></td>
</tr>
<tr>
<td>Smooth Muscle Abs</td>
<td>3-5 DAYS</td>
<td></td>
</tr>
<tr>
<td>Anti-Endomysial (IgG) antibody</td>
<td>1 WEEK</td>
<td></td>
</tr>
<tr>
<td><strong>INTERNAL TESTS</strong></td>
<td><strong>Turnaround time</strong></td>
<td><strong>Urgent Service</strong></td>
</tr>
<tr>
<td>------------------------------------------</td>
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</tr>
<tr>
<td>Anti-MPO antibody</td>
<td>2-3 DAYS (or as required)</td>
<td>On Request</td>
</tr>
<tr>
<td>Anti-PR3 antibody</td>
<td>2-3 DAYS (or as required)</td>
<td>On Request</td>
</tr>
<tr>
<td>Anti-Ribosomal P-protein antibodies</td>
<td>4-6 WEEKS</td>
<td></td>
</tr>
<tr>
<td>Anti-Nucleosome antibody</td>
<td>4-6 WEEKS</td>
<td></td>
</tr>
<tr>
<td>Anti-Pneumococcal antibodies</td>
<td>2-4 weeks</td>
<td></td>
</tr>
<tr>
<td>ASOT</td>
<td>2-3 DAYS</td>
<td></td>
</tr>
<tr>
<td>Complement - C3, C4</td>
<td>Next day</td>
<td>On Request</td>
</tr>
<tr>
<td>Complement, C1q</td>
<td>4-6 WEEKS</td>
<td></td>
</tr>
<tr>
<td>Complement CH100 &amp; AP100</td>
<td>4-6 WEEKS</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>2-4 WEEKS</td>
<td></td>
</tr>
<tr>
<td>MBL</td>
<td>1 MONTH</td>
<td></td>
</tr>
<tr>
<td>C1 inhibitor</td>
<td>4-6 WEEKS</td>
<td></td>
</tr>
<tr>
<td>C1 inhibitor Function</td>
<td>4 months</td>
<td></td>
</tr>
<tr>
<td>Mast Cell Tryptase</td>
<td>1 MONTH</td>
<td></td>
</tr>
<tr>
<td>Total IgE</td>
<td>1 WEEK</td>
<td></td>
</tr>
<tr>
<td>Allergen specific IgE</td>
<td>1 WEEK</td>
<td></td>
</tr>
<tr>
<td>Specific IgGs</td>
<td>1 WEEK</td>
<td></td>
</tr>
<tr>
<td>IgG Subclasses</td>
<td>6 WEEKS</td>
<td></td>
</tr>
<tr>
<td>Myositis Screen</td>
<td>4-6 WEEKS</td>
<td></td>
</tr>
<tr>
<td>NMDA</td>
<td>3 WEEKS</td>
<td></td>
</tr>
</tbody>
</table>