## Departmental Information

<table>
<thead>
<tr>
<th>Department</th>
<th>Information</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Transfusion</td>
<td>Clinical Guidelines</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Laboratory Information</td>
<td>96</td>
</tr>
<tr>
<td>Haematology &amp; Coagulation &amp; Flow Cytometry</td>
<td>Clinical Guidelines</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Laboratory Information</td>
<td>99</td>
</tr>
<tr>
<td>Chemical Pathology</td>
<td>Clinical Guidelines</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Laboratory Information</td>
<td>108</td>
</tr>
<tr>
<td>Immunology</td>
<td>Clinical Guidelines</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Laboratory Information</td>
<td>128</td>
</tr>
<tr>
<td>Microbiology</td>
<td>Clinical Guidelines</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Laboratory Information</td>
<td>140</td>
</tr>
<tr>
<td>Histopathology, Cytology, Neuropathology &amp; Molecular Pathology</td>
<td>Clinical Guidelines</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Laboratory Information</td>
<td>146</td>
</tr>
<tr>
<td>NHISSOT</td>
<td>Clinical Guidelines</td>
<td>71</td>
</tr>
<tr>
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<td>Laboratory Information</td>
<td>159</td>
</tr>
</tbody>
</table>

See next page for detailed table of contents.
1 INTRODUCTION .................................................................................................................. 7
1.1 UPDATES OF USER’S HANDBOOK .................................................................................. 7

2 TESTING GUIDELINES ......................................................................................................... 8
  2.1 BLOOD TRANSFUSION & HAEMOVIGILANCE ................................................................. 8
  2.1.1 Type & Screen (TS) Specimen Collection .................................................................. 8
  2.1.2 Procedure for taking a Type & Screen Specimen ....................................................... 9
  2.1.3 The TS Specimen ........................................................................................................ 10
  2.1.4 Detection of antibody(s) in a TS Specimen ............................................................... 13
  2.1.5 Cost of Blood Components and Products .................................................................. 13
  2.1.6 Transfusion Information Leaflets For Patients ......................................................... 14
  2.2 HAEMATOLOGY .............................................................................................................. 16
  2.2.1 Thrombophilia Screening ......................................................................................... 16
  2.2.2 ESR .......................................................................................................................... 17
  2.2.3 Lupus Anticoagulant ............................................................................................... 18
  2.2.4 Haematology Molecular Tests .................................................................................. 19
  2.3 CHEMICAL PATHOLOGY ............................................................................................ 20
  2.4 IMMUNOLOGY ............................................................................................................... 21
  2.4.1 Rheumatoid Factor .................................................................................................. 21
  2.4.2 Anti-Cyclic Citrullinated Peptide antibodies (CCP) .................................................. 22
  2.4.3 Connective Tissue Disease (CTD) Screen ............................................................... 22
  2.4.4 Anti-Nuclear Factor (ANF) by immunofluorescence ............................................... 24
  2.4.5 Anti-Double-Stranded-DNA Antibodies ................................................................. 25
  2.4.6 Anti-ENA (Extractable Nuclear Antigen) Antibodies .............................................. 26
  2.4.7 Anti-Nucleosome Antibodies .................................................................................. 28
  2.4.8 Anti-Histone Antibodies .......................................................................................... 29
  2.4.9 Anti-Ribosomal-P-Protein antibodies ....................................................................... 29
  2.4.10 Anti-Neutrophil Cytoplasm Antibodies (ANCA) Anti-Myeloperoxidase Antibodies (Anti-MPO) Anti-Proteinase 3 Antibodies (Anti-PR3) ................................................. 30
  2.4.11 Anti-Glomerular Basement Membrane Antibodies (Anti-GBM) ................................ 33
  2.4.12 Anti-Cardiolipin Antibodies (IgG and IgM) ............................................................... 34
  2.4.13 Antibodies to Beta 2 Glycoprotein I ...................................................................... 36
  2.4.14 Anti-Smooth Muscle Antibodies ............................................................................. 37
  2.4.15 Anti-Liver-Kidney Microsomal (LKM) Antibodies .................................................. 38
  2.4.16 Anti-Mitochondrial Antibody & M2 subtyping ....................................................... 39
  2.4.17 Anti-Gastric-Parietal Cell Antibodies (Anti-GPC) .................................................... 40
  2.4.18 Anti-Intrinsic Factor Antibodies ............................................................................ 40
  2.4.19 Anti Thyroid Peroxidase Antibodies (anti-TPO) ..................................................... 41
  2.4.20 Anti-Adrenal Antibodies ........................................................................................ 42
  2.4.21 Anti-Tissue Transglutaminase Antibodies (anti-tTG) ............................................... 42
  2.4.22 IgA Anti-Endomyosal Antibodies (EMA) ................................................................ 43
  2.4.23 Anti-Neuronal Antibodies – Anti-Hu & anti-Yo ......................................................... 44
  2.4.24 Anti-Skin Antibodies ............................................................................................... 45
  2.4.25 Total IgE and Allergen Specific IgE ......................................................................... 46
  2.4.26 Complement - C3 and C4 ....................................................................................... 47
2.4.27 Complement Function CH100 and AP100 ........................................... 49
2.4.28 Complement C1 Esterase Inhibitor (C1INH) ....................................... 49
2.4.29 C1 Inhibitor Function ........................................................................... 51
2.4.30 Anti-Streptolysin-O Titre (ASOT) .......................................................... 51
2.4.31 Mast Cell Tryptase ................................................................................. 52
2.4.32 Anti-Pneumococcal Antibodies .............................................................. 53
2.4.33 Specific IgGs .......................................................................................... 54
2.4.34 Mannose Binding Lectin (MBL) .............................................................. 55
2.4.35 Myositis Screen ...................................................................................... 56
2.4.36 Scleroderma Blot .................................................................................... 58
2.4.37 IgG Subclasses ....................................................................................... 59
2.4.38 Anti-PLA2R Antibodies ......................................................................... 60
2.4.39 Query Test .............................................................................................. 60
2.4.40 Direct Immunofluorescence (DIF) on Skin Biopsies ............................... 61

2.5 MICROBIOLOGY ......................................................................................... 63
2.5.1 General Sample Collection Guidelines .................................................. 63
2.5.2 Guidelines for Routine Specimens ......................................................... 63
2.5.3 Serological Investigations ........................................................................ 67

2.6 HISTOPATHOLOGY/CYTOPATHOLOGY/NEUROPATHOLOGY .................. 68
2.6.1 Current Best Practice for Renal Biopsies ................................................. 68
2.6.2 Handling of Tissue after Biopsy has been taken ........................................ 68
2.6.3 Coroners’s Post Mortem ........................................................................... 68

2.7 NHISST .................................................................................................. 71
2.7.1 HLA Antigens ......................................................................................... 71
2.7.2 Graft Rejection ......................................................................................... 71

3 LABORATORY SERVICES PROVIDED .................................................................. 73

3.1 GENERAL INFORMATION ........................................................................ 73
3.1.1 Location of Department ........................................................................... 73
3.1.2 Contacting the Department/Telephone Numbers ..................................... 73
3.1.3 Department Opening Hours ..................................................................... 77
3.1.4 Consent ..................................................................................................... 78
3.1.5 Specimen Collection Guidelines & Order Of Draw ................................... 78
3.1.6 Specimen Labelling .................................................................................. 84
3.1.7 Specimen Request Forms ......................................................................... 84
3.1.8 Specimen Acceptance Criteria ................................................................. 85
3.1.9 Specimen Tubes & Containers .................................................................. 87
3.1.10 Delivery of Specimens for Analysis ....................................................... 88
3.1.11 Specimen Reception Process ................................................................. 89
3.1.12 Test Results ............................................................................................ 89
3.1.13 Attendance at Phlebotomy: ................................................................. 90
3.1.14 Specimen Referral ................................................................................... 91
3.1.15 Specimen Transportation Guidelines ..................................................... 91
3.1.16 Specimen Storage Conditions ................................................................. 92

3.2 DATA PROTECTION POLICY .................................................................. 93
3.3 TIME LIMITS FOR REQUESTING ADDITIONAL EXAMINATIONS ............ 93
3.4 REPEAT EXAMINATION DUE TO ANALYTICAL FAILURE .......................... 94
3.5 **Uncertainty of Measurement (UM)** .......................................................... 94
3.6 **Accreditation/Quality Standards** .......................................................... 94
3.7 **Complaints** ......................................................................................... 95
3.8 **Blood Transfusion** ............................................................................. 96
  3.8.1 **Repertoire of Test Services** ................................................................. 96
  3.8.2 **Components/Products Available From the Blood Bank** .................. 97
  3.8.3 **Specialised Tests Referred to the IBTS** .............................................. 98
3.9 **Specialised Tests Referred to the IBTS** .................................................. 98
  3.9.1 **Clinical Advice for Blood Transfusion Department** .......................... 98
3.10 **Haematology** ..................................................................................... 99
  3.10.1 **Repertoire of Haematology Tests** .................................................... 99
  3.10.2 **Repertoire of Flow Cytometry Tests** ............................................... 101
  3.10.3 **Repertoire of Coagulation Tests** ...................................................... 102
  3.10.4 **Repertoire of Haematology Molecular Tests** ................................. 105
  3.10.5 **Clinical Advice & Laboratory Test Interpretation** ......................... 105
3.11 **Requests for Additional Analysis** ......................................................... 106
  3.11.1 **Requests for Additional Analysis** .................................................... 106
3.12 **Critical Values** .................................................................................. 107
3.13 **Chemical Pathology** ........................................................................ 108
  3.13.1 **Services Offered** ............................................................................ 108
  3.13.2 **Clinical Contact for Medical Indications/ Clinical Advice** .............. 108
  3.13.3 **Requests for Additional Tests** ......................................................... 108
  3.13.4 **Table D: Profiles and their Components** ...................................... 109
  3.13.5 **Sample Requirements for Toxicology** .......................................... 109
  3.13.6 **Therapeutic Drug Monitoring (TDM) samples** ............................. 109
3.14 **Molecular Testing** ............................................................................. 109
3.15 **Tumour Marker Analysis** .................................................................. 110
3.16 **Investigation of Multiple Myeloma** .................................................. 110
3.17 **Changes To Lipid Profile for Cardiovascular Risk Assessment** ........ 110
3.18 **Externally Referred Tests** ................................................................. 111
  3.18.1 **Reports from External Laboratories** ............................................. 111
3.19 **Fertility Clinics** ................................................................................ 111
3.20 **Critical Phonping Limits** ................................................................. 111
  3.20.1 **Repertoire of Test Services – Routine Chemistry** .......................... 113
  3.20.2 **Repertoire of Test Services – Toxicology** ....................................... 125
  3.20.3 **Catecholamines and Metabolites Reference Ranges** ........................ 127
  3.20.4 **Endocrinology Reference Ranges** .................................................. 127
3.21 **Immunology** .................................................................................... 128
  3.21.1 **Clinical Service** ............................................................................ 128
  3.21.2 **Laboratory Service** ........................................................................ 128
  3.21.3 **Out-of-Hours Service** .................................................................... 128
  3.21.4 **Repertoire of Tests & Test Profiles** ............................................... 129
3.22 **Microbiology** .................................................................................. 140
  3.22.1 **Repertoire of Test Services** ............................................................ 140
  3.22.2 **General Notes** ................................................................................ 143
  3.22.3 **Key Factors Affecting Turn Around Times** .................................... 143

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**Page 5 of 180**
1 INTRODUCTION

This user guide is designed to enable Laboratory users to obtain the maximum benefits from the services provided by the Clinical Directorate of Laboratory Medicine in Beaumont Hospital.

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

1.1 UPDATES OF USER’S HANDBOOK

This Handbook is available on the Hospitals Internet site, and will be updated on a regular basis. If you have any suggestions for improvements please contact the Laboratory Manager or Quality Manager.

Please note the most up to date version of this manual will be available online. It is policy within the Clinical Directorate of Laboratory Medicine to notify external users of updates to this manual by email.

Changes between revisions of the user guide will be highlighted in grey text to alert users of changed information.
2 TESTING GUIDELINES

2.1 BLOOD TRANSFUSION & HAEMOVIGILANCE

2.1.1 Type & Screen (TS) Specimen Collection

Collection of a correctly labelled blood specimen from the intended recipient is critical to safe blood transfusion. Only Registered Medical Doctors, Registered General Nurses and Phlebotomists, who have received instructions (e-learning), are permitted to take Type & Screen (TS) specimens.

The mandatory training requirements for staff involved in taking Type and Screens are as followings:

Doctors: complete the Safe Transfusion Practice and the Blood Components and Indications for Use courses on the e-learning website www.learnbloodtransfusion.org.uk and attend blood transfusion education sessions when offered. The e-learning courses must be repeated every 2 years.

Nurses complete the Safe Transfusion Practice course on the e-learning website www.learnbloodtransfusion.org.uk and attend our 1½ hour Blood Transfusion Education programme in the Centre of Education. Both these courses must be repeated every 2 years.

Phlebotomists are provided with the relevant training by the Haemovigilance Officers.

Patient Identification

All patients must be allocated a unique identification number referred to in this document as a History/Patient Number, (or their unique identification number as used in St. Francis Hospice) which should remain unchanged for the duration of hospitalisation and should be reused in all subsequent hospital admissions. For external facilities refer to local guidelines on patient identification policy.

Positive patient identification must be carried out by a Registered Nurse, Doctor or Phlebotomist before taking a blood specimen for Type and Screen. A TS specimen must only be taken from a patient who is wearing an ID Bracelet. No ID Bracelet = No TS.

If the patient is unable to state their name, etc, then verify the patient’s first name, surname, date-of-birth and History Number/Patient Number in the patient’s Healthcare Record/Emergency Department notes, with the patient’s ID Band and verify these details with parent/guardian, if present.
2.1.2 Procedure for taking a Type & Screen Specimen

Most reactions causing major morbidity or death result from errors in patient or specimen identification. Incorrectly labelled or illegible Type & Screen specimens or request forms will not be processed and will be discarded. Beaumont Hospital Blood Bank staff is acting correctly in refusing to accept a request for pre-transfusion testing when either the request form or the specimen is incorrectly or inadequately labelled (McClelland, 2001).

A TS specimen will not be accepted by the hospital Blood Bank unless it is accompanied by a TS request form that contains the following information, handwritten or in barcode or addressograph format:

- Surname.
- Forename, (initials are not acceptable).
- History/Patient Number
- St. Francis Hospice will have a unique identification number and will be prefixed by SFH e.g. SFH MRN.
- Date of birth.
- Signature of the person taking the TS specimen

Other desirable information includes:

- Gender
- Ward/location
- Consultant’s name.

Request form must be signed, timed and dated in the relevant section, by the person taking the specimen, including the bleep number and/or the Irish
Medical Council number (IMC) where applicable. (An Electronic signature is acceptable when using the Blood Track PDA device. The Blood Track label can be placed on the request form in the section, “must be completed by specimen taker”)

Medical staff should complete the series of questions regarding special requirements of the patient. If there are any queries with regards to the special requirements of a patient, please contact the Haematology team or consult the relevant section of the Blood Transfusion Guidelines: “Indications for the use of Blood Components and Products for Beaumont Hospital and St. Francis Hospice”.

The doctor should obtain the patient’s obstetric and recent transfusion history and must review the patient’s chart where applicable for previous history of antibody(s) or reactions. This information should be documented on the TS request form.

A TS is valid for only 3 days where a patient has been pregnant or transfused in the last 3 months. It will only be extended by the Medical Scientist following confirmation by a doctor that a patient has not had a red cell transfusion or been pregnant in the last 3 months.

2.1.3 The TS Specimen

Staff involved in pre-transfusion sampling must be familiar with standard precautions, Beaumont Hospital, and St Francis Hospice (SFH) guidelines on infection control and local guidelines for the prevention of infection related to the use of ‘sharps’.

7.5ml TS Specimen Bottle & Label

Ask the patient to state his/her surname, first name and date of birth and verify these details using the patient's hospital ID Bracelet and check that these correspond with the details in the patient’s Health Care Record.

Do not proceed if there are any discrepancies or omissions.

The TS specimen is collected with the Sarstedt Monovette® Blood Collection system. The specimen bottle is red and marked “EDTA - FOR BLOOD BANK” For adults use the 7.5.ml specimen bottle, for children use the 2.7ml specimen bottle.
2.1.3.1 Procedure for taking a TS specimen-USING a Blood Track™ PDA device

Completion of Type & Screen Request Form:

Order Type & Screen “TS” test on PIPE and print a computer-generated label which has “TS” on the lower left corner

- Affix the “TS” computer-generated label to box in the upper right side of the Type & Screen Request Form and answer questions about “Consultant” and “Gender”. If there is no computer available, hand-write the answers.
- If a doctor is completing the form he/she should answer all questions under the heading “All questions to be completed by doctor”.

Taking the Type and Screen Specimen

- The full procedure must be completed by the same staff member, one specimen at a time.
- The BloodTrack label printer must be attached to the BloodTrack PDA before going to the patient.
- Check that patient is wearing an ID Band. (No ID Band, no Type & Screen).
- Go to patient with Type & Screen Request Form and Type & Screen Specimen bottle. The label on the specimen bottle must not have any handwriting or printed label, at this stage.
- Positive Patient Identification must be done, ie, with T&S form in hand, ask the patient to state (if able) his/her full name and date-of-birth and verify these details with the patient's ID Band and Type & Screen form. If the patient is unable to state their name, etc, then verify the patient’s full name, date-of-birth and History Number/Patient Number in the patient's Healthcare Record/Emergency Department notes, with the patient’s ID Band and verify these details with parent/guardian/nurse/carer, if present.
- If there are any discrepancies, do not take the specimen and inform the patient’s nurse who should organise a correct ID Band.
- Take the specimen from the patient now.
- With the stylus on the Blood Track PDA TAP “Collect Sample”.
- Scan your Staff ID badge.
- Scan the 2D barcode on the patient’s ID Band.
- Tap “next”.
- Complete all reminders by ticking the boxes with the stylus and then tap “Next”.
- The PDA will prompt you to “Print Collection Label”. The PDA must be connected to the portable label printer. Select “2” labels for printing.
- Place 1 label on the specimen bottle now.
- Place 1 label in the specimen taker area on the lower right of the Type & Screen Request Form
- Tap “Done” to complete the process
2.1.3.2 Contingency plan for taking a TS specimen when the BloodTrack PDAs are not available for use:

Completion of Type & Screen Request Form:

Order Type & Screen “TS” test on PIPE and print a computer-generated label which has “TS” on the lower left corner

- Affix the “TS” computer-generated label to box in the upper right side of the Type & Screen Request Form and answer questions about “Consultant” and “Gender”. If there is no computer available, hand-write the answers.
- If a doctor is completing the form he/she should answer all questions under the heading “All questions to be completed by doctor”.

Taking the Type and Screen Specimen

The specimen bottle must be hand-written with patient details, while with the patient, and labelled with the following information from the patient’s ID Bracelet. These are the specimen acceptance criteria and there must be no discrepancies between the patient information provided on the Type & Screen request form and the specimen.

- Surname
- Forename, (initials are not acceptable).
- History/Patient Number
- St. Francis Hospice will have a unique identification number (no more than a five digit number) and will be prefixed by SFH e.g. SFH MRN.
- Date of birth.
- Signature of person taking the specimen and bleep number if applicable. By signing the specimen bottle, the person taking the specimen is confirming the patient's identity.
- Date and time specimen taken.

There is a zero tolerance on specimen acceptance in Blood Transfusion. No amendments to specimen or forms are permitted.

Place in Bio-hazard bag and transport to Hospital Blood Bank via the pneumatic chute system or via Portering Services as soon as possible after taking the specimen.

A TS specimen must not be greater than 24 hours old prior to testing in the hospital Blood Bank. A positive antibody screen indicates the presence of an allo-antibody, and an antibody identification procedure must be used to identify the specificity of the antibody. In certain circumstances a repeat Type & Screen specimen may be required for additional serological investigations.

A serum specimen (clotted) is also required in the investigation of a suspected transfusion reaction investigation.
2.1.4 Detection of antibody (s) in a TS Specimen

- Clinically significant antibodies are capable of causing patient morbidity due to accelerated destruction of a significant proportion of transfused red cells.

- Where an antibody has been detected in the TS specimen an Antibody Notification Form is sent to the relevant clinical area via the pneumatic chute system. Two special blood bank notice stickers are attached to the form which indicates what antibody(s) is present and that 24 hours notice must, when possible be given to the hospital Blood Bank if red blood cells are required.

- For external locations, the blood bank will inform the relevant location by telephone and fax a copy of the antibody notification form to be placed in the patient’s healthcare record.

- This sticker is placed on the front of the patient's old style Medical Chart under Drugs and Sensitivities. For patients with new style Healthcare Records, the sticker should be found inside the front cover under “Allergies/Alert or Adverse Drug Reactions”. Always check the patient’s chart/records for these stickers.

**BLOOD BANK SPECIAL NOTE**

Patients Plasma contains **ALLOANTIBODIES**

Please give 24 hours notice prior to transfusion or surgery

BT1

2.1.5 Cost of Blood Components and Products

<table>
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<tr>
<th>Blood component</th>
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<td>Red Blood Cells</td>
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<tr>
<td>Platelets</td>
<td>€825.76</td>
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<tr>
<td>*Solvent Detergent Plasma (SDP)</td>
<td>€108</td>
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<tr>
<td>Granulocytes</td>
<td>€290</td>
</tr>
<tr>
<td>Octaplex (500IU)</td>
<td>€325</td>
</tr>
<tr>
<td>Octaplex (1000IU)</td>
<td>€650</td>
</tr>
<tr>
<td>Riastap 1g (Fibrinogen)</td>
<td>€440</td>
</tr>
<tr>
<td>Berinert-P (C1 Esterase inhibitor)</td>
<td>€550</td>
</tr>
<tr>
<td>Novoseven (activated FVIIa)</td>
<td>1mg = €663.76</td>
</tr>
<tr>
<td></td>
<td>2mg = €1659.40</td>
</tr>
<tr>
<td></td>
<td>5mg = €3318.79</td>
</tr>
<tr>
<td>Wilate (Human factor VIII and human von Willebrand factor (VWF))</td>
<td>500IU = €306</td>
</tr>
<tr>
<td>Blood component</td>
<td>Cost (2012)</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td></td>
<td>1000IU = €612</td>
</tr>
<tr>
<td>Advate (Recombinant human coagulation factor VIII)</td>
<td>500IU = €260</td>
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<tr>
<td></td>
<td>1000IU = €520</td>
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<tr>
<td>Alprolix (factor IX)</td>
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<td>1000IU =</td>
</tr>
<tr>
<td>Rhophylac (Anti-D 2ml/300μg)</td>
<td>€83.53</td>
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<tr>
<td>Albumin</td>
<td>5% = €68.25</td>
</tr>
<tr>
<td></td>
<td>20% = €49.50</td>
</tr>
</tbody>
</table>

*octaplasLG®, will replace Solvent Detergent Plasma (SDP). It is available in 200ml bags in A, B, O and AB Blood groups. This will be introduced in the coming months (Q4 2013).

### 2.1.6 Transfusion Information Leaflets For Patients

**Consent**

In a situation where a patient requires a transfusion of a blood component/product as part of their medical treatment, verbal consent must first be obtained by the medical team. In addition, written information on the procedure should be given to the patient as per Beaumont Hospital Policy in relation to obtaining Patient’s Consent, July 2005. This policy deals with issues such as adults who refuse treatment for religious or other reasons, Parental Refusal to Consent to treatment of a Child, consent in emergency/life threatening situations, foster children, adults with learning difficulties and other specific information e.g. unconscious emergency patients.

**Information Leaflets**

In addition to obtaining verbal consent, the provision of transfusion information leaflets in Beaumont Hospital, St Joseph’s Hospital and the Rockfield Unit may help patients to understand the transfusion process and may lessen some of the anxieties which they may have.

St Francis Hospice should provide their patients with similar information leaflets.

The leaflet should be given to the patient or the patient’s guardian by a Registered Medical Doctor or Registered General Nurse and offer to help answer any questions that the patient may have. It should also be documented in the patient’s Health Care Record that these information leaflets have been provided to the patient. Patients may for moral, ethical and religious reasons may against medical advice refuse to receive a transfusion of blood components/products. It should also be documented in the patient’s Health Care Record.
The following blood transfusion information leaflets are available in Beaumont Hospital:

- Blood Transfusion – Information for Patients and Families (MR514)
- Children Receiving a Blood Transfusion – Information for Parents (MR515)

The leaflets are available from Beaumont Hospital’s Supplies Department and should be stocked in each clinical area.
2.2 Haematology

2.2.1 Thrombophilia Screening


These guidelines advise “Indiscriminate testing for heritable thrombophilia in unselected patients presenting with a first episode of venous thromboembolism is not indicated”

In particular, screening is not recommended for patients with arterial thrombosis (although, testing for a lupus anticoagulant may be appropriate) Thrombophilia screening is expensive and labour-intensive. Therefore, it should be targeted at patients’ where the results are most likely to influence management decisions.

The following guidelines should identify the majority of these patients:

**Potential Indications**

- Patients with a family history of any of inherited thrombophilia
- Patients with a family history of venous thrombo-embolism (VTE)
- Patients who have developed a venous thrombosis with no obvious precipitating cause or at a relatively young age (<50 years of age)
- Women with a history of recurrent miscarriages should be screened for the presence of antiphospholipid antibodies.
- Neonates with purpura fulminans and adults developing skin necrosis in association with vitamin-K deficiency (i.e. warfarin therapy)
- Other requests will be dealt with on a cases by case basis after discussion

**Thrombophilia Screening - Timing**

- Screening should be done as soon as any of the risk factors above are identified
- Thrombophilia screening should not be done during the acute phase after the patient presents with a thrombosis.
- Patients should be tested after the acute event and after any anticoagulation therapy (at least 2 weeks post warfarin therapy).
- Protein C and S results are affected by warfarin as both are vitamin K dependant proteins and are therefore reduced by warfarin.
- Lupus tests should not be performed if the patient is receiving therapeutic doses of UFH or new Direct Oral Anticoagulants e.g Dabigatran, Rivaroxaban and Apixaban.
WHAT TESTS SHOULD BE DONE?
- Protein C
- Protein S
- Antithrombin III
- APCR +/- Factor V Leiden
- Prothrombin gene mutation
- Lupus anticoagulant
- Anticardiolipin anti-IgG and IgM

WHAT SAMPLES ARE REQUIRED?
- Complete the Request Form with the Patient’s name, DOB, Episode Number, Ward/Location and Thrombophilia Screen (TPSC) requested
- Include patient relevant clinical information on the form
- Date and sign
- Take FOUR 2.9 mL Tri-sodium citrate 9NC (green) samples and
- Take ONE 2.7 mL EDTA (pink) sample
- Send the samples to the Coagulation Laboratory
- Please note: Anti Cardiolipin Antibodies are processed by Immunology and are not part of this screen.

2.2.2 ESR

ESR is clinically indicated in the following circumstances only:
- Temporal Arteritis/Polymyalgia
- Connective Tissue Diseases
- Lupus

In all other cases, C - reactive protein (CRP) is the preferred test.
2.2.3 Lupus Anticoagulant

Test Requirements:
- LA tests should not be performed if the patient is receiving therapeutic doses of UFH. (Coagulation times of mixing tests can still be prolonged due to the presence of Heparin)
- Limited literature is available for the effect of LMWH; screening for LA in patients treated with LMWH is possible. It should, however be noted that the effect on LA assays may vary depending on the ratio between FXa to FIIa activity in each LMWH preparation.
- LA testing is not recommended in patients receiving Vitamin K antagonists. To avoid misinterpretation, it is recommended to test for LA when the patient has discontinued Warfarin for 1-2 weeks.
- Alternatively, if the INR is between 1.5 - 3.0, a 1:1 dilution of patient plasma and PNP can be considered prior to LA testing. (The LA titre will however be diluted 2-fold)
- The effect of direct thrombin or FXa inhibitors on LA assays is unknown.

Revised guidelines by the British Committee for Standards in Haematology are as follows:

Choice of Test:
- Two tests based on different principles must be performed.
- Dilute Russel Viper Venom (DRVV) should be the first test considered
- The second test must be a sensitive APTT (low phospholipids and silica as activator).
- LA should be considered as positive if one of the two tests gives a positive result.

Confirmatory Test:
- Confirmatory test(s) must be performed by increasing the concentration of the phospholipid of the screening test.

Mixing Studies:
- Ideally Pooled Normal Plasma (PNP) should be prepared in house, alternatively commercial or frozen PNP can be used.
- A 1:1 proportion of patient: PNP should be used without preincubation.
- Cut off may be determined by the ICA defined according to the equation

\[
ICA = \frac{\text{Test } 50:50 \text{ PTTLA } - \text{PNP PTTLA}}{\text{Test PTTA}} \times 100
\]
**INTERPRETATION OF RESULTS**

Expression of Results;
- Tests should be reported with a quantitative result, whereby tests are expressed as ratio of patient-to-PNP for all procedures
- In addition an interpretative comment that indicates whether there is the presence or absence of LA should be included.


**2.2.4 Haematology Molecular Tests**

**2.2.4.1 Factor V Leiden and Prothrombin G20210A Mutation**

- Requests for the Prothrombin G20210A mutation and Factor V Leiden mutation are routinely ordered as part of a Thrombophilia screen (See section 2.2) however they may also be ordered as individual tests.
- All requests for Factor V Leiden MUST also have an Activated Protein C Resistance (APCR) ordered in order to be processed.
- The Molecular Test Request Form HAEMG-LF-084 must be completed. This can be obtained from the Beaumont Hospital website under the Haematology Department.
- One 2.7 mL EDTA and one Tri-sodium citrate 9NC (green) sample is required for Factor V Leiden/APCR.
- One 2.7 mL EDTA sample is required for a Prothrombin G20210A
- Results are reported as either positive or negative.

**2.2.4.2 JAK2 V617F Mutation**

- Requests for JAK2 V617F mutation must be sanctioned by the Haematology team. HAEMG-LF-084 Molecular Test Request Form must be completed. This can be obtained from the Beaumont Hospital website under the Haematology Department.
- ONE 2.7 mL EDTA (pink) sample is required
- Results are reported as “JAK2 V617F mutation Detected” or “JAK2 V617F mutation Not Detected”.
2.3 **CHEMICAL PATHOLOGY**

See section 3.13 below
2.4 IMMUNOLOGY

2.4.1 Rheumatoid Factor

**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.
2.4.2 Anti-Cyclic Citrullinated Peptide antibodies (CCP)

**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

2.4.3 Connective Tissue Disease (CTD) Screen

**INDICATIONS**
- Inflammatory arthritis
- Suspected vasculitis/ connective tissue disease
- Photosensitive/other typical skin rash
- Pleural/pericardial effusions.
- Query autoimmune haemolytic anaemia, ITP, leucopenia
- Renal impairment, proteinuria, haematuria
- Unexplained CNS disease

The CTD Screen by EliA was introduced in February 2014 as an alternative method for the detection of anti-nuclear antibodies (also referred to as ANF or ANA). The CTD screen tests for anti-DNA and clinically relevant anti-ENA such as anti-Ro, anti-La, anti-Sm, and anti-RNP. CTD screen by EliA is carried
out as part of the vasculitis screen panel, the inflammatory arthritis antibodies panel and for “stand-alone” ANF and connective tissue disease screen requests. For assessment of liver autoimmune diseases, the liver antibodies panel is recommended and the ANF component of this panel will remain tested using immunofluorescence (IIF) method.

Our validation analysis confirmed that the CTD screen assay has comparable performance with the immunofluorescence method for ANF in screening for anti-DNA, and clinically relevant anti-ENA including anti-Ro, anti-La, anti-Sm, and anti-RNP. As the number of anti-Scl-70 sera in our validation panel was small, we recommend that anti-Scl-70 is specifically requested in addition to CTD screen if scleroderma is clinically suspected. Additionally if myositis is clinically suspected, we recommend that anti-Jo-1 is specifically requested in addition to CTD screen. With the introduction of the CTD method for ANF analysis the table below outlines the appropriate test requests as guided by clinical history.

<table>
<thead>
<tr>
<th>Clinical indication</th>
<th>Suggestions</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connective tissue disease screen</td>
<td>CTD screen C3 C4</td>
<td>Positive CTD screen samples will be tested for ENA and DNA</td>
</tr>
<tr>
<td>Excluding connective tissue disease in patients with low clinical suspicion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scleroderma</td>
<td>CTD screen ENA (for anti-Scl-70)</td>
<td>If scleroderma suspected, consider rheumatology opinion, regardless of results</td>
</tr>
<tr>
<td>Myositis</td>
<td>CTD screen ENA (for anti-Jo1)</td>
<td>If negative Jo-1 but clinical suspicion high with a raised CK, please discuss with laboratory (details as below)</td>
</tr>
<tr>
<td>Raynaud’s with suspicion of connective tissue disease</td>
<td>CTD screen, C3, C4 HEP2 ANF</td>
<td>HEP2ANF: ANF will be tested by indirect Immunofluorescence on HEP 2 cells</td>
</tr>
<tr>
<td>Monitoring lupus</td>
<td>DNA, C3, C4</td>
<td></td>
</tr>
<tr>
<td>Patients with negative CTD screen but strong clinical suspicion</td>
<td>Liaise with lab : ANF by immunofluorescence is available if clinically indicated</td>
<td><a href="mailto:immunologydepartment@beaumont.ie">immunologydepartment@beaumont.ie</a> Lab extension 2635 Clinical team bleep 797</td>
</tr>
<tr>
<td>Autoimmune liver disease</td>
<td>Liver antibodies panel ANF component is tested by immunofluorescence on HEP 2 cells</td>
<td></td>
</tr>
</tbody>
</table>

In most cases, a positive CTD screen result would also yield a positive result for anti-DNA and/or anti-ENA. Therefore, for internal (Beaumont) specimens and
specimens from GPs, the lab will automatically test for anti-DNA and anti-ENA on all positive CTD screen results. Serum samples with positive CTD screen results from external hospitals will be stored for 4 weeks to facilitate add-on requests for further testing. Further testing can be requested by contacting the department via email to immunologydepartment@beaumont.ie including term “Lab test” in subject line or by fax to 01-8093145.

Once a diagnosis of connective tissue disease has been made, repeated measurement of CTD (or 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.

**INTERPRETATION OF CTD RESULTS**

**Negative CTD:** CTD screen negative makes connective tissue disease unlikely.

**Equivocal CTD:** CTD screen equivocal. If connective tissue disease clinically suspected, suggest further testing for anti-ENA and anti-DNA.

**Positive CTD:** CTD screen positive. An internal audit showed that most patients with positive CTD screen are subsequently found to have a positive anti-DNA or / and anti-ENA. These follow-on tests will be automatically added to internal (Beaumont) and GP specimens.

**2.4.4 Anti-Nuclear Factor (ANF) by immunofluorescence**

**INDICATIONS**

- Autoimmune liver disease
- Suspected vasculitis/ connective tissue disease**

ANF is one of the main serological markers for autoimmune hepatitis and most data in the literature is based on the immunofluorescence method. Therefore we have retained the immunofluorescence method (using Hep2 cells as substrate) as the ANF method for autoimmune liver disease panel.

**From February 2014, we introduced the CTD screen by EliA as the method for the detection of anti-nuclear antibodies for connective tissue disease and vasculitis screens. Please refer to section 2.4.3 for information on CTD screen (page 33).**
INTERPRETATION OF RESULTS

Negative ANF: ANF is the commonest autoantibody found in autoimmune hepatitis but a negative ANF does not exclude the diagnosis.

Positive ANF (titre >1:80): A positive ANF is one of the serological markers for autoimmune hepatitis. Results should be interpreted within the context of clinical history, imaging and other laboratory parameters.

 PATTERNS OF ANF

Both the homogenous and the speckled pattern are commonly seen in patients with autoimmune hepatitis. Anti-nucleolar pattern which is typically associated with scleroderma, is also frequently seen in autoimmune hepatitis.

Anti-Centromere antibody pattern is seen in about 13% of patients with primary biliary cirrhosis. Anti-centromere antibody is also typically found in CREST syndrome (Calcinosis, Raynaud’s phenomenon, Oesophageal dysmotility, Sclerodactyly & Telangiectasia).

2.4.5 Anti-Double-Stranded-DNA Antibodies

INDICATIONS
- Strong clinical suspicion of SLE
- Positive CTD Screen
- Strongly positive ANF
- Follow-up of known SLE patients

INTERPRETATION OF RESULTS

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. 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. In September 2014 dsDNA by Crithidia Luciliae IIF was repatriated as a confirmatory assay for new patients with dsDNA EliA results >10 IU/mL. The dsDNA Crithidia assay is highly specific but is less sensitive than the EliA method for dsDNA antibodies. The EliA method is highly sensitive but has reduced specificity, possibly related to detection of low-affinity antibodies. The IIF method is less useful for monitoring disease activity & patients will continue to be monitored using the EliA assay.
Negative EliA 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.

Equivocal EliA Result (10-30 IU/mL): Most patients with non-inflammatory disorders have values less than 30 IU/mL. At this level, if connective tissue disease remains a clinical possibility we suggest repeating serology in 3-6 months to exclude an evolving process, unless an alternative diagnosis is established in the meantime.

Negative DNA by crithidia & DNA EliA <30 IU/mL: Equivocal anti-dsDNA by EliA with negative crithidia is of uncertain clinical significance & may not reflect lupus. If lupus is still part of the differential diagnoses suggest retesting in 3-6 months.

Negative DNA by crithidia & DNA EliA >30 IU/mL: Positive anti-dsDNA antibodies by EliA but negative crithidia has low specificity for lupus compared with dual positivity by dsDNA EliA & dsDNA crithidia. If there is a strong clinical suspicion of lupus please discuss.

Positive DNA by crithidia: Positive anti-dsDNA by EliA & crithidia is consistent with lupus in the appropriate clinical context.

2.4.6 Anti-ENA (Extractable Nuclear Antigen) Antibodies

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

**INDICATIONS**

- ANF positive 1:400 or greater
- Clinical suspicion of SLE/CTDs with ANF of 1:100/1:200
- Clinical & Biochemical evidence Polymyositis
- Suspected Sjogren’s syndrome
- DLE/Subacute cutaneous lupus
- Congenital heart block – test mother & child
INTERPRETATION OF RESULTS

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.

2.4.7 **Anti-Nucleosome Antibodies**

**INDICATIONS**

- Strong clinical suspicion of SLE
- Suspected Sjogrens syndrome
- Suspected Systemic Sclerosis

**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.
2.4.8 Anti-Histone Antibodies

**INDICATIONS**
- Suspected drug-induced SLE (90% Positive)
- Felty's Syndrome (70% Positive)
- Juvenile Chronic Arthritis

**INTERPRETATION OF RESULTS**

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.

2.4.9 Anti-Ribosomal-P-Protein antibodies

**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 OF RESULTS**

**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.
2.4.10 Anti-Neutrophil Cytoplasm Antibodies (ANCA)  
Anti-Myeloperoxidase Antibodies (Anti-MPO)  
Anti-Proteinase 3 Antibodies (Anti-PR3)

Urgent service and plasmapheresis 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.

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 EliA 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 EliA 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 ANCAs 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 EliA 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.

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.
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.

Note: Since 03/10/11 the assay for MPO & PR3 antibodies has changed to a new sensitive assay. 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>

#### 2.4.11 Anti-Glomerular Basement Membrane Antibodies (Anti-GBM)

Urgent service and Plasmapheresis monitoring available.

**INDICATIONS**

- Pulmonary Haemorrhage
- Acute Renal Failure
- Haematuria of renal origin

**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 (>10 U/mL): Suggestive of anti-GBM disease. Urgent renal consultation should be arranged, and renal biopsy is usually indicated.

Equivocal (7-10 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.

2.4.12 Anti-Cardiolipin Antibodies (IgG and IgM)

**INDICATIONS**

- *Arterial or venous thrombosis*
- Pregnancy associated Morbidity:
  - Recurrent miscarriage (x 3)
  - Mid or third trimester fetal loss
- Severe pre-eclampsia or intrauterine growth retardation requiring delivery before 36 weeks
- Known SLE
- Thrombocytopenia
- Ischaemic stroke <50 years
- Transverse myelopathy
- Mesenteric infarction
- Myocardial infarction in the absence of risk factors

**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

________________________________________________________________________

Page 34 of 180
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
2.4.13 Antibodies to Beta 2 Glycoprotein 1

INDICATIONS

Suspected Antiphospholipid syndrome
See Section 2.4.12

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 \( \geq 10 \) 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-\( \beta \)2 glycoprotein antibodies

INTERPRETATION OF RESULTS

**Negative:** Normal

**Positive Anti B2 glycoprotein IgG Antibodies >8:** The 99th centile for IgG anti-beta 2 glycoprotein 1 antibodies in an evaluation of over 1100 Beaumont patients was 8 U/mL.

The 99th centile in studies including 470 apparently healthy subjects by the assay manufacturer was 17.7 U/mL.

The uncertainty of measurement of the assay is 18%.
Therefore 2 measurements of IgG anti-beta 2 glycoprotein 1 antibodies greater than 22 taken a minimum of 12 weeks apart constitutes a definite laboratory criterion for the diagnosis of APS.

Please discuss patients in whom 2 measurements of IgG anti-beta 2 glycoprotein 1 antibodies taken a minimum of 12 weeks apart are between 10 & 22 U/mL.

Positive Anti B2 glycoprotein IgM Antibodies >6: The 99th centile for IgM anti-beta 2 glycoprotein 1 antibodies in an evaluation of over 1100 Beaumont patients was 6 U/mL.

The 99th centile in studies including 470 apparently healthy subjects by the assay manufacturer was 5.7 U/mL.

The uncertainty of measurement of the assay is 25%.

Therefore 2 measurements of IgM anti-beta 2 glycoprotein 1 antibodies greater than 8 taken a minimum of 12 weeks apart constitutes a definite laboratory criterion for the diagnosis of APS.

2.4.14 Anti-Smooth Muscle Antibodies

INDICATIONS
- Persistently abnormal Liver Function Tests
- Other signs of chronic liver disease
- Investigation of hypergammaglobulinaemia

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.
2.4.15 Anti-Liver-Kidney Microsomal (LKM) Antibodies

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 patient's 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.
2.4.16 Anti-Mitochondrial Antibody & M2 subtyping

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.

**INDICATIONS**
- Persistently abnormal Liver Function Tests
- Other signs of chronic liver disease
- Investigation of hypergammaglobulinaemia
- Pruritis

**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.
2.4.17 Anti-Gastric-Parietal Cell Antibodies (Anti-GPC)

**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.

2.4.18 Anti-Intrinsic Factor Antibodies

**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.
2.4.19 Anti Thyroid Peroxidase Antibodies (anti-TPO)

**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**

In November 2014 we changed the method for anti-TPO antibodies from ELISA to EliA. Both assays use the same units but the EliA method uses a different reference range for reporting results, these are included on all reports. Both methods are calibrated to the same International Standard (MRC 66/687) with results given in International Units (IU/mL).

Changes to basic parameters of the assay:

<table>
<thead>
<tr>
<th></th>
<th>Negative result</th>
<th>Equivocal Result</th>
<th>Positive Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OLD ELISA ASSAY</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IU/mL</td>
<td>&lt;50</td>
<td>50-75</td>
<td>&gt;75</td>
</tr>
<tr>
<td><strong>CURRENT EliA ASSAY</strong></td>
<td>&lt;25</td>
<td>25-35</td>
<td>&gt;35</td>
</tr>
</tbody>
</table>

Negative (Anti-TPO < 25 IU/ml): Autoimmune thyroid disease unlikely.

Equivocal (Anti-TPO 25-35 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 > 35 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.
2.4.20 Anti-Adrenal Antibodies

**INDICATIONS**

- Hypocortisolaemia
- Other autoimmune endocrinopathy
- Hyperpigmentation

**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.

2.4.21 Anti- Tissue Transglutaminase Antibodies (anti-tTG)

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.

**INDICATIONS**

- Suspected coeliac disease
- Malabsorption (including low iron, Vit B12 or albumin)
- Anaemia
- Gastrointestinal symptoms
- Down's syndrome (increased risk of coeliac disease)
- IDDM (increased risk of coeliac disease)
- Dermatitis Herpetiformis
- Osteoporosis & Osteomalacia
- Peripheral Neuropathy
- Unexplained Infertility
- Unexplained weight loss
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 (<4 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 4-10 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 EliA will be further tested for EMA antibodies by indirect immunofluorescence.

**2.4.22 IgA Anti-Endomysial Antibodies (EMA)**

**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.

2.4.23 Anti-Neuronal Antibodies – Anti-Hu & anti-Yo

INDICATIONS

- Suspected paraneoplastic neurological syndromes, Esp – acute or subacute cerebellar syndromes
- Encephalomyelitis
- Sensory & autonomic neuropathy
- Axial ataxia
- Opsoclonus-myoclonus

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/Dr Khalib.
**INTERPRETATION OF RESULTS**

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, rhabdosarcoma 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.

**2.4.24 Anti-Skin Antibodies**

**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 OF RESULTS**

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.

2.4.25 Total IgE and Allergen Specific IgE

**INDICATIONS – TOTAL IgE**
- Suspected allergic bronchopulmonary aspergillosis (ABPA)
- 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 (sIgE) should be requested for limited number of allergens suggested by history. Disease specific profiles of suggested allergens are listed in Section 3.21.4.1.

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 sIgE testing may be ordered after discussion with Senior laboratory or Medical staff.

**INTERPRETATION OF RESULTS**

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.

2.4.26 Complement - C3 and C4

**INDICATIONS**
- Diagnosis of suspected immune complex disease
- Monitoring immune complex disease including cryoglobulinaemia and SLE
- Angioedema (without urticaria)
- Glomerulonephritis
- Suspected anaphylactoid reaction eg to IVIg, colloid infusions

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/Dr Khalib.

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 OF RESULTS**

**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.
2.4.27 Complement Function CH100 and AP100

**INDICATIONS**
- Immune complex disease such as SLE
- Recurrent infections
- Immune complex disease with recurrent infections
- Family history of complement deficiency or any of the above

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 OF RESULTS**

**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.

2.4.28 Complement C1 Esterase Inhibitor (C1INH)

**INDICATIONS**
- Angioedema of skin, gastrointestinal or respiratory tract without Urticaria

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 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 recommended 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 C4 concentrations <0.2 g/L 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.

In September 2014 the method for measurement of C1 inhibitor was changed from RID to nephelometry on the BNII. The validation studies demonstrated excellent correlation between the two methods. However the BNII method has a different reference range & units. Changes to basic parameters of the assay are outlined in the table below:

<table>
<thead>
<tr>
<th>C1 Inhibitor</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old Assay</td>
<td>150-350 mg/L</td>
</tr>
<tr>
<td>Current Assay</td>
<td>0.21-0.39 g/L</td>
</tr>
</tbody>
</table>

**INTERPRETATION OF RESULTS**

C1 INH Low (<0.15 g/L): Significant reduction in C1 inhibitor may be due to consumption, but deficiency cannot be excluded. Please discuss. C1 inhibitor should be measured if patient has angioedema, abdominal pain or low C4.

C1 INH Borderline (0.15-0.21 g/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. However please discuss if patient has angioedema or low C4.

C1 INH Normal (0.21-0.39 g/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 (> 0.39 g/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.
2.4.29 C1 Inhibitor Function

**INDICATIONS**
- Suspected hereditary angioedema
- Angioedema without urticaria AND low C4 during an attack

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 OF RESULTS**

**Normal (>/= 68%):** Normal C1 inhibitor function excludes hereditary angioedema Types 1 and 2 and acquired C1 inhibitor deficiency.

**C1 Inhibitor Function Equivocal (41-67%):** 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 (</=40%):** 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.

2.4.30 Anti-Streptolysin-O Titre (ASOT)

**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 OF RESULTS**

**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.

### 2.4.31 Mast Cell Tryptase

**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 OF RESULTS**

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.

**2.4.32 Anti-Pneumococcal Antibodies**

**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. Hib, Tetanus and Diptheria antibody testing is not performed in Beaumont & are sent to referral laboratories for analysis. It is Directorate policy not refer samples for external hospitals/other institutions.

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 OF RESULTS**

**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.

2.4.33 Specific IgGs

**INDICATIONS**
- Suspected APBA
- Suspected extrinsic allergic alveolitis e.g. Farmer’s Lung or Bird Fancier’s Lung

2.4.33.1 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 OF RESULTS**

**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.

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

**Interpretation**
Normal Result (<22 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.

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

**Interpretation**
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.

2.4.34 Mannose Binding Lectin (MBL)

**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.

**REQUIREMENTS FOR REFERRAL SAMPLES**
Dispatch fresh serum samples to Immunology Laboratory in Beaumont **on same day** as collection.

OR
Samples must be **frozen within 24hrs of collection** (-20°C or below) & dispatched frozen & sent to the Immunology Laboratory in Beaumont.
Haemolysed, Hyperlipemic, Heat-treated or Contaminated Samples are unsuitable for analysis. 

**Note:** Samples received which do not meet these requirements will not be processed.

**INTERPRETATION OF RESULTS**

**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.

### 2.4.35 Myositis Screen

**INDICATIONS**

- Suspected dermatomyositis or polymyositis
- Suspected idiopathic myositis

The myositis screen includes antibodies to Mi-2α, Mi-2β, Ku, PM-Scl 100, PM-Scl 75, SRP, Ro-52, T1F1γ, MDA5, NXP2, SAE1 and the anti – synthetase antibodies; Jo-1, PL-7, PL-12, EJ, and OJ.

**INTERPRETATION OF RESULTS**

**Normal Value:** Negative

**Positive Anti-Mi-2α Antibody:** This antibody is highly specific for dermatomyositis. It can be found in 15% – 20% of dermatomyositis patients and in 8%- 12% idiopathic myositis. Please correlate with clinical details.

**Positive Anti-Mi-2β Antibody:** This antibody is highly specific for dermatomyositis. It can be found in 15% – 20% of dermatomyositis patients and in 8%- 12% idiopathic myositis. Please correlate with clinical details. This antibody may be associated with malignancy induced dermatomyositis. Please correlate with clinical details.

**Positive Anti-Ku Antibody:** This antibody can be associated with myositis, scleroderma, SLE or overlap syndromes. Please correlate with clinical details.

**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. Please correlate with clinical details.

**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. Please correlate with clinical details.

Positive Anti SRP Antibody: Antibodies against the Signal Recognition Particle (SRP) occur in 4% - 5% of myositis patients. Please correlate with clinical details.

Positive Anti Ro-52 Antibody: Anti-Ro positivity detected on Immunoblot. Antibodies to Ro52 are not Lupus specific & can be detected in samples from patients suffering from myositis, scleroderma, Sjogrens & other autoimmune diseases. Please correlate with clinical details.

Positive Anti TIF1γ Antibody: This antibody is highly specific for dermatomyositis. It can be found in approximately 15% of patients with dermatomyositis. Anti-TIF1-gamma positive dermatomyositis has been strongly associated with malignancy. Please correlate with clinical details.

Positive Anti MDA5 Antibody: This antibody occurs in 13-26% of patients with dermatomyositis, in particular amyopathic dermatomyositis and dermatomyositis associated with interstitial lung disease. Please correlate with clinical details.

Positive NXP2 Antibody: This antibody occurs in 18-25% of patients with juvenile dermatomyositis. It is associated with calcinosis and severe disease. It is rare in adult onset dermatomyositis where it may be associated with malignancy. Please correlate with clinical details.

Positive Anti SAE1 Antibody: This antibody is highly specific for dermatomyositis. It can be found in approximately 8% of patients with dermatomyositis. It may occur in dermatomyositis associated with interstitial lung disease. Please correlate with clinical details.

Positive Anti Jo-1 Antibody: Anti-Jo-1 is associated with the anti-synthetase syndrome – polymyositis, Raynaud's and interstitial lung disease. Please correlate with clinical details.

Positive Anti – PL-7 Antibody: This antibody occurs in 3 - 6 of patients with anti-synthetase syndrome. Please correlate with clinical details.

Positive PL-12 Antibody: This antibody occurs in up to 3% of patients with anti-synthetase syndrome. Please correlate with clinical details.

Positive anti – EJ Antibody: This antibody occurs in 1% of patients with anti-synthetase syndrome. Please correlate with clinical details.

Positive anti OJ Antibody: This antibody occurs in 1% of patients with anti-synthetase syndrome. Please correlate with clinical details.
2.4.36 Scleroderma Blot

**INDICATIONS**

- Suspected Systemic sclerosis

The Scleroderma Immunoblot screens for antibodies against the Systemic Sclerosis associated antigens Scl-70, CENP A, CENP B, RP11, RP155, Fibrillarin, NOR90, Th/To, PM-Scl100, PM-Scl75, Ku, PDGFR and Ro-52.

**INTERPRETATION OF RESULTS**

**Normal Value:** Negative

**Positive Anti-Scl-70 Antibody:** 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. Please correlate with clinical details.

**Positive Anti-CENP A Antibody:** This antibody is found in patients with Limited Cutaneous Systemic Sclerosis (lcSSc), & pulmonary arterial hypertension. Please correlate with clinical details.

**Positive Anti-CENP B Antibody:** This antibody is found in patients with Limited Cutaneous Systemic Sclerosis (lcSSc), & pulmonary arterial hypertension. Please correlate with clinical details.

**Positive Anti-RP11 Antibody:** This antibody is a RNA Polymerase III subunit, associated with Diffuse Cutaneous Systemic Sclerosis (dcSSc), renal crisis, synovitis & tendon friction rubs. Please correlate with clinical details.

**Positive Anti-RP155 Antibody:** This antibody is a RNA Polymerase III subunit, associated with Diffuse Cutaneous Systemic Sclerosis (dcSSc), renal crisis, synovitis & tendon friction rubs. Please correlate with clinical details.

**Positive Anti-Fibrillarin Antibody:** This antibody is found in patients with Diffuse Cutaneous Systemic Sclerosis (dcSSc), renal crisis, cardiac involvement. Please correlate with clinical details.

**Positive Anti-NOR90 Antibody:** This antibody is found in patients with mild internal organ involvement. Please correlate with clinical details.

**Positive Anti-Th/To Antibody:** This antibody is found in patients with Limited Cutaneous Systemic Sclerosis (lcSSc), pulmonary fibrosis & renal crisis. Please correlate with clinical details.

**Positive Anti-PM-Scl100 Antibody:** This antibody is found in patients with an overlap syndrome with a combination of symptoms associated with
polymyositis/ dermatosynovitis and systemic sclerosis. Please correlate with clinical details.

Positive Anti-PM-Scl75 Antibody: This antibody is found in patients 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. Please correlate with clinical details.

Positive Anti-Ku Antibody: This antibody is found in patients with myositis, scleroderma, SLE or overlap syndromes. Please correlate with clinical details.

Positive Anti-PDGFR Antibody: Platelet-derived growth factor receptor (PDGFR) antibodies are hypothesized to have a pathogenic role in Systemic Sclerosis however this requires further investigation. Please correlate with clinical details.

Positive Anti-Ro-52 Antibody: Anti-Ro positivity detected on Immunoblot. Antibodies to Ro52 are not Lupus specific & can be detected in samples from patients suffering from myositis, scleroderma, Sjogrens & other autoimmune diseases. Please correlate with clinical details.

2.4.37 IgG Subclasses

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 OF RESULTS

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.

### 2.4.38 Anti-PLA2R Antibodies

**INDICATIONS**
- Patients with idiopathic membranous nephropathy.
- As part of risk assessment of disease recurrence post renal transplantation.

**INTERPRETATION OF RESULTS**

Normal Value: Negative

The reference ranges applicable to the assay are outlined in the table below:

<table>
<thead>
<tr>
<th>Classification</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>&lt;14 RU/mL</td>
</tr>
<tr>
<td>Borderline</td>
<td>≥14-&lt;20 RU/mL</td>
</tr>
<tr>
<td>Weak Positive</td>
<td>20 - 30 RU/mL</td>
</tr>
<tr>
<td>Positive</td>
<td>&gt;30 RU/mL</td>
</tr>
</tbody>
</table>

Anti-PLA2R antibodies have been reported in up to 70% of patients with primary membranous nephropathy and antibody levels appear to correlate with disease activity. In patients with ESRD secondary to membranous nephropathy waiting for renal transplant, positivity for anti-PLA2R antibodies has also been associated with an increased risk of disease recurrence. Close correlation with clinical history and histological findings is advised.

### 2.4.39 Query Test

**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.
2.4.40 Direct Immunofluorescence (DIF) on Skin Biopsies

**INDICATIONS**

DIF should be considered when a skin biopsy is being taken for the following conditions:

- Blistering skin disorders – such as pemphigus & pemphigoid
- Dermatitis Herpetiformis
- Lupus Erythematosus
- Vasculitis

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.

**INTERPRETATION OF RESULTS**

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>
2.5 MICROBIOLOGY

2.5.1 General Sample Collection Guidelines

- Collect specimens aseptically in appropriate CE-marked leak-proof containers and transport in sealed plastic bags.
- A sufficient volume of material must be submitted.
- Swabs in transport media are acceptable for throat, eye, ear, vaginal and urethral specimens. Otherwise, pus, fluid or tissue is preferable to a swab.
- Swabs with special transport media are available where applicable, e.g., for viral and chlamydia investigation.
- If a diagnosis of a viral haemorrhagic fever (Lassa, Ebola, Marburg, Congo-Crimean fever), or CJD is suspected, the consultant microbiologist must be informed before any specimens are collected.
- If a potentially cytotoxic specimen is being sent, the chief or senior medical scientists in microbiology must be informed.
- Samples must be delivered to the laboratory as soon as possible. If this is not possible, store specimens in fridge until they can be transported.

2.5.2 Guidelines for Routine Specimens

PUS

Pus sent in sterile containers give the best results for both Gram stain and culture and is essential for the diagnosis of TB or actinomycosis. If a swab is taken, it should be sent in transport medium after it has been thoroughly soaked in the pus or exudate.

ULCERS

For the best results, ulcers should be cleaned with sterile saline to remove surface contamination, prior to obtaining the sample.

EYES

- Discharging eyes should be swabbed for bacterial culture in the usual way.
- When viral conjunctivitis or corneal lesions are suspected, a swab must be collected using viral transport medium.
- If fungal or amoebic infections are suspected, please contact the clinical microbiology team.

THROAT SWABS

- Even though viruses account for over 70% of sore throats, the most common bacterial cause of sore throat in this country is group A β-haemolytic streptococcus.
- Throat swabs should be taken from the tonsillar region.
• If a throat swab is being taken for other pathogens e.g. C. diphtheriae, N. gonorrhoea or N. meningitidis, it must be clearly requested.
• If whooping cough (pertussis) is suspected, please send a nasopharyngeal swab.
• Specimens for virology should be taken early in the course of a suspected viral illness. Virus transport medium should be used.

**FAECES - ENTERIC PATHOGENS**

• Testing for enteric pathogens is not part of a routine septic screen and faeces specimens should only be sent when gastrointestinal infection is suspected.
• Faeces investigation for enteric pathogens is only performed on specimens which take the shape of the container. ([www.hpsc.ie](http://www.hpsc.ie))
• It is important that clinical details or suspected diagnoses are included on the request form. Relevant information includes: travel history, prolonged diarrhoea, antibiotic use and suspected outbreak. Investigations for pathogens such as Yersinia, Vibrio, or Aeromonas etc. are only performed if indicated by clinical details.
• Specimen may be passed into a clean, dry, disposable bedpan or similar container and transferred into an appropriate CE-marked leak-proof container and place in sealed plastic bags.
• Please note the possibility of Norovirus infection and state whether vomiting is a feature or whether an outbreak is suspected. Please send a separate specimen for Norovirus testing, as this test is performed by an external laboratory.

**FAECES – OVA AND PARASITES**

• The patient’s travel history or other relevant clinical details must be provided.
• Three specimens should be collected over no more than a 10-day period. It is recommended that specimens are collected every other day.
• Unless the patient has severe diarrhoea or dysentery, no more than one specimen should be examined within a single 24-hour period, as shedding of cysts and ova tends to be intermittent.
• If E. histolytica or G. lamblia is suspected and the first three samples are negative, ideally four additional samples should be submitted at weekly intervals.
• Microscopy for Cryptosporidium oocytes is performed on the following:
  ➢ On request
  ➢ Children <16 years old
  ➢ When ova and parasite examination is requested

**FAECES - CLOSTRIDIUM DIFFICILE**

• Testing for Clostridium difficile is performed on all faecal samples except in the following cases:
  ➢ Patients less than 2 years of age
Specimens that do not take the shape of the container
➢ If specimen was positive for *C. difficile* within the last 14 days, These criteria are in compliance with national guidelines (www.hpsc.ie)

**FAECES - HELICOBACTER PYLORI**
- Freshly collected samples should be sent to the laboratory for testing.
- *H. pylori* testing is not carried out on blood samples in the laboratory

**When to send a stool specimen:** Send a stool specimen to the laboratory when there are ≥3 liquid or very loose stools per day. There may be other symptoms suggestive of infectious diarrhoea e.g., abdominal pain or discomfort, nausea, faecal urgency, tenesmus, fever, blood or mucus in stools.

**How many samples to send:** One stool specimen is normally all that is required for routine testing. As microscopy for parasites is less sensitive, please send 3 specimens (but no more than 3) on different days, as some parasites are excreted intermittently. If a worm is excreted, please send the worm and faeces sample.

**How much to send:** Please fill the specimen container to between ¼ and ½ full. Please do not fill to the brim.

**URINE SPECIMENS**

**In the elderly (>65 years):**
- Do not send urine for culture in asymptomatic elderly patients with a positive dipstick.
- Only send urine for culture if signs of urinary tract infection, especially dysuria, fever >38°C or new incontinence. A change in colour or odour of urine is not a sufficient indication for sending urine in the absence of clinical symptoms.
- Do not treat asymptomatic bacteriuria in the elderly, as it is very common. Treating it does not reduce mortality nor prevent symptomatic episodes, but increases the risk of antimicrobial side effects, antibiotic resistance and *C. difficile* infection.
- Rapid transport, or measures to preserve the sample aid reliable laboratory diagnosis. Delays and storage at room temperature allow organisms to multiply, which generate results that do not reflect the true clinical situation.

**What type of specimen should you send?**

Send a mid-stream specimen of urine (MSU) where possible. Patients should be instructed to pass a little urine into the toilet first, then pass enough urine into the specimen container to half fill it and finish urinating into the toilet. Obtain about 10 ml of urine in a sterile universal container tighten the lid and transport to the laboratory without delay. Specimens should be processed within 4 hours. If transport to the laboratory has to be delayed, the specimen can be stored at 4°C for up to 48 hours.
Urine specimens for TB
Urine specimens should be collected in the early morning on three consecutive days in a CE-marked leak-proof container (that does not contain boric acid), and placed in a sealed plastic bag. If there are no appropriate containers for a whole Early Morning Urine (EMU) sample, a midstream EMU sample is an acceptable, but not ideal alternative.

Respiratory Specimens
Sputum for culture and sensitivity:

- A good quality purulent or mucopurulent sputum specimen should be obtained, preferably before antimicrobial therapy, although antimicrobial therapy should not be delayed unnecessarily while awaiting a sputum specimen.
- The specimen should be transported to the laboratory within 2 hours.
- Salivary specimens are unsuitable and as such are not processed.
- If transport is delayed up to 24 hours, refrigeration is preferable to storage at ambient temperature. Specimens are not processed if they are >48 hours old at time of receipt in laboratory.

Sputum for investigation of Mycobacterium spp.:

- Sputum specimens should be relatively fresh (less than 1 day old) to minimise contamination. Purulent specimens are best.
- Two to three samples of ≥5mL should be collected approximately 8-24 hours apart with at least one from early morning.
- Samples taken early morning (that is, shortly after patient waking) have the greatest yield.
- When the cough is dry, physiotherapy, postural drainage or inhalation of nebulised saline (‘sputum induction’) before expectoration may be helpful.

High Vaginal Swabs:
Obtain a high vaginal swab by use of a speculum and a trans swab and submit to the laboratory.

Cervical / Endocervical Swabs:
Use a speculum without lubricant. Wipe the cervix clean of vaginal secretions and mucus. Gently insert a swab into the endocervical canal and rotate to obtain any exudate and submit to the laboratory.

Molecular Testing:
for Chlamdia trachomatis, Neisseria gonorrhoeae and Trichomonas vaginalis
Samples should be collected in the APTIMA unisex Swab Specimen Collection Kit for endocervical and male urethral swab specimens and the Urine Collection
kit for male and female urine specimens. All are available from the NVRL on request and the test is sent directly to the NVRL by the general practitioner.

Swab and urine specimens are stable at room temperature for 60 and 30 day post collection respectively.

2.5.3 Serological Investigations

HIV- Viral loads and Hepatitis C PCR.
- For HIV viral load, blood should be collected in an EDTA blood collection tube.
- For hepatitis C PCR, a serum sample is required.
- Hepatitis C PCR and HIV viral load investigations should be sent to the laboratory immediately for processing. The serum must be frozen within 6 hours of taking the patient’s blood.
- Specimens are transported at -20˚C by courier each Friday to the NVRL.

Antibody Detection.
- In order to establish a diagnosis of acute or recent viral infection by serology, viral specific IgM needs to be detected.
- Before laboratory investigations are performed, paired sera must be submitted. The first should be taken as early as possible in the illness, and the second 14-21 days later and a four-fold rise in titre is required to confirm recent infection.
- A single specimen of serum is required to determine immune status or past infection.
- For serological investigations, a serum specimen of more than 1ml is required. One container of clotted blood should be sent to the NVRL.
- For results enquiries, please phone the NVRL 01- 7161354.
- Printed reports are distributed to the requesting clinician and are not available in the Department of Microbiology.

Viral Screening
- Samples for routine viral investigations are transported to the NVRL twice daily by courier: 9.30am and 2pm.
- Please use the appropriate NVRL request form.
- Clotted blood is the specimen of choice for most other external investigations.
- Please include relevant clinical details, travel history (including destinations visited and travel dates), complete demographics and inform laboratory if urgent.
2.6 **HISTOPATHOLOGY/CYTOPATHOLOGY/NEUROPATHOLOGY**

2.6.1 **Current Best Practice for Renal Biopsies**

Two cores of tissue should be taken to ensure that there are sufficient numbers of glomeruli for examination – not less than 10 for light microscopy and immunofluorescence. This applies to native and allograft kidneys. Both cores can be placed in the same container.

2.6.2 **Handling of Tissue after Biopsy has been taken.**

Tissue must be fresh in order to allow immunological assessment to be performed. In Beaumont Hospital biopsies are carried out in the X-Ray Dept. by one of the Radiologists. The biopsy cores are placed in a universal container which is at least half full of normal saline. The container is placed in a biohazard bag and the Renal Biopsy Request form which should have been filled in by the Nephrology team on the ward prior to transfer of the patient to X-Ray is placed in the outer pouch of the bag.

2.6.3 **Coroners’ Post Mortem**

In all cases the Information Sheet on Post-Mortem Examination (Lab 360A) should be given to families. ([http://dms.beaumont.ie/sections/medical/procedures-for-medical1263](http://dms.beaumont.ie/sections/medical/procedures-for-medical1263))

Circumstances where a death should be reported to the Coroner are listed below.

If an autopsy is required, the clinical staff must inform the Anatomical Pathology Technician at extension 2679 or Mortuary Service Co-Ordinator at extension 8180. Information relating to consent is available on request.

For "consented" autopsies (so called non-Coroners or "House Cases") it is the responsibility of the individual who requests the autopsy to ensure the completed consent form (LAB 358B), patient case notes and a concise clinical summary are delivered to the Mortuary/Pathology in order for the autopsy to be performed. Case should be discussed with Pathologist where possible. (Ext 2638)

In the case of deaths outside normal working hours, the individual who obtained consent for autopsy must ensure that the relevant documentation is given to the Anatomical Pathology Technician or Autopsy/Mortuary Manager (Ext 8354) the following morning.
In Coroner’s cases it is the responsibility of the clinical team to notify the Coroner and to ensure that the Coroner Autopsy Post Mortem Examination Form (LAB 357B) is completed.

**DEATHS WHICH MUST BE REPORTED TO THE CORONER**

(a) Deaths occurring at home or other place of residence:
- Where the deceased was not attended by a doctor during the last illness;
- Where the deceased was not seen and treated by a doctor within one month prior to the date of death;
- Where death was sudden or unexpected;
- Where death may have resulted from an accident (regardless of length of time between injury and death), suicide or homicide;
- Where the cause of death is unknown or uncertain;
- Where concerns are expressed by any person in relation to a death.
- Where the cause of death is suspected to be CJD.

(b) Deaths occurring in hospital:
- Deaths occurring in the accident and emergency department and individuals dead on arrival at hospital;
- Deaths occurring within 24 hours of admission;
- Where a patient dies before a diagnosis is made and the general practitioner is also unable to certify the cause;
- When death occurred while a patient was undergoing an operation or under anaesthesia or within 24 hours of same;
- Where death occurred during or as a result of any procedure;
- Where any question of negligence or misadventure arises in relation to the treatment of the deceased;
- Where death resulted from an industrial disease;
- Where death was due to neglect or lack of care (including self neglect);
- Where death occurred in a Mental Hospital;
- Where death may have resulted from an accident (regardless of length of time between injury and death), suicide or homicide.
- Where a patient has MRSA, C. Diff. or VRE if this is a contributing factor
- Where a patient is resident in a long stay unit or nursing home (e.g. Rockfield Unit)
- Where the cause of death is suspected to be CJD.

(c) A death is reported to the coroner by a member of the Garda Siochana:
- Where death may have resulted from an accident, suicide or homicide;
- Where death occurred in suspicious circumstances;
Where death is unexpected or unexplained;
Where a dead body is found;
Where there is no doctor who can certify the cause of death.

(d) Other Circumstances
- Sudden infant deaths;
- Where a body is to be removed out of Ireland.

A detailed list of reportable deaths is available in the "The Role of the Coroner in Death Investigation", a copy of which is available on request.

It is the responsibility of the most senior member of the medical staff attending the patient to ensure that the death is reported to the Coroner.
2.7 NHISST

2.7.1 HLA Antigens

HLA (Human Leucocyte Antigen) typing refers to the techniques for identifying the ‘tissue type’ of a patient. A person’s tissue type is defined by the presence or absence of different antigens or ‘markers’ on their cell surfaces. In solid organ transplantation the major HLA antigens involved are HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ and HLA-DP.

HLA-A, HLA-B and HLA-C (Class I) antigens are found on all nucleated cells in the body including all the tissues in the kidney, heart, lung and liver and on platelets.

The HLA-DR, HLA-DP and HLA-DQ (Class II) antigens are normally present on a more restricted range of cells in the body. These cells include B cells, macrophages, activated T Cells and endothelium. HLA class II expression can be induced on cells, which do not normally express these molecules during infections and episodes of inflammation including during organ reperfusion.

The mismatches between the donor tissue type and the recipient tissue type are a major stimulus leading to the patient’s immune system recognising the ‘foreign tissue’ and rejecting the transplanted organ. While HLA mismatches provoke a very strong immune stimulus, other differences between donor and recipient can also stimulate a clinically relevant immune response.

Following a blood transfusion, pregnancy, a failed graft or infection, patients can develop antibodies to HLA antigens. If patients receive an organ bearing HLA antigens to which they have circulating preformed antibodies, they may lose the organ due to antibody mediated rejection. These HLA antibodies complicate finding a suitable organ for the recipient and hence a major focus of the H&I department is active antibody screening and identification.

2.7.2 Graft Rejection

Transplant rejection occurs when the recipient’s immune system attacks the transplanted organ. This is because the immune system recognises the transplant as foreign and attempts to destroy it. The immune response during rejection is mediated mainly through T cells but can also involve antibodies (humoral immune response). There are four categories of rejection: Hyperacute, acute humoral rejection, acute cellular rejection and chronic allograft dysfunction (or chronic allograft nephropathy in the case of renal transplantation). Chronic allograft dysfunction may result from both immunological and non-immunological insults to the transplanted organ.
Hyperacute rejection is a rapid process which occurs immediately following transplant. It is mediated by pre-formed antibodies that react with many different antigens expressed on the transplanted organ. The result of hyperacute rejection is rapid destruction of the transplanted organ which must be removed immediately. With modern H&I techniques, this type of rejection would not be expected.

Acute rejection (AR) typically occurs in the sensitised patient and its onset is usually a few days to 4 weeks after transplant. Occasionally AR can result in delayed graft function. Antibody mediated rejection (AMR) may be seen in non-sensitised patients due to de novo(new) production of donor specific antibodies post transplant. Early diagnosis is essential as aggressive treatment with plasma exchange and/or augmented immunosuppression to remove donor specific antibodies can salvage the graft in most cases, although long term graft outcome is usually significantly compromised.

Chronic allograft dysfunction can occur from 6 months to many years post transplant. In renal transplantation it is characterised by progressive deterioration of graft function, proteinuria and specific histological appearances. Recent data has shown that post transplant production of donor specific antibodies is associated with an earlier onset of chronic allograft dysfunction. Demonstration of complement deposition in these grafts indicates that antibodies are involved in damaging the graft, at least in a large proportion of patients. There is no consensus on effective treatments for this form of graft injury, which is the commonest cause of long term graft failure.
3 LABORATORY SERVICES PROVIDED

3.1 GENERAL INFORMATION

3.1.1 Location of Department

The Clinical Directorate of Laboratory Medicine is located between the lower ground and ground floors of Beaumont Hospital.

The postal address of the Directorate is:
Clinical Directorate of Laboratory Medicine
Beaumont Hospital
PO Box 1297
Beaumont Road
Dublin 9

Visitors to any 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 relevant Laboratory.

3.1.2 Contacting the Department/Telephone Numbers

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<td>– Mater Misericordiae Hospital</td>
<td>Fax</td>
<td>01-8032985</td>
</tr>
<tr>
<td></td>
<td>Urgently via Switch</td>
<td>01-8032000</td>
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<tr>
<td>Liver/Pancreas Transplant Co-ordinators</td>
<td>Office</td>
<td>01-2094131</td>
</tr>
<tr>
<td>– St Vincent’s Hospital</td>
<td>Fax</td>
<td>01-2213407</td>
</tr>
<tr>
<td></td>
<td>E-Mail</td>
<td><a href="mailto:liver.transplant@stvincents.ie">liver.transplant@stvincents.ie</a></td>
</tr>
<tr>
<td></td>
<td>Urgently via Switch</td>
<td>01-2774000</td>
</tr>
</tbody>
</table>

**HEALTHLINK SYSTEM**

| GENERAL ENQUIRIES/TEST RESULT ISSUES         | Project Manager   | 01-8825720                  |
| Project Manager  | 01-8825720   | info@healthlink.doh.ie    |

### 3.1.3 Department Opening Hours

The Clinical Directorate of Laboratory Medicine is open 8am to 8pm, Monday to Friday. There is no routine Saturday/Sunday/Bank Holiday service. Immunology laboratory hours are from 9.00 am to 5.00pm, on Monday to Friday.

Blood Transfusion laboratory hours are from 08:00am to 5.00pm, on Monday to Fridays. After 5pm, it is emergency on call service, Blood Transfusion/Haematology laboratory can be contacted on bleep 252/ Biochemistry Bleep 251 Only a limited Histopathology/Cytopathology/Neuropathology service is provided between 8am to 9am and 5pm to 8pm

NHISSOT Laboratory hours are from 8:00am to 600pm Monday to Friday. After 6pm, it is an emergency on call service. The laboratory is closed on Saturday, Sunday and Bank Holidays

There is no clerical support outside Mon-Fri 09:00-17:00

Please ensure samples arrive in the laboratory as early as possible in the working day.
Arrangements are put in place each year regarding the specific services available over the Easter, Christmas and New Year periods and issued to service users with reports.

3.1.4 Consent

All procedures carried out on a patient need the informed consent of the patient. For most routine procedures, consent can be inferred when the patient presents himself or herself with a request form and willingly submits to the collecting procedure e.g. venepuncture. Patients in a hospital bed should normally be given the opportunity to refuse. Special procedures, including more invasive procedure, or those with an increased risk of complications to the procedure will need a more detailed explanation and in some cases, written consent. In emergency situations, consent might not be possible; under these circumstances it is acceptable to carry out necessary procedures, provided they are in the patient’s best interest. The requirement for consent for individual tests performed is outlined in the relevant section of this laboratory manual.

3.1.5 Specimen Collection Guidelines & Order Of Draw

3.1.5.1 Patient Preparation

Patients should adhere strictly to any conditions which are required prior to and during primary sample collection. Caregivers and phlebotomists should ensure that patients are informed of the procedure required for specialist primary sample collection and that they have the required equipment e.g. 24hr urine collection containers. For further information on patient preparation for primary sample collection, please contact the relevant laboratory using the contact details provided in section 3.1.2 above.

3.1.5.2 Venepuncutre Instructions

The collection of a venous sample means the identification of the best vein to source the sample. The arm veins are normally the first choice for a phlebotomist. The most commonly used veins are the cephalic, medial cubital or basilic veins.

1. The Limb should be supported on a pillow or armrest of a phlebotomy chair.
2. Apply the tourniquet 2 – 3 inches above the selected site.
3. Wear your disposable gloves, cleanse the patients skin with a mediswab.
4. Anchor the vein using manual traction below the site of entry. The vein should feel firm and slightly bouncy.
5. Insert the needle with the bevel facing upwards and the needle at 15° angle.
6. There should be a flashback of blood to denote a vein has been accessed.
7. The needle should be held firmly between your thumb and fingers to allow the change of the different tubes onto the needle.
8. When all blood specimens have been obtained, release the tourniquet, detach the last tube and now remove the needle smoothly and quickly.
9. Apply pressure to the venous site for as long as required. This avoids a haematoma forming.
10. Dispose of the used needle immediately into the sharps bin. Do not recap the needle.

The blood bottles must now be labelled correctly and any special requirements adhered to.

3.1.5.3 Blood Sample Order of Draw

Samples must be drawn in the order as tabulated below, to avoid any cross contamination of samples.

Never pour blood from one tube into another. The preservative in the first tube could contaminate the second tube; this can greatly affect results and potentially compromise patient care.

Refer to the Test Library for information on sample requirements and the number of tubes required. Tubes CANNOT be used / shared across different platforms because of the risks involved in sample re-labelling.

The brown and white cap samples must be stood upright to clot as soon as the bottles are filled to ensure that the clot forms in the base of the tube and not the lid. The yellow and pink bottles must be inverted gently to ensure complete mixing. Place all the labelled samples into the bio-hazard bag attached to the patient request form and seal.
Please note: The order of draw is in line with approved standards. Please refer to test information available under relevant department guidelines below.

3.1.5.4 24-Hour Urine Collection: General Information for Patients:

You will receive
- A large plastic container in which to store urine.
- A request form with your details on it.
- A plastic bag in which to return your collection and request form.

1. You may need more than one storage container to contain all of your urine for the 24-hour period.
2. Make sure each storage container is labelled with your full name and hospital number written on it. **If your container is not labelled properly, you may be asked to repeat the 24-hour collection.**
3. Keep your storage container cool throughout the 24-hour collection period until you bring it back
4. For certain collections, a blood sample may need to be taken within the 24 hour collection period; you will be informed if this is the case.

**How to collect your sample.**
1. Start the 24-hour urine test by urinating directly into the toilet. Do not save this urine.
2. After you urinate, write the date and time on your storage container, **this is the start of your test.** Write this time & date on the container.
3. For the next 24 hours, collect all your urine into your storage container.
4. Exactly 24 hours after you started the test, urinate one last time and place the urine in your storage container. **This is the end of your test.** Write the date and time the test ended on your storage container.
5. If you need to use more than one container during the 24-hour period, use one container at a time. When it is full, collect your urine in the next container.
6. Please bring the urine to the hospital as soon as possible. To prevent leaks, make sure the lid is on tightly, and that the container is transported upright inside a plastic bag.
7. If you are an inpatient, your nurse will tell you what time to begin and end the collection and will set up more containers, as needed. If you have questions about the procedure, please ask.

**3.1.5.5 24-Hour Urine Collection (Acidified): Information for Patients**

HCl can cause burns and irritate the respiratory system. It is designated harmful and corrosive and bears the following hazard warnings.

![Hazard Warning: Harmful](image)

![Hazard Warning: Corrosive](image)

You will receive
- A large plastic container with acid in which to store urine.
- A request form with your details on it.
- A plastic bag in which to return your collection and request form.

1. You may need more than one storage container to contain all of your urine for the 24-hour period.
2. Make sure each storage container is labelled with your full name and hospital number written on it. **If your container is not labelled properly, you may be asked to repeat the 24-hour collection.**
3. Keep your storage container in a cool place throughout the 24-hour collection period and until you return it to the laboratory.
4. For certain collections, a blood sample may need to be taken within the 24 hour collection period; you will be informed if this is the case.

**How to handle acid safely.**
1. Your storage container is supplied with a small volume of acid, do not throw this out.
2. You should open the container in a well ventilated area as fumes may escape from the acid.
3. Do not urinate directly into an acidified container.
4. Pour the urine slowly down the inside wall of the container, trying not to splash the acid.
5. Close the lid and swirl the container gently, to mix the acid and the urine.
6. Repeat steps 2–4 each time you add urine to the container.
7. Should you spill any acid on your skin, wash it off at once with plenty of running water.
8. If you experience soreness or reddening of your skin, as a result of a splash, consult your doctor & take these instructions with you.
9. Keep the container in a safe place and out of the reach of children at all times.

How to collect your sample.
1. Start the 24-hour urine test by urinating directly into the toilet. Do not save this urine.
2. After this urination, write the date and time on your storage container, **this is the start of your test**.
3. For the next 24 hours, collect all your urine into your storage container.
4. Exactly 24 hours after you started the test, urinate one last time and collect this urine in your storage container. **This is the end of your test.** Write the date and time the test ended on your storage container.
5. If you need to use more than one container during the 24-hour period, use one container at a time. When it is full, collect your urine in the next container.
6. Please bring the urine to the hospital as soon as possible. To prevent leaks, make sure the lid is on tightly, and that the container is transported upright inside a plastic bag.
7. If you are an inpatient, your nurse will tell you what time to begin and end the collection and will set up more containers, as needed. If you have questions about the procedure, please ask.

**3.1.5.6 Mid-Stream Urine**

Male: Clean the glans penis with soap and water. Commence micturition and when a few ml of urine has been passed, introduce a widemouthed container into the stream.

Females: If the patient is able to collect urine without assistance from the nursing staff, instruct them as follows:
1. Separate the labia and with cotton wool or a sponge moistened with water, wipe the vulva from the front to the back. Disinfectants must not be used.
2. With the labia still separated allow some urine to pass into the toilet, and then, without stopping, allow some to pass into a sterile container.
3. Pass the remaining urine into the toilet.

3.1.5.7 Swabs

Collect the specimen by passing the swab twice over the relevant area. Label and send to the laboratory as soon as possible after collection.

3.1.5.8 Endocervical Swab for GC Culture

Clean the cervical os with a large sterile swab and discard. Insert a new swab into the endocervix and rotate 360 degrees. Swab the external os 360 degrees if os stenosed.

3.1.5.9 Sputum

Instruct the patient to remove dentures, rinse mouth and gargle with tap water and not with antiseptic mouthwash. Instruct the patient to expectorate saliva or postnasal discharge and discard, before expectorating a deep lung sputum sample into a specimen container. Specimens must be submitted in a wide-mouthed container and sent to the laboratory without delay.

3.1.5.10 Stool Samples

Stool specimens should be collected in a clean container with a secure lid, labeled, and sent to the laboratory as soon as possible after collection.

3.1.5.11 Disposal of Materials Used

Dispose of all clinical waste must be in accordance with National Guidelines.

- Universal precautions must be adhered to at all times.
- Gloves must be worn at all times.
- Gloves must be changed after each patient.
- Needles must not be recapped after use.
- Dispose of sharps in a suitable sharps container.
- Dispose of all clinical waste into yellow bag.
3.1.6 Specimen Labelling

The following details must be recorded clearly on specimen containers:

- Name
- Date of Birth
- Medical Record Number where available
- Date and time of specimen collection
- For 24 hour urine collections the date and time that the collection commenced and finished
- For Histopathology/Cytopathology/Neuropathology, anatomical location of specimen. If multiple specimens on the patient are taken, the specimen containers must be individually labelled as to the site of origin.
- For Microbiology, nature and site of specimen

3.1.7 Specimen Request Forms

Specimens must be accompanied by a fully completed Beaumont Hospital External User/GP request form. Approved request forms are distributed by First Direct Couriers on behalf of Beaumont Hospital. The Hospital does not have supplies of request forms. An example is shown below.
When ordering, submit separate samples for each laboratory department. Refer to the individual sections of this manual to ascertain number of specimens required for each sub-laboratory area. If there any problems please contact the department for clarification.

The following details must be recorded on the request form

- Name of Patient
- Date of Birth
- Hospital/Practice Name/Address
- Requesting Clinician
- Tests requested
- Clinical details (where appropriate/relevant and including details of recent antimicrobial therapy)
  
  NOTE: For ESR requests, full clinical details are required
- Specimen Type for Histopathology/Cytopathology/Neuropathology
- Nature and exact body site and source of the specimen for Microbiology

The following details should also be recorded on the request form:

- Patients Address (and previous address where applicable)
- A current Episode number or medical record number if available
- Gender (this may have a bearing on a reference range)
- Date of collection/time
- Drawing doctor’s or phlebotomist’s signature
- Contact number also an out-of-hours contact number.
- When requesting a thromboexact specimen to be tested, please indicate on the request form that this sample needs to be processed in addition to the FBC.

Note: It is imperative that contact details of the requesting doctor and/or location of the patient are attached to the test request so that critical results can be phoned immediately.

NHIS SOT request forms for HLA typing and HLA Antibody screening can be obtained by emailing crossmatch@beaumont.ie

3.1.8 Specimen Acceptance Criteria

The name on the request form and accompanying specimen(s) must match e.g. do not use Pat on one and Patrick or Patricia on other. Please ensure that writing is legible - BLOCK CAPITALS. The requesting clinician is responsible for the correct labelling of specimens and request cards. Incorrectly or inadequately
labelled specimens are not accepted by the laboratory and will be returned to the source of origin.

Specimens/request will be rejected in the following situations:

Request Form:
- Request form illegible
- No request form received
- Name, date of birth or address missing from request form
- No requesting clinician/clinician address stated on request form
- No test requested on request form
- Uncontrolled request form received

Specimen:
- Leaking Specimen unsuitable
- Unlabelled specimen
- No suitable sample received for test requested
- Name or date of birth missing on specimen
- Specimen illegible
- Sample not suitable for analysis (e.g. vomit or MRSA on axilla)
- Incorrect transport media/container used (e.g. viral swab sent for C&S, MRSA requested on Chlamydia swab)
- Specimen Clotted, Underfilled, Overfilled or Haemolysed
- ESR requested with lack of clinical details
- HbA1c is analysed in the Biochemistry Laboratory. Patients requiring a FBC and HbA1c will require 2 EDTA 2.7mL samples sent with the test request.
- One specimen submitted for CD4 and G6PD: In the event whereby 1 EDTA sample is received for CD4 and G6PD analysis, the G6PD will be given priority and the CD4 request rejected
- Aged Specimens:
  - Coagulation samples must be <8 hours old. In samples greater than 8 hours old. The clotting factors begin to deteriorate which lead to inaccurate results, with the exception of patients on Warfarin. In such cases, samples are stable for 24 hours.
  - D-Dimer/Fibn: Request for D-Dimer add-on, must be <8 hours old post sample collection
  - ESRs should be < 6 hours old. Samples >6 hours can lead to a false lowering of results.
  - Reticulocyte samples must be < 24 hours old.
  - FBC: EDTA samples must be <24hours
  - Blood film preparation: samples must be <8 hours old
Flow Cytometry: CD4 & Lymphocyte subsets must be <24 hours old.
Malaria: samples must be <2 hours old. External patients must attend A/E or the Phlebotomy Outpatients if Malaria is suspected

In the case of a sample being rejected, the requesting clinician will be informed by means of a completed Specimen Rejection Form. A written record of all discarded samples is kept in the laboratory.
Note: Only External INR requests that are rejected will be telephoned.

3.1.9 Specimen Tubes & Containers

With the exception of swabs, specimen tubes and containers are available from Beaumont Hospital Stores Department. Contact number: 01 809 3030. All orders must be accompanied by a requisition form. These are also available from the Stores Department.

Swabs are available for collection from Pathology Reception every Friday afternoon from 2 – 4pm only, on a walk-in basis. Supplies of non-standard phlebotomy accessories are available to purchase from Sarstedt, 053-9144922, www.sarstedt.com.

Sarstedt brand tubes are ESSENTIAL as their size and shape are compatible with our laboratory analysers. Tubes supplied by other hospital laboratories are not compatible with the requirements of Beaumont Hospital. It is important to check expiry dates on all tubes. Tubes MUST BE FILLED to ensure the appropriate concentration of any anticoagulant. Do not use Paediatric tubes.

BLOOD SAMPLES
- 4.9mL BROWN cap tubes (serum sample) contain an inert gel, when the sample clots and is centrifuged in the laboratory the gel acts to preserve the sample. REF #04.1935.001
- 4.9 mL WHITE tubes are plain tubes; these contain no preservative (serum sample). The blood clots naturally in the tube. REF #04.1934.001.001
- 7.5mL WHITE tubes are plain tubes; these contain no preservative (serum sample). The blood clots naturally in the tube. REF #01.1601.001 – required for Immunology requests only.
- 2.7mL YELLOW tubes contain Fluoride Oxalate (plasma sample), a preservative that inhibits glycolysis from occurring in vitro, thus preventing falsely low glucose results. REF #04.1903.001
• 2.7mL PINK potassium EDTA tubes contain EDTA preservative (whole blood/plasma sample), which removes calcium to prevent clotting. REF #05.1167.001 (Length = 66mm, Diameter = 11mm). Please **do not use** Sarstedt Code:04.1917 (Length = 75mm, Diameter = 13mm) as these bottles are not suitable on our analyser or filing system.

• 2.9mL GREEN tube contains Tri-sodium citrate 9NC (whole blood sample) which chelates Calcium and prevents the clotting process.

• 2.7mL RED tube contains 0.82mg Magnesium/mL (whole blood sample) which is used to obtain a platelet count when the EDTA sample has reported as clumped. These tubes cannot be obtained from stores. They must be obtained from the Haematology Laboratory by contacting 01-8092703.

Never pour blood from one tube into another. The preservative in the first tube will contaminate the second tube; this can greatly affect results.

**Urine Samples**
Both 24 hour urine collections and random spot urine samples are analysed in the laboratory.

• Random spot urine samples are collected into approved yellow screw-capped Sarstedt containers (CE Marked); these contain no preservative. REF #75.9922.745

• A 24 hour urine collection is either taken in a plain 3L container or an acidified 3L container, depending on the test required. Pre-acidified containers with either 50% acid or concentrated acid are available from phlebotomy. If known in advance that the patient has an unusually large output, please request 2 containers for the test. Results are normally expressed per 24 hour period. Where two tests are desired, each requiring a different container, two separate 24 hour collections must be obtained. If in doubt please contact the relevant laboratory prior to commencement of the test.

**Aptima GenProbe Collection Devices**

Aptima GenProbe Collection Devices (swabs and urine containers) are only available from the NVRL. Contact number: (01) 7161354

**3.1.10 Delivery of Specimens for Analysis**

Specimens can be delivered directly to Pathology Specimen Reception or posted to the relevant laboratory department. If posting specimens, the guidelines outlined in section 3.1.15 on page 91 must be adhered to.
3.1.10.1 GP Courier Service

A courier collects samples from each GP practice within the Beaumont Hospital catchment area. The samples are brought to Pathology Reception where request forms are reviewed, required tests are ordered and samples are labelled prior to analysis. The final courier delivery to Pathology reception is 1:30pm Monday to Friday.

3.1.11 Specimen Reception Process

Samples are received in the central pathology reception where they are distributed to the relevant laboratory department. Where appropriate, specimens are centrifuged. This process separates the cells from the serum/plasma. Samples left unseparated for a number of hours / overnight (sample 'on cells'), causes a gradual leakage of red cell contents and produces spurious results for some assays including potassium, phosphate, magnesium and transaminases. Therefore accurate information on date and time of sampling is very important and saves many unnecessary phone calls to busy clinicians.

3.1.12 Test Results

Written reports are issued for all tests performed. Departmental 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 where appropriate.

If you have any queries in relation to a report, please contact the relevant laboratory area to discuss the result. Feedback from users about difficulty with reports helps us to improve the service. Contact details are available on page 73 of this manual.

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. We need to have a single point of contact for this to ensure that all non-conformities are corrected in the same way.

Beaumont Hospital participates in the Healthlink service, which provides the secure transfer of patient results over the internet. This service is available free of charge to all GPs and is highly recommended. Beaumont Hospital uploads patient reports to the Healthlink service 4 times during a 24 hour period. Report up-loads to Healthlinks are at 13.00, 16.00, 18.00 and 24.00.
If you are interested in accessing this service please contact The National Healthlink Project. Tel. (01) 8825606. Email info@healthlink.doh.ie

PLEASE NOTE: It is the responsibility of the laboratory to ensure that tests are performed to the highest possible standard and reported in the time specified within this User Manual. It is the responsibility of the requesting clinician to follow up on the test results.

3.1.12.1 Critical Values

Results falling outside defined alert limits will be telephoned to the appropriate ward/ personnel. Given the hundreds of specimens received each day, sample analysis often continues into the 'out-of-hours' period, it is vital that the laboratory has a mobile phone contact number for each GP so that urgent results can always be phoned.

Reports that are critical to care, requiring immediate attention will be phoned to the requesting practice as soon as they are authorised. To avoid inappropriate phone calls it is essential that the time and date of sample draw is clear on both the sample and request form. Chemical Pathology Samples that are received that were not drawn on the day of delivery to the department will have all labile tests reported as ‘on cells’.

Copies of patient reports are available from the Pathology Office, 8092507.

Beaumont Hospital has no access to the Healthlinks service.

3.1.13 Attendance at Phlebotomy:

An online appointment system for Phlebotomy is in place for the GP phlebotomy clinic. GP patients and family members of patients can go to www.beaumont.ie and select the ‘Patient Information’ link to make a blood test appointment. Alternateively, GPs can go to www.swiftqueue.com/gp to make an appointment for a patient after registering using the code ‘sw1ft45’.

Telephone appointments can be made between 10.30am and 12.30pm Monday to Friday at 01-2910993 (standard local call rates), for a limited period of time. Outside these times, telephone appointments can be made by calling 1517 345 333. NOTE: This is a premium rate service with calls charged at €2.03 inclusive of VAT (calls from some mobiles may be higher with a maximum cost of €2.50).

A Phlebotomy Appointments Online User Guide is available on the hospital website.
3.1.14 Specimen Referral

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 referral laboratories in use is available from the Directorate on request. The Directorate does not refer samples for GPs or other external units, we are not funded for this service. We will advise users of suggested suitable referral laboratories.

3.1.15 Specimen Transportation Guidelines

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 will not be accepted for analysis – another specimen must be collected.

Send specimens in the bag attached to the request form. Up to 10 specimens may be placed in the bag. It is the responsibility of referring hospitals to ensure that packaging complies with relevant legislation.

The international regulations for the transport of infectious materials by any mode of transport are based upon the recommendations of the United Nations Committee of Experts on the Transport of Dangerous Goods (UN), The Universal Postal Union (UPU), the International Civil Aviation Organisation (ICAO) and the International Air Transport Association (IATA) have also incorporated the UN Recommendations in their respective regulations.

The specimen should be placed in watertight containers containing 10% Neutral Buffered Formalin (volumes larger than 125ml should not be transported by post but hand delivered to the laboratory), the lid must be securely closed to avoid leakages. Patient's details entered on container and request form as above. Specimens must be packaged in a UN-approved packaging system (UN3373/4GU/Class 6.2/ 05 GB) which consists of three layers:
1. Primary Receptacle: a labeled primary watertight, leak-proof receptacle containing the specimen. The receptacle is wrapped in enough absorbent material to absorb all fluid in case of breakage.

2. Secondary Receptacle: A second durable, watertight leak-proof container to enclose and protect the primary receptacle(s). Several wrapped primary receptacles may be placed in one secondary receptacle. Sufficient additional absorbent material must be used to cushion multiple primary receptacles.

3. Outer Packaging: The secondary container is placed in an outer shipping package which protects its contents from outside influences such as physical damage and water while in transit.

4. Both the recipient's and the sender's name and address must be shown on the packaging so that contact can be made in the event of a leakage.

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.

If diagnostic specimens in 10% formalin are posted the following guidelines and instructions must be adhered to:

**Please note:** Glass specimen tubes are not acceptable due to Health and Safety regulations. Please refer to page 87 for correct specimen tubes to be used.

**3.1.16 Specimen Storage Conditions**

- Store blood samples at room temperature, unless otherwise specified. Note that blood samples stored in a refrigerator may have falsely elevated results e.g. potassium. The exception to this is FBC samples which may be stored in a refrigerator for up to 24 hours (however, should there be a delay in an FBC reaching the laboratory the sample must be <24 hours old in order for it to be processed)

- 24 hour urine collections should be refrigerated throughout the collection and brought to the laboratory ASAP.

- Samples for auto antibody crossmatches for NHISSTOT should reach the laboratory within 24 hours' and **should not** to be refrigerated. Addition of test requests to existing samples is not recommended due to issues of sample integrity. Contact individual laboratory for advice and to book in the samples for testing.
• Malaria tests must be examined within 2 hours of sample collection. Therefore, it is recommended that patients attend the Phlebotomy Department in Beaumont Hospital for sample collection.

• In most cases, if delays are unavoidable, microbiology specimens can be preserved by refrigeration at 2-8°C in a designated specimen fridge, as this maintains the viability of the pathogens present and prevents the overgrowth of non-pathogenic bacteria. This is of particular importance if quantitative or semi-quantitative culture is required, for example during microbiological analysis of sputum and urine. Exceptions to this include:
  1) Blood cultures should be promptly
  2) CSF should be held at room temperature.
  3) Samples specifically for the isolation of *Neisseria gonorrhoea.* (i.e. cervical or urethral specimens) should be stored at room temperature.

3.2 **Data Protection Policy**

The Clinical Directorate of Laboratory Medicine complies with the policy of the HSE regarding the legislation pertaining to the rights of the patient and staff and to act in an ethical and responsible manner in maintaining the security and integrity of all personal information. The Directorate retains the following information in relation to each test request received, for a minimum of 30 years, in order to ensure patient history is maintained and that sufficient information is available to staff responsible for the interpretation and reporting of results from the laboratory:

1. Patient full name
2. Patient Address
3. Patient medical record number/episode number
4. Patient date of birth
5. For each specimen: date/time of collection, date/time of receipt in the laboratory and date/time of report, specimen type, priority.
6. Clinical information provided by clinicians
7. The results and where appropriate, interpretation of each test requested.
8. Requesting clinician and address

3.3 **Time Limits for Requesting Additional Examinations**

Please note that verbal requests for any examinations must be followed by a fully completed request form, faxed request or by email request in order for results to be issued. Request forms must be received within the timeframe outlined for each department below.
3.4 **Repeat Examination Due to Analytical Failure**

In the event of an analytical failure, if the system returns to normal within the test cut-off time, the samples are processed accordingly. However, if this time exceeds the test cut-off limit, the users are notified and repeat samples are requested, where applicable.

3.5 **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. The uncertainty associated with any assay performed in the laboratory is available on request.

3.6 **Accreditation/Quality Standards**

Beaumont Hospital Clinical Directorate of Laboratory Medicine’s current scope of Accreditation to ISO15189 is available from the INAB website, [http://www.inab.ie/directoryofaccreditedbodies/laboratoryaccreditationmedicaltesting/225MT.pdf](http://www.inab.ie/directoryofaccreditedbodies/laboratoryaccreditationmedicaltesting/225MT.pdf)
The H&I Department is accredited by EFI (European Federation for Immunogenetics).

3.7 COMPLAINTS

A verbal complaint may be made to any member of staff. In any case, there may be a resolution at point of contact or the case may be of a serious nature that requires further action. All complaints (verbal or written) are recorded directly onto Q-Pulse, and are classified as per Non-conformity procedure. The medical significance of each complaint is decided upon by the departmental Consultant Pathologist. The Head of Department or Laboratory Manager may deal with the complaint depending on its severity. Records of complaints are maintained for periods as defined in schedule for record retention.

If a complaint cannot be resolved at local level it will be forwarded to the hospital’s Patient Liaison officer.
### 3.8 Blood Transfusion

#### 3.8.1 Repertoire of Test Services

<table>
<thead>
<tr>
<th>Test</th>
<th>Specimen Container</th>
<th>Minimum Volume</th>
<th>When Available</th>
<th>TAT</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type &amp; Screen</td>
<td>Adult: 7.5ml Specimen bottle labelled: “EDTA - FOR BLOOD BANK” for Type &amp; Screen.</td>
<td>2.5ml</td>
<td>Routine Requests Mon-Fri 08.00hrs to 17.00hrs, Restricted Service on Sat 09.00 to 13.00</td>
<td>Routine specimens’ minimum of 1.5 hours to process where no antibody is detected in the specimen.</td>
<td>TS specimens should be taken using the Blood Track™ PDA device. When the Blood Track™ PDA device is not available the patient details must be handwritten on the specimen bottle and should be sent to the hospital Blood Bank as soon as possible after taking the specimen.</td>
</tr>
<tr>
<td>Cold Reactive Antibodies*</td>
<td>7.5ml EDTA specimen bottle.</td>
<td>Minimum volume of 2.5ml.</td>
<td>Referred to the IBTS, Monday to Friday 08.00hrs to 17.00hrs</td>
<td>2-5 days as per Primary Specimen User Manual IBTS 2009</td>
<td>Should be sent to the hospital Blood Bank as soon as possible after taking the specimen.</td>
</tr>
<tr>
<td>Direct Antiglobulin Test</td>
<td>EDTA Specimen.</td>
<td>Minimum volume of 2.5ml (Use FBC bottle).</td>
<td>Mon-Fri 0800hrs-1700hrs Sat 0900hrs-1300hrs</td>
<td>Routine specimens 30 minutes</td>
<td>Should be sent to the hospital Blood Bank as soon as possible after taking the specimen.</td>
</tr>
<tr>
<td>Transfusion Reaction Investigation</td>
<td>7.5ml EDTA Specimen.+</td>
<td>Minimum volume of 2.5ml.</td>
<td>Mon-Fri 0800hrs-1700hrs Sat 0900hrs-1300hrs Urgent requests at any time</td>
<td>Depends on the complexity of investigation. Delays will occur if referred to IBTS for investigation</td>
<td>Should be sent to the hospital Blood Bank as soon as possible after taking the specimens.</td>
</tr>
<tr>
<td>ABO Antibody titration</td>
<td>7.5ml EDTA Specimen.</td>
<td>Minimum volume of 2.5ml.</td>
<td>Mon-Fri 0800hrs-1700hrs Sat 0900hrs-1300hrs</td>
<td>Approx 2 hours (where time permits)</td>
<td></td>
</tr>
</tbody>
</table>

*Specimens referred to the IBTS for antibody investigation, serological crossmatch or/and Cold Reactive Antibody, the results of these tests are not covered by the scope of Beaumont Hospital Blood Bank Department ISO15189 accreditation.
### 3.8.2 Components/Products Available From the Blood Bank

<table>
<thead>
<tr>
<th>Component/Product</th>
<th>When Available</th>
<th>Special Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cells</td>
<td>Routine Requests Mon-Fri 0800hrs to 1700 hrs Sat 0900 to 1300hrs Urgent requests at any time</td>
<td>With valid TS (no antibody) Phone request 20 minutes before red cells are required for transfusion *Patients with known antibody(s)-TS is required at least 24 hours before compatible red cells are available</td>
</tr>
<tr>
<td>Platelets</td>
<td>Routine &amp; Urgent</td>
<td>Platelets supplied by the IBTS nationally. Orders should be placed prior to 1400 hrs during routine working hours</td>
</tr>
<tr>
<td>SD Plasma</td>
<td>Routine &amp; Urgent</td>
<td>Phone request 30 minutes before plasma is required where blood group has been established.</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>On Request</td>
<td>Place order ASAP following consultation with the Haematology team, who will place initial order with IBTS.</td>
</tr>
<tr>
<td>Fibrinogen Concentrate</td>
<td>Routine &amp; Urgent</td>
<td>Phone request 30 minutes in advance of use.</td>
</tr>
<tr>
<td>Specific Coagulation Factors (Prothrombin Complex Concentrate, activated Factor VIIa, Factor VIII, Factor VIII + vWF, Factor IX)</td>
<td>On Request</td>
<td>Discussion with Haematology Medical Team required</td>
</tr>
<tr>
<td>Anti-D Immunoglobulin</td>
<td>On request</td>
<td>Following consultation with the Haematology Team.</td>
</tr>
<tr>
<td>Albumin</td>
<td>Routine &amp; Urgent</td>
<td>Phone request in 30 minutes advance.</td>
</tr>
<tr>
<td>C1-Esterase Inhibitor</td>
<td>Routine &amp; Urgent</td>
<td>Following consultation with the Immunology Team, phone request 30 minutes in advance. On call hours contact Haematology team.</td>
</tr>
<tr>
<td>Paedipacks</td>
<td>Routine &amp; Urgent</td>
<td>24 hours notice to be given to the IBTS before red cells required for top-up transfusions for infants less than 1 year old.</td>
</tr>
<tr>
<td>Neonatal Red Cells</td>
<td>Routine &amp; Urgent</td>
<td>24 hours notice to be given to the IBTS before red cells required for surgical procedures for infants less than 1 year.</td>
</tr>
<tr>
<td>Neonatal blood components/products (Platelets &amp; SDP)</td>
<td>Routine &amp; Urgent</td>
<td>Available from the IBTS via the Hospital Blood Bank following consultation with the Haematology Team.</td>
</tr>
</tbody>
</table>
3.8.3 Specialised Tests Referred to the IBTS

3.9 Specialised Tests Referred to the IBTS

<table>
<thead>
<tr>
<th>Test Referred</th>
<th>Specimen Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Leucocyte Antigen (HLA Typing)</td>
<td>5-10mls EDTA / Citrate</td>
</tr>
<tr>
<td>Screening for HLA Antibodies</td>
<td>5-10mls clotted</td>
</tr>
<tr>
<td>Screening for Platelet Allo-antibodies</td>
<td>5-10mls clotted</td>
</tr>
<tr>
<td>Human Platelet Antigen Typing (HPA)</td>
<td>5mls EDTA</td>
</tr>
<tr>
<td>Post Transfusion Purpura</td>
<td>5-10mls Clotted + 5 ml EDTA,</td>
</tr>
<tr>
<td>Transfusion Related Acute Lung Injury (TRALI)</td>
<td>Discuss with the IBTS*</td>
</tr>
</tbody>
</table>

* In these circumstances, requests should be confirmed by the patient’s consultant with the IBTS medical personnel prior to referring a specimen. Dispatch forms and specimens to the Haematology Secretary, Pathology Laboratories, Haematology Secretary Lower Ground Floor. Specimens are transported by courier at 10:00 hrs and 14:30 hrs Monday to Friday to the IBTS, urgent specimens can be referred outside these times.

3.9.1 Clinical Advice for Blood Transfusion Department

The Blood Transfusion Department has two specific Consultants appointed to Beaumont Hospital who is available for consultation and specialist clinical advice on blood transfusion issues. In the situation that the Consultant is unavailable to give advice on blood transfusion related issues, the Specialist Register in Haematology will deputise and provide the relevant support.
3.10 HAEMATOLOGY

Same day turnaround times refer to results being available to the requesting clinician on the same working day. Results are available on ward look-up or on Healthlink. Clinicians receiving results by post will incur an added delay.

3.10.1 Repertoire of Haematology Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Specimen Container</th>
<th>Minimum/Container Volume</th>
<th>Adult Reference Range (See Reports for Paediatric Ranges)</th>
<th>TAT</th>
<th>Comment</th>
</tr>
</thead>
</table>
| Full Blood count         | EDTA (pink capped) | 2.7ml standard 1.2 ml    | Hb 13-17.5 g/dL 11.5-16.5 g/dL 11.7-16.0 *  PCV 0.37-0.54 L/L 0.335-0.54 L/L 0.37-0.54*  RCC 4-6.5 x 10^12/L 3.8-5.8 x 10^12/L 4.0-6.5*  RDW 12.0-13.6% 12.1-14.3%  MCV 79 -96 fL  MCH 27 -32 pg  RDW 12.1-14.3%  PLTS 140 -400 x 10^9/L  WBC 4.0 -11 x 10^9/L  Neut 2.0 -7.5 x 10^9/L  Lymph 1.0 -4.0 x 10^9/L  Mono 0.2- 1.0 x 10^9/L  Eosin 0.04 - 0.4 x 10^9/L  Baso 0.01 - 0.1 x 10^9/L | 1 Working Day | 7.5ml and 10ml EDTA samples are not acceptable
|                           |                    | 1.2 ml paediatric        |                                                            |          | *Women >50 years                                                        |
| Platelet Check           | 0.82mgMg^{2+}/mL   | 2.7mL                    | 140 -400 x 10^9/L                                          | 1 Working Day | Arrange in advance with laboratory to obtain sample tube. Please write on the request form
| (Thromboexact)           | (Red)              |                          |                                                            |          |                                                                         |

*Women >50 years

Page 99 of 180
<table>
<thead>
<tr>
<th>Test</th>
<th>Specimen Container</th>
<th>Minimum/Container Volume</th>
<th>Adult Reference Range</th>
<th>TAT</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>See Reports for Paediatric Ranges</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR</td>
<td>Trisodium citrate</td>
<td>3.5 ml must be filled to the line</td>
<td>Male: 1-12 mm/hr  Female: 1-20 mm/hr</td>
<td>1 Working Day</td>
<td>that a platelet count from a Thromboexact tube is required</td>
</tr>
<tr>
<td></td>
<td>4NC/purple</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reticulocyte Count</td>
<td>EDTA (pink capped)</td>
<td>2.7ml standard 1.2 ml paediatric</td>
<td>Retic: 0.3 – 2.0% Retic (Abs) 20 – 80 x10⁹/L</td>
<td>1 Working Day</td>
<td>Addressograph label must only be placed over the manufacturer’s label on the bottle.</td>
</tr>
<tr>
<td>Haptoglobins</td>
<td>Clotted sample (white)</td>
<td>7.5ml standard 2.7 ml paediatric</td>
<td>0.3-2.0g/L</td>
<td>8 working days</td>
<td></td>
</tr>
<tr>
<td>Infectious mononucleosis Screen</td>
<td>EDTA (pink capped)</td>
<td>2.7ml standard 1.2 ml paediatric</td>
<td>Negative</td>
<td>1 Working Day</td>
<td></td>
</tr>
<tr>
<td>Blood film examination</td>
<td>EDTA (pink capped)</td>
<td>2.7ml standard 1.2 ml paediatric</td>
<td>N/A</td>
<td>5 Working Days</td>
<td>Sample must be &lt;8 hrs old. Please specify that a blood film is required on the request form.</td>
</tr>
<tr>
<td>Malaria: Rapid Diagnostic Tests (RDT)</td>
<td>N/A</td>
<td>N/A</td>
<td>Negative</td>
<td>N/A</td>
<td>Samples are required in the Laboratory &lt; 2 hours post sample collection. Therefore, patients must attend the Hospital Phlebotomy/A&amp;E Department for sample collection.</td>
</tr>
<tr>
<td>Blood Film</td>
<td>N/A</td>
<td>N/A</td>
<td>Negative</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Sickle solubility Screen</td>
<td>EDTA (pink capped)</td>
<td>2.7ml Standard</td>
<td>Negative</td>
<td>1 Working Day</td>
<td>For Urgent pre- Anaesthetic screen contact Laboratory. If Haemoglobin Electrophoresis is required, please specify on the request form.</td>
</tr>
</tbody>
</table>
3.10.2 Repertoire of Flow Cytometry Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Specimen Container</th>
<th>Minimum/Container Volume</th>
<th>Reference Range</th>
<th>TAT</th>
<th>Comment</th>
<th>Mnemonic</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4</td>
<td>EDTA (pink capped)</td>
<td>2.7ml Standard</td>
<td>502-1749 Cells/ul</td>
<td>2 Working days</td>
<td>Samples must be &lt;24 hours old. Only processed Monday to Friday. Must be Received in Laboratory before 3pm on a Friday.</td>
<td>CD4</td>
</tr>
<tr>
<td>Lymphocyte Subsets</td>
<td>EDTA (pink capped)</td>
<td>2.7ml Standard</td>
<td>CD3#797-2996Cells/ul</td>
<td>2 Working days</td>
<td>Samples must be &lt;24 hours old. Only processed Monday to Friday. Must be Received in Laboratory before 3pm on a Friday.</td>
<td>LY_SUB</td>
</tr>
<tr>
<td>Lymphoid Screening Tube</td>
<td>EDTA (pink capped)</td>
<td>2.7ml Standard</td>
<td>N/A</td>
<td>Written report: EDTA Samples must be &lt;24 hours old. Verbal report: Sodium Heparin (orange capped - BMA) with 1ml RPMI must be &lt;24 hours old</td>
<td>LST</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium Heparin (orange capped - BMA) with 1ml RPMI</td>
<td>2.7ml Standard</td>
<td></td>
<td>10 working days</td>
<td>Written report: EDTA Samples must be &lt;24 hours old. Verbal report: Sodium Heparin (orange capped - BMA) with 1ml RPMI must be &lt;48 hours old</td>
<td>LY_PRO</td>
</tr>
<tr>
<td>Lymphoproliferative Panel</td>
<td>EDTA (pink capped)</td>
<td>2.7ml Standard</td>
<td>N/A</td>
<td>Written report: EDTA Samples must be &lt;24 hours old. Verbal report: Sodium Heparin (orange capped - BMA) with 1ml RPMI must be &lt;48 hours old</td>
<td>AL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium Heparin (orange capped - BMA) with 1ml RPMI</td>
<td>2.7ml Standard</td>
<td></td>
<td>10 working days</td>
<td>Written report: EDTA Samples must be &lt;24 hours old. Verbal report: Sodium Heparin (orange capped - BMA) with 1ml RPMI must be &lt;48 hours old</td>
<td>AL</td>
</tr>
<tr>
<td>Acute Leukaemia Panel</td>
<td>EDTA (pink capped)</td>
<td>2.7ml Standard</td>
<td>N/A</td>
<td>Written report: Must be arranged in advance with prior consultation with the lab. Containers are only</td>
<td>AL</td>
<td></td>
</tr>
</tbody>
</table>
### 3.10.3 Repertoire of Coagulation Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Specimen Container</th>
<th>Number of Samples</th>
<th>Minimum Volume</th>
<th>Reference Range</th>
<th>TAT</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation Screen</td>
<td>Trisodium citrate 9 NC/2.9 mL</td>
<td>1</td>
<td>Must be filled to the line</td>
<td>PT: 12 – 15 seconds</td>
<td>1 working Day</td>
<td>Sample must be &lt;8 hours old</td>
</tr>
<tr>
<td>Prothrombin Time</td>
<td>(green capped)</td>
<td></td>
<td></td>
<td>APTT: 24 – 38 seconds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activated Partial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thromboplastin Time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>Specimen Container</td>
<td>Number of Samples</td>
<td>Minimum Volume</td>
<td>Reference Range</td>
<td>TAT</td>
<td>Comment</td>
</tr>
<tr>
<td>-----------------</td>
<td>--------------------</td>
<td>-------------------</td>
<td>----------------</td>
<td>-----------------</td>
<td>--------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>INR</td>
<td>Trisodium citrate 9 NC/2.9 mL (green capped)</td>
<td>1</td>
<td><strong>Must</strong> be filled to the line</td>
<td>Different ranges depending on the reason the patient was put on Warfarin</td>
<td>1 working Day</td>
<td>INR only requests are stable for 24 hrs</td>
</tr>
<tr>
<td>Warfarin office INR</td>
<td>Trisodium citrate 9 NC/2.9 mL (green capped)</td>
<td>1</td>
<td><strong>Must</strong> be filled to the line</td>
<td>Therapeutic Range is dependent on clinical condition</td>
<td>1 working Day</td>
<td>Warfarin Office contact no. 01-8092083 WINRs are stable for 24 hrs</td>
</tr>
<tr>
<td>D-Dimer</td>
<td>Trisodium citrate 9 NC/2.9 mL (green capped)</td>
<td>1</td>
<td><strong>Must</strong> be filled to the line</td>
<td>&lt;0.5 g/ml</td>
<td>1 working Day</td>
<td>Sample must be &lt;8 hours old</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Trisodium citrate 9 NC/2.9 mL (green capped)</td>
<td>1</td>
<td><strong>Must</strong> be filled to the line</td>
<td>1.9 – 3.8 g/L</td>
<td>1 working Day</td>
<td>Sample must be &lt;8 hours old</td>
</tr>
<tr>
<td>Mixing study</td>
<td>Trisodium citrate 9 NC/2.9 mL (green capped)</td>
<td>2</td>
<td><strong>Must</strong> be filled to the line</td>
<td>Corrected to within the PT and APTT normal ranges</td>
<td>1 week</td>
<td>Tests done in batches. For urgent requests, contact the laboratory in the morning, may be able to facilitate testing that day.</td>
</tr>
<tr>
<td>Factor Assays</td>
<td>Trisodium citrate 9 NC/2.9 mL (green capped)</td>
<td>2</td>
<td><strong>Must</strong> be filled to the line</td>
<td>FII 70-120 U/dL</td>
<td>Case dependent, maximum 14 days</td>
<td>Tests done in batches. For urgent requests, contact the laboratory in the morning, may be able to facilitate testing that day.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FV 70-120 U/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FVII 55-170 U/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FVIII 60-200 U/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FIX 60-140 U/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FX 70-120 U/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FXI 60-140 U/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FXII 60-151 U/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombophilia screen</td>
<td>Trisodium citrate 9 NC/2.9 mL (green capped)</td>
<td>4</td>
<td><strong>Must</strong> be filled to the line</td>
<td>See individual requests: APCR, PC, FPS, AT3 and L.A.</td>
<td>4 weeks.</td>
<td>Batch tested. The Thrombophilia screen (TPSC) includes the following tests: PT, APTT, FIBN, D-DIMER, LA, AT3,PC, FPS, APCR 5LEIDEN* and PT_MUT*.</td>
</tr>
<tr>
<td>Test</td>
<td>Specimen Container</td>
<td>Number of Samples</td>
<td>Minimum Volume</td>
<td>Reference Range</td>
<td>TAT</td>
<td>Comment</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>--------------------</td>
<td>-------------------</td>
<td>----------------</td>
<td>-----------------------</td>
<td>------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Protein C</td>
<td>Trisodium citrate 9 NC/2.9 mL (green capped)</td>
<td>1</td>
<td>Must be filled to the line</td>
<td>70 – 145 U/dl</td>
<td>4 weeks.</td>
<td>Hence, these tests do not need to be ordered on an individual basis. See below for TAT for 5Leiden and PT_MUT. Batch tested. Patient must be off warfarin for a minimum of 2wks to perform this assay.</td>
</tr>
<tr>
<td>Free Protein S</td>
<td>Trisodium citrate 9 NC/2.9 mL (green capped)</td>
<td>1</td>
<td>Must be filled to the line</td>
<td>Males: 60-148 U/dL Females: 50-115 U/dL</td>
<td>4 weeks.</td>
<td>Batch tested. Patient must be off warfarin for a minimum of 2wks to perform this assay.</td>
</tr>
<tr>
<td>Antithrombin III</td>
<td>Trisodium citrate 9 NC/2.9 mL (green capped)</td>
<td>1</td>
<td>Must be filled to the line</td>
<td>80 – 132 U/dl</td>
<td>4 weeks.</td>
<td>Batch tested</td>
</tr>
<tr>
<td>Activated protein C resistance</td>
<td>Trisodium citrate 9 NC/2.9 mL (green capped)</td>
<td>1</td>
<td>Must be filled to the line</td>
<td>Negative</td>
<td>4 weeks</td>
<td>Batch tested</td>
</tr>
<tr>
<td>Von Willebrand factor</td>
<td>Trisodium citrate 9 NC/2.9 mL (green capped)</td>
<td>2</td>
<td>Must be filled to the line</td>
<td>46-146 U/dl</td>
<td>Case dependent, maximum 14 days</td>
<td></td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>Trisodium citrate 9 NC/2.9 mL (green capped)</td>
<td>1</td>
<td>Must be filled to the line</td>
<td>DRVV-S: &lt;1.17 NORMRAT: &lt;1.21 PTT-LA: &lt;1.24 ICA RAT: &lt;9.72</td>
<td>4 weeks</td>
<td>Batch tested. Patients must not be on Unfractionated Heparin, or new Direct Oral Anticoagulants e.g Dabigatran, Rivaroxaban and Apixaban and if on Warfarin, the INR must be &lt;3.0 to perform this assay.</td>
</tr>
<tr>
<td>Thrombin time</td>
<td>Trisodium citrate 9 NC/2.9 mL (green capped)</td>
<td>1</td>
<td>Must be filled to the line</td>
<td>&lt; 21 seconds</td>
<td>2 weeks</td>
<td></td>
</tr>
</tbody>
</table>
### 3.10.4 Repertoire of Haematology Molecular Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Specimen Container</th>
<th>Number of Samples</th>
<th>Minimum/Container Volume</th>
<th>Reference Range</th>
<th>TAT</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V Leiden mutation (5Leiden)</td>
<td>EDTA sample (pink)</td>
<td>1</td>
<td>2.7ml Standard</td>
<td>Negative</td>
<td>5 weeks</td>
<td>Only tested if APCR is positive or family history.</td>
</tr>
<tr>
<td>Prothrombin (G20210A) mutation</td>
<td>EDTA sample (pink)</td>
<td>1</td>
<td>2.7ml Standard</td>
<td>Negative</td>
<td>5 weeks</td>
<td></td>
</tr>
<tr>
<td>JAK2 V617F mutation</td>
<td>EDTA sample (pink)</td>
<td>1</td>
<td>2.7ml Standard</td>
<td>Not Detected</td>
<td>4 weeks</td>
<td>Must be sanctioned by the Haematology Team and the Molecular Test Request Form HAEMG-LF-084 must be completed. This form can be obtained from the Beaumont Hospital website, under Haematology Dept.</td>
</tr>
</tbody>
</table>

### 3.10.5 Clinical Advice & Laboratory Test Interpretation

Interpretation of Laboratory Tests / procedures may be obtained by phoning any of the telephone numbers in section 3.1.2 and asking for the Chief Medical Scientist or by requesting a senior member of staff 09:00-17:00 Mon-Fri excluding Bank Holidays.
3.11 REQUESTS FOR ADDITIONAL ANALYSIS

3.11.1 Requests for Additional Analysis

Verbal requests for additional examinations from GPs will be reviewed on a case-by-case basis and are dependent on suitable specimen availability and the appropriateness of the test request. Processing of GP verbal requests must be preceded by a faxed fully completed request form for the additional test.

Provided a suitable sample is available verbal requests will be accepted for tests. Refer to table below for test cut-off times when requested to add a test to a sample already received in the Laboratory. Ensure that the correct sample requirements are met when taking an add-on request i.e. the sample has been received/ correct anti-coagulant/ the sample is not too old for analysis.

3.11.1.1 Test Cut-Off Times

<table>
<thead>
<tr>
<th>Test</th>
<th>Test Cut-off Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBC</td>
<td>&lt;24 hours</td>
</tr>
<tr>
<td>Blood Film preparation</td>
<td>&lt;8 hours</td>
</tr>
<tr>
<td>Platelet Exact for platelet clumping</td>
<td>&lt;24 hours</td>
</tr>
<tr>
<td>Reticulocyte</td>
<td>&lt;24 hours</td>
</tr>
<tr>
<td>ESR</td>
<td>&lt;6 hours</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>&lt;8 days once stored @ 2-8°C</td>
</tr>
<tr>
<td>Malaria</td>
<td>&lt;2 hours</td>
</tr>
<tr>
<td>IM</td>
<td>&lt;24 hours. Stable for 3 days if stored @ 2-8°C</td>
</tr>
<tr>
<td>Sickle Screen</td>
<td>&lt;14 days if stored @ 2-8°C</td>
</tr>
<tr>
<td>PNH</td>
<td>&lt;48 hours if stored at @ 2-8°C</td>
</tr>
<tr>
<td>Flow Cytometry: Lymphocyte Subset Analysis &amp; CD4</td>
<td>&lt;24 hours</td>
</tr>
<tr>
<td>Flow Cytometry: Lymphoproliferative Panel, T-Panel, Acute Leukaemia Panel</td>
<td>Peripheral Blood &lt;24 hours</td>
</tr>
<tr>
<td>Lymphoid Screening Tube</td>
<td>Bone Marrow &lt;48 hours.</td>
</tr>
<tr>
<td>Coagulation Samples</td>
<td>&lt;8 hours</td>
</tr>
<tr>
<td>INR/WINR</td>
<td>&lt;24 hours</td>
</tr>
<tr>
<td>Factor V Leiden, Prothrombin G20210A and JAK2 V617F mutation</td>
<td>&lt;7 days once stored at 2-8°C</td>
</tr>
</tbody>
</table>

In cases where the Haematology consultants have reviewed a blood film, these are reported under ‘REF_FILM’. ‘BF’ and ‘REF_FILM’ can only be ordered in the Laboratory.
3.12 Critical Values

- GP results are available on Healthlink.
- Results falling outside defined alert limits are telephoned to the appropriate ward/personnel.
- Note: This may not be possible due to an inability to contact the relevant clinical personnel out of hours. In such cases, the critical alert value will be telephoned the following day.

The following table is a list of these results that will be phoned:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result to Be Phoned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>&lt;7.0g/dL &amp; &gt;19.0g/dL</td>
</tr>
<tr>
<td>PLT</td>
<td>&lt;50 x 10^9/L</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>&lt;0.75 x 10^9/L</td>
</tr>
<tr>
<td>IM</td>
<td>Positive</td>
</tr>
<tr>
<td>Malaria Screen</td>
<td>Positive</td>
</tr>
<tr>
<td>Sickle Screen</td>
<td>Positive</td>
</tr>
<tr>
<td>INR/WINR</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>D-Dimer</td>
<td>&gt;5µg/ml</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>&lt;1.0 g/l</td>
</tr>
<tr>
<td>Flow Cytometry</td>
<td>New Leukaemia patients</td>
</tr>
<tr>
<td>FBC</td>
<td>Results indicating possible leukaemia i.e. numerous flags (especially blast flag), increase WCC, DIFF vote-out or very abnormal, plt&lt;100 and low Hb.</td>
</tr>
</tbody>
</table>

Note: It is imperative that contact details of the requesting doctor and/or location of the patient are attached to the test request so that critical results can be phoned immediately.

INR/WINR >5.0 are communicated to relevant clinical staff as per Hospital Policy: PPCC-HAEM-11
3.13 CHEMICAL PATHOLOGY

3.13.1 Services Offered

GPs and external users have the same access to Chemical Pathology services as hospital doctors with the exception of a specific qualification for tumour markers, androgens, infertility screens, assessment of ovulation and menopausal status.

The Chemical Pathology Department provides a comprehensive list of tests that can be described as follows:

- General biochemistry, including test profiles for renal, liver, bone, cardiac, muscle, lipid disorders and glucose homeostasis.
- Immunoassay tests of thyroid, gonadal, adrenal and pituitary function, haematinics, therapeutic drug monitoring.
- Urine tests for total protein and albumin, calcium, phosphate, magnesium and uric acid.
- Biochemical tests for phaeochromocytoma, neuroblastoma and carcinoid tumours including urinary fractionated catecholamines, metanephrines and 5HIAA
- Blood toxicology and urine drugs of abuse screens are available, but not for Medico legal purposes.
- Additional Toxicology tests that are available include: cyanide, ethylene glycol, methanol, paraquat. Please contact the laboratory for further information if any of these are required.

3.13.2 Clinical Contact for Medical Indications/ Clinical Advice

For test results, information on test requirements or to request additional tests please contact the relevant laboratory.

For Medical Indications, clinical advice and interpretation, contact Prof. Bill Tormey (Consultant Chemical Pathologist) on 809 2676 or 087 2544646.

During working hours clinical advice can also be obtained by contacting the Specialist Registrar, 8092666.

3.13.3 Requests for Additional Tests

Samples are retained in the Department for 48 hours and are validated for testing only up to this time. Requests for additional analysis must be made to the laboratory within this validity period. The laboratory will advise on the suitability of the sample for additional testing if appropriate. Please note that verbal requests for additional testing are not acceptable and all requests for
further testing must be discussed with the relevant laboratory, availability of
suitable sample ascertained and a fax outlining the request must then be faxed to
Pathology reception (809 3217) for the attention of the relevant chemical
pathology staff member following the discussion.

3.13.4 Table D: Profiles and their Components

<table>
<thead>
<tr>
<th>Description</th>
<th>Mnemonic</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea And Electrolytes (Renal)</td>
<td>U/E</td>
<td>Urea, Na, K. Cl Creatinine</td>
</tr>
<tr>
<td>Liver Function Test</td>
<td>LFT/ γGT</td>
<td>Bilirubin, ALT, Alkaline Phosphatase, γGT</td>
</tr>
<tr>
<td>Lipids - fasting</td>
<td>HDL</td>
<td>Cholesterol, Triglyceride, HDL, non-HDL cholesterol, calculated LDL</td>
</tr>
<tr>
<td>Bone profile</td>
<td>CPMA</td>
<td>Ca, Phos Mag, Albumin</td>
</tr>
<tr>
<td>Glucose tolerance test</td>
<td>GTT</td>
<td>Fasting Glucose and 2 Hour sample</td>
</tr>
<tr>
<td>Multiple myeloma screen</td>
<td>SPE &amp; UPE</td>
<td>Serum and urine electrophoresis</td>
</tr>
<tr>
<td>Pituitary Screen</td>
<td>PITSCR</td>
<td>Cortisol, GH, TFT, PRL, FSH, LH, TESTO, IGF-1</td>
</tr>
<tr>
<td>Thyroid function test</td>
<td>TFT</td>
<td>FreeT4 and TSH</td>
</tr>
</tbody>
</table>

3.13.5 Sample Requirements for Toxicology.

- Blood toxicology and urine drugs of abuse screens are available, but **not for Medico-legal purposes**.
- Additional tests that are available include: cyanide, ethylene glycol, methanol, paraquat. Please contact the toxicology laboratory for further information if any of these are required.

3.13.6 Therapeutic Drug Monitoring (TDM) samples

- All samples must be drawn into a white cap serum tube, 4.9mL.
- Samples should be taken immediately prior to next dose – trough sample.
- **Digoxin**: samples must be taken pre-dose or at least 6 hours post-dose.
- **Lithium**: samples must be collected 12 hours post dose.

3.14 Molecular Testing

For molecular tests please contact: Department of Clinical Genetics at Our Lady’s Children’s Hospital, Crumlin directly on 01-409 6739.

Further information is available on: www.genetics.ie.
3.15 TUMOUR MARKER ANALYSIS

Tumour markers are not used to diagnose disease. They are used to monitor / follow-up known disease states. If a patient is attending Beaumont Public Hospital for Oncology treatment, we can accept samples for Tumour marker analysis, at the request of the attending clinician and this MUST be indicated on the request form – or a copy of the Consultant's request included.

Patients that are being treated and followed-up at other facilities must have tumour markers analysed at that laboratories as there can be very significant variations in results with the different assay platforms.

3.16 INVESTIGATION OF MULTIPLE MYELOMA

Serum and urine electrophoresis are the first line tests in the investigation of MM. Immunoglobulins G, A & M are not appropriate.

3.17 CHANGES TO LIPID PROFILE FOR CARDIOVASCULAR RISK ASSESSMENT

The National Institute for Health and Care Excellence (NICE) in the UK has recently published it’s guidelines on lipid modification therapy for both primary and secondary prevention of cardiovascular disease (CVD). The aim of these guidelines is to identify those with a 10-year risk of CVD of 10% or greater, and to assist you with your patient assessment, they promote the use of an on-line risk assessment tool known as QRISK2 (available at www.qrisk.org).

They also recommend the use of non-fasting bloods for the assessment of blood lipids in the initial assessment of patients. This change from fasting to non-fasting lipids is based upon several studies showing non-fasting lipids to be at least as good as fasting lipids, and in some studies it proved better than fasting samples. Therefore, our lipid profile has been adjusted to take account of this change, and will now be reported as Total Cholesterol, HDL-Cholesterol, Triglyceride and a calculated test, Non-HDL-Cholesterol, which replaces the older method used to calculate the LDL-Cholesterol concentration. LDL-Cholesterol will still be reported.

This does not require a fasting sample; a fasting sample is generally only required where the diagnosis of Primary Familial Hypercholesterolaemia is being considered. These changes should prove much more convenient for patients as they will no longer need to fast overnight.

Overall, the guidelines make a number of recommendations relating to CVD risk assessment, modification of blood lipids, and the primary and secondary prevention of CVD. The full guidelines are available on-line at www.nice.org.uk/guidance/cg181.
3.18 EXTERNALLY REFERRED TESTS

Samples are not referred to external laboratories for General Practitioners or other external users of our services as we are not funded to provide this service.

If a clinician of Beaumont Public Hospital requests a GP to organise a test not provided in Beaumont Hospital we will refer the sample out if the request from the clinician is sent with the request or noted very clearly on the request form.

3.18.1 Reports from External Laboratories

Such reports are issued on paper only. They will not be on Healthlinks. However, they will be available electronically within Beaumont Hospital to the requesting clinician.

3.19 FERTILITY CLINICS

We do not provide specialist testing services for patients attending fertility clinics.

3.20 CRITICAL PHONING LIMITS

- Results falling outside defined alert limits are telephoned to the appropriate ward/ personnel.

- Note: This may not be possible due to an inability to contact the relevant clinical personnel out of hours. In such cases, the critical alert value will be telephoned the following day.

The Following Table is a list of these results that will be phoned:

<table>
<thead>
<tr>
<th>Test</th>
<th>Less Than</th>
<th>Greater Than</th>
<th>Units</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>120</td>
<td>150</td>
<td>mmol/L</td>
<td>If new event</td>
</tr>
<tr>
<td>Potassium</td>
<td>2.5</td>
<td>6.5</td>
<td>mmol/L</td>
<td>If new: 5.7 – 6.4 phone next day to GP</td>
</tr>
<tr>
<td>Urea</td>
<td>-</td>
<td>30: if &lt; 16yr: 10</td>
<td>mmol/L</td>
<td>If new event &amp; external patient</td>
</tr>
<tr>
<td>Creatinine</td>
<td>-</td>
<td>400: if&lt; 16yr: 200</td>
<td>µmol/L</td>
<td>If new event &amp; external patient</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.5</td>
<td>25.0</td>
<td>mmol/L</td>
<td>If new event (Not DFT)</td>
</tr>
<tr>
<td>Calcium (adj)</td>
<td>1.8*</td>
<td>3.5</td>
<td>mmol/L</td>
<td>* report with albumin</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.3</td>
<td>-</td>
<td>mmol/L</td>
<td>If new event</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.4</td>
<td>-</td>
<td>mmol/L</td>
<td>If new event</td>
</tr>
<tr>
<td>CK</td>
<td>-</td>
<td>5000</td>
<td>IU/L</td>
<td>If new &amp; external patient</td>
</tr>
<tr>
<td>Amylase</td>
<td>-</td>
<td>450</td>
<td>IU/L</td>
<td>If new event</td>
</tr>
<tr>
<td>CRP</td>
<td>-</td>
<td>300</td>
<td>mg/L</td>
<td>If new &amp; external patient</td>
</tr>
<tr>
<td>Digoxin</td>
<td>-</td>
<td>2.5</td>
<td>µg/L</td>
<td></td>
</tr>
<tr>
<td>Phenobarbitone</td>
<td>-</td>
<td>70</td>
<td>mg/L</td>
<td></td>
</tr>
<tr>
<td>Phenytoin</td>
<td>-</td>
<td>25</td>
<td>mg/L</td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>Less Than</td>
<td>Greater Than</td>
<td>Units</td>
<td>Conditions</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------</td>
<td>--------------</td>
<td>----------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>AST</td>
<td>-</td>
<td>600</td>
<td>IU/L</td>
<td>If new event</td>
</tr>
<tr>
<td>ALT</td>
<td>-</td>
<td>525</td>
<td>IU/L</td>
<td>If new event</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td></td>
<td>25</td>
<td>mg/L</td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>All results</td>
<td>mg/L</td>
<td></td>
<td>External hospitals, if &lt; 24 hrs old</td>
</tr>
<tr>
<td>Theophylline</td>
<td>-</td>
<td>25</td>
<td>mg/L</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td>20.0</td>
<td>mmol/L</td>
<td>If new event, check Na, K on ABL</td>
</tr>
<tr>
<td>pH</td>
<td>7.2</td>
<td>7.6</td>
<td></td>
<td>If new event</td>
</tr>
<tr>
<td>Lithium</td>
<td>-</td>
<td>1.5</td>
<td>mmol/L</td>
<td></td>
</tr>
</tbody>
</table>

The following results will be telephoned on the next working day to all service users.

<table>
<thead>
<tr>
<th>Test</th>
<th>Less Than</th>
<th>Greater Than</th>
<th>Units</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol</td>
<td>50</td>
<td>1000</td>
<td>nmol/L</td>
<td>Not DFT</td>
</tr>
<tr>
<td>Ethanol</td>
<td>All results</td>
<td></td>
<td>mg%</td>
<td>External hospitals</td>
</tr>
<tr>
<td>Ferritin</td>
<td></td>
<td>5000</td>
<td>ng/mL</td>
<td>If new event</td>
</tr>
<tr>
<td>Folate</td>
<td>1.5</td>
<td></td>
<td>µg/L</td>
<td>If new event</td>
</tr>
<tr>
<td>FT4</td>
<td>5.5</td>
<td>30</td>
<td>pmol/L</td>
<td>If new event</td>
</tr>
<tr>
<td>Prolactin</td>
<td></td>
<td>2000</td>
<td>mIU/L</td>
<td>If new event</td>
</tr>
<tr>
<td>PSA</td>
<td></td>
<td>40</td>
<td>ng/mL</td>
<td>If new event</td>
</tr>
<tr>
<td>Salicylate</td>
<td>All results</td>
<td>mg/L</td>
<td></td>
<td>External hospitals, if &lt; 24 hrs old</td>
</tr>
<tr>
<td>TSH</td>
<td></td>
<td>30</td>
<td>mIU/L</td>
<td></td>
</tr>
<tr>
<td>Testosterone Female</td>
<td></td>
<td>5</td>
<td>nmol/L</td>
<td></td>
</tr>
</tbody>
</table>
### 3.20.1 Repertoire of Test Services – Routine Chemistry

<table>
<thead>
<tr>
<th>Test</th>
<th>Sample Required</th>
<th>Specimen Container</th>
<th>Minimum Volume</th>
<th>Reference Range/ Unit of Measurement</th>
<th>TAT</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HIAA</td>
<td>24 hour Urine collection</td>
<td>Pre-acidified (50% acid)</td>
<td>N/A</td>
<td>&lt; 50 μmol/24hr</td>
<td>12 working days</td>
<td>If the patient is &lt; 15 years old, please contact the laboratory [(01) 7977333] for information regarding sample collection. Age-related reference ranges will accompany results for paediatric samples.</td>
</tr>
<tr>
<td>Adrenocorticotrophic Hormone (ACTH)</td>
<td>EDTA Plasma on Ice</td>
<td>Pink</td>
<td>4.9mL</td>
<td>7.2 – 63.3 pg/mL</td>
<td>168 hours</td>
<td>Patient must attend Beaumont Hospital phlebotomy for sample collection as the sample is labile sent to lab immediately on ice as immediate separation is required.</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>24 hour Urine collection</td>
<td>Pre-acidified (50% acid)</td>
<td>N/A</td>
<td>See Table Below</td>
<td>12 working days</td>
<td>If the patient is &lt; 15 years old, please contact the laboratory (01- 7977333) for information regarding sample collection. Age-related reference ranges will accompany results for paediatric samples</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9 mL</td>
<td>Female: &lt; 35 I.U./L Male: &lt; 50 I.U./L</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9 mL</td>
<td>35 – 50 g/L</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Albumin Creatinine Ratio</td>
<td>Spot urine sample</td>
<td>Plain MSU</td>
<td>N/A</td>
<td>mg/mmol Creatinine Males: &lt;2.5 Females: &lt;3.5</td>
<td>24 hours</td>
<td>For diabetic patients only</td>
</tr>
<tr>
<td>Test</td>
<td>Sample Required</td>
<td>Specimen Container</td>
<td>Minimum Volume</td>
<td>Reference Range/Unit of Measurement</td>
<td>TAT</td>
<td>Comment</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-----------------</td>
<td>------------------------------</td>
<td>----------------</td>
<td>-------------------------------------</td>
<td>-------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Alkaline Phosphatase (Total Alk Phos)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9 mL</td>
<td>30 - 130 I.U./L (Adult)</td>
<td>24 hours</td>
<td>Children and adolescents would be expected to have ALP levels 2-3 times the adult reference range quoted.</td>
</tr>
<tr>
<td>Amylase</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>22-80 I.U./L</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Amylase Urine</td>
<td>Spot urine sample</td>
<td>Plain container</td>
<td>N/A</td>
<td>Units: IU/L</td>
<td>24 hours</td>
<td>Reference ranges are not available for this spot urine test, results must be considered in conjunction with the age, sex and hydration status of the patient</td>
</tr>
<tr>
<td>Angiotensin Converting Enzyme (ACE)</td>
<td>Serum</td>
<td>Plain (WHITE cap)</td>
<td>4.9mL</td>
<td>8 – 65 U/L</td>
<td>7 working days</td>
<td></td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>Female &lt; 35 I.U./L Male &lt; 50 I.U./L</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>B12 (Vitamin)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>Normal 160-600 ng/ml Deficient &lt;130 ng/ml Indeterminate 130-159 ng/ml</td>
<td>72 hours</td>
<td></td>
</tr>
<tr>
<td>Bicarbonate (TCO2)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>22 – 29 mmol/L</td>
<td>24 hours</td>
<td>Fresh sample required – patient should attend Beaumont Hospital phlebotomy for sample collection.</td>
</tr>
<tr>
<td>Bilirubin - total</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>&lt; 21 μmol/L</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Bilirubin – conjugated (conj. bili)</td>
<td>Serum</td>
<td>Brown cap wrapped in foil to exclude light</td>
<td>4.9mL</td>
<td>&lt;3.4μmol/L</td>
<td>72 hours</td>
<td>Only analysed if total bilirubin is elevated. Patient must attend Beaumont Hospital phlebotomy so that a fresh sample can be taken and protected from light.</td>
</tr>
<tr>
<td>Calcium</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>2.20 – 2.60 μmol/L</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Calcium 24 Hour Urine</td>
<td>24 hour Urine collection</td>
<td>No Preservative</td>
<td>N/A</td>
<td>2.5 - 7.5 mmol/24hrs</td>
<td>72 hours</td>
<td>Container available from Phlebotomy Department</td>
</tr>
<tr>
<td>Test</td>
<td>Sample Required</td>
<td>Specimen Container</td>
<td>Minimum Volume</td>
<td>Reference Range/ Unit of Measurement</td>
<td>TAT</td>
<td>Comment</td>
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</tr>
<tr>
<td>Carbamazepine Serum</td>
<td>Serum</td>
<td>Plain (WHITE cap)</td>
<td>4.9mL</td>
<td>4.0 – 12.0 mg/L</td>
<td>24 hours</td>
<td>Recommendations of the ACB October 2006 Samples should be taken immediately prior to next dose.</td>
</tr>
<tr>
<td>Catecholamines (Noradrenaline, Adrenaline &amp; Dopamine) 24 hour Urine collection Pre-acidified (50% acid)</td>
<td>N/A</td>
<td>See Table Below</td>
<td>12 working days</td>
<td></td>
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</tr>
<tr>
<td>Chloride Serum</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>95 – 108 mmol/L</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Cholesterol Serum</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>&lt; 5.0 mmol/L</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Cortisol AM (8-10AM) Serum</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>185 – 624 nmol/L</td>
<td>72 hours</td>
<td></td>
</tr>
<tr>
<td>Creatine Kinase Serum (CK)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>&lt;210 I.U./L males &lt;170 I.U./L females</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Creatinine Serum</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>Male: 64-104μmol/L Female: 49-90μmol/L Paeds: 21-65μmol/L</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Creatinine Urine 24 Hour Serum</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>8800 – 17600 μmol/ 24hrs</td>
<td>72 hours</td>
<td></td>
</tr>
<tr>
<td>Creatinine Clearance (GFR)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>80 – 125 mL/min (adults)</td>
<td>72 hours</td>
<td>Blood creatinine level also required for GFR calculation.</td>
</tr>
<tr>
<td>Cyclosporin A Whole blood</td>
<td>EDTA (PINK cap)</td>
<td>2.6mL</td>
<td>N/A</td>
<td>168 hours</td>
<td>Trough level sample.</td>
<td></td>
</tr>
<tr>
<td>Dehydroepiandrosterone Sulphate (DHEAS) Serum Plain (WHITE cap)</td>
<td>N/A</td>
<td>N/A</td>
<td>48 hours</td>
<td>Age and gender specific ranges are applied to individual reports.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dexamethasone overnight</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9 mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>Sample Required</td>
<td>Specimen Container</td>
<td>Minimum Volume</td>
<td>Reference Range/ Unit of Measurement</td>
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</tr>
<tr>
<td>Digoxin</td>
<td>Serum</td>
<td>Plain (WHITE cap)</td>
<td>4.9mL</td>
<td>0.8 – 2.0 µg/L</td>
<td>24 hours</td>
<td>With hypokalaemia toxicity may occur within the therapeutic range.</td>
</tr>
<tr>
<td>Dopamine</td>
<td>24 hour Urine</td>
<td>Pre-acidified (50% acid)</td>
<td>N/A</td>
<td>See Table Below</td>
<td>12 working days</td>
<td>If the patient is &lt;15 years, please contact the laboratory [(01)7977333)] for information regarding sample collection. Age-related reference ranges will accompany results for paediatric samples.</td>
</tr>
<tr>
<td>Ferritin</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>Female: 11 – 307ng/mL Male: 24 - 336ng/mL</td>
<td>72 hours</td>
<td></td>
</tr>
<tr>
<td>Folate</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>3.1 – 19,9µg/L from a European population. WHO: &lt; 4.0 µg/L is deficient</td>
<td>72 hours</td>
<td>Affected by light and recent food intake.</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone (FSH)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>Male: 1 – 12 mIU/mL Female: Mid-follicular: 1 – 10 Mid-cycle: 4 – 23 Mid-luteal: 1 – 8 Post-menopausal 30-120</td>
<td>72 hours</td>
<td></td>
</tr>
<tr>
<td>Free Thyroxine (fT4)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>7.0 – 16.0 pmol/L</td>
<td>72 hours</td>
<td></td>
</tr>
<tr>
<td>Gamma-glutamyl transferase (GGT)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>&lt; 58 I.U./L males &lt; 38 I.U./L females</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Glucose - fasting</td>
<td>Plasma</td>
<td>Fluoride Oxalate (YELLOW cap)</td>
<td>2.7mL</td>
<td>3.6 – 6.0 mmol/L</td>
<td>24 hours</td>
<td>Fast for at least 10 hours.</td>
</tr>
<tr>
<td>Glucose- random</td>
<td>Plasma</td>
<td>Fluoride Oxalate (YELLOW cap)</td>
<td>2.7mL</td>
<td>N/A</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>Sample Required</td>
<td>Specimen Container</td>
<td>Minimum Volume</td>
<td>Reference Range/Unit of Measurement</td>
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</tr>
<tr>
<td>Growth Hormone (hGH)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>0 - 5µg/L</td>
<td>Up to 15 working days</td>
<td>Fasting sample required. hGH test most useful in dynamic tests where states of hypoglycaemia or hyperglycaemia are induced.</td>
</tr>
<tr>
<td>HbA1C</td>
<td>Whole blood</td>
<td>EDTA (PINK cap)</td>
<td>2.7mL</td>
<td>IFCC: 20–42 mmol/mol Reference range for people without diabetes. The target range for patients with diabetes will be set by the clinician</td>
<td>12 working days</td>
<td></td>
</tr>
<tr>
<td>High Density Lipoprotein (HDL) Cholesterol</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>1.00 – 1.70 mmol/L</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>HMMA (VMA)</td>
<td>24 hour Urine collection</td>
<td>Pre-acidified (50% acid)</td>
<td>&lt; 45 µmol/24 hr (Adult)</td>
<td>12 working days</td>
<td>If the patient is &lt;15 years, please contact the laboratory [(01)7977333]] for information regarding sample collection. Age-related reference ranges will accompany results for paediatric samples.</td>
<td></td>
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<tr>
<td>HVA</td>
<td>24 hour Urine collection</td>
<td>Pre-acidified (50% acid)</td>
<td>&lt; 40 µmol/24 hr (Adult)</td>
<td>12 working days</td>
<td>If the patient is &lt;15 years, please contact the laboratory [(01)7977333]] for information regarding sample collection. Age-related reference ranges will accompany results for paediatric samples.</td>
<td></td>
</tr>
<tr>
<td>HFE Haemochromatosis</td>
<td>Whole blood</td>
<td>EDTA (PINK cap)</td>
<td>2.7mL</td>
<td>N/A</td>
<td>6weeks</td>
<td>Must be accompanied by completed consent form (CP-LF-0068)</td>
</tr>
<tr>
<td>Test</td>
<td>Sample Required</td>
<td>Specimen Container</td>
<td>Minimum Volume</td>
<td>Reference Range/Unit of Measurement</td>
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<tr>
<td>IGF1</td>
<td>Serum</td>
<td>White Cap</td>
<td>4.9mL</td>
<td>1-7 Days: 26</td>
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<td>8-15 Days: 41</td>
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<td>1 Yr: 55-327</td>
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<td>2 Yr: 51-303</td>
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<td>3 Yr: 49-289</td>
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<td>4 Yr: 49-283</td>
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<td>5 Yr: 50-286</td>
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<td>6 Yr: 52-297</td>
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<td>7 Yr: 57-316</td>
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<td>8 Yr: 64-345</td>
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<td>9 Yr: 74-388</td>
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<td>10 Yr: 88-452</td>
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<td>11 Yr: 111-551</td>
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<td>12 Yr: 143-693</td>
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<td>13 Yr: 183-850</td>
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<td>14 Yr: 220-972</td>
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<td>15 Yr: 237-996</td>
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<td>16 Yr: 226-903</td>
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<td>17 Yr: 193-731</td>
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<td>18 Yr: 163-584</td>
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<td>19 Yr: 141-483</td>
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<td>20 Yr: 127-424</td>
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<td>21-25 Yr: 116-358</td>
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<td>26-30 Yr: 117-329</td>
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<td>31-35 Yr: 115-307</td>
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<td>36-40 Yr: 109-284</td>
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<td>41-45 Yr: 101-267</td>
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<td>46-50 Yr: 94-252</td>
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<td>51-55 Yr: 87-238</td>
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<td>56-60 Yr: 81-225</td>
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<td>61-65 Yr: 75-212</td>
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<td>66-70 Yr: 69-200</td>
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<td>71-75 Yr: 64-188</td>
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<td>76-80 Yr: 59-177</td>
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<td>81 – 85 Yr: 55-166</td>
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<td></td>
<td>Up to 15 working days.</td>
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<td>Test</td>
<td>Sample Required</td>
<td>Specimen Container</td>
<td>Minimum Volume</td>
<td>Reference Range/Unit of Measurement</td>
<td>TAT</td>
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</tr>
<tr>
<td>Iron</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>Male: 12.5 – 32.0 μmol/L Female: 10.7 - 32.2 μmol/L</td>
<td>24 hours</td>
<td>DRAMATICALLY increased by haemolysis or if sample is left on cells overnight.</td>
</tr>
<tr>
<td>Lactate Dehydrogenase (LDH)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>208 – 378 I.U./L</td>
<td>24 hours</td>
<td>Therapeutic range: Up to 1.3 in acute mania. Severe Toxicity likely if level &gt; 2.0</td>
</tr>
<tr>
<td>Lithium</td>
<td>Serum</td>
<td>Plain (WHITE cap)</td>
<td>4.9mL</td>
<td>0.4 – 1.0 mmol/L</td>
<td>24 hours</td>
<td>Therapeutic range: Up to 1.3 in acute mania. Severe Toxicity likely if level &gt; 2.0</td>
</tr>
<tr>
<td>Luteinising Hormone (LH)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>Male: 1 – 9 mIU/mL Female: Mid-follicular 2-12 Mid-cycle 15-100 Mid-Luteal 1-13</td>
<td>72 hours</td>
<td>Therapeutic range: Up to 1.3 in acute mania. Severe Toxicity likely if level &gt; 2.0</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>0.70 – 1.00 mmol/L</td>
<td>24 hours</td>
<td>Increased by haemolysis or if sample is left on cells overnight.</td>
</tr>
<tr>
<td>Magnesium 24 Hour Urine</td>
<td>24 hour Urine collection</td>
<td>No Preservative</td>
<td>6.0 – 8.5 mmol/24hr</td>
<td>72 hours</td>
<td>Container available from Phlebotomy Department</td>
<td></td>
</tr>
<tr>
<td>Metanephrine (Total)</td>
<td>24 hour Urine collection</td>
<td>Pre-acidified (50% acid)</td>
<td>See Table Below</td>
<td>12 working days</td>
<td>Container available from Phlebotomy Department</td>
<td></td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>24 hour Urine collection</td>
<td>Pre-acidified (50% acid)</td>
<td>N/A</td>
<td>See Table Below</td>
<td>12 working days</td>
<td>If the patient is &lt;15 years, please contact the laboratory [(01)7977333)] for information regarding sample collection. Age-related reference ranges will accompany results for paediatric samples. Samples should be taken immediately prior to next dose.</td>
</tr>
<tr>
<td>Test</td>
<td>Sample Required</td>
<td>Specimen Container</td>
<td>Minimum Volume</td>
<td>Reference Range/Unit of Measurement</td>
<td>TAT</td>
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</tr>
<tr>
<td>Normetanephrine (Total)</td>
<td>24 hour collection Urine</td>
<td>Pre-acidified (50% acid)</td>
<td>N/A</td>
<td>See Table Below</td>
<td>12 working days</td>
<td>Container available from Phlebotomy Department</td>
</tr>
<tr>
<td>Oestadiol</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>Male: &lt; 173 pmol/L</td>
<td>72 hours</td>
<td>see Table Below</td>
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<tr>
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<td>Female:</td>
<td></td>
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<td></td>
<td>Mid-follicular: 99 - 448</td>
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<td>Mid-cycle: 349 - 1590</td>
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<td>Mid-luteal: 180 - 1068</td>
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<td>200 - 800</td>
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<td>Post-menopausal &lt; 147</td>
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<td></td>
</tr>
<tr>
<td>Osmolality</td>
<td>Plasma</td>
<td>Lithium Heparin</td>
<td>4.9mL</td>
<td>275 – 295 mOsm/Kg</td>
<td>24 hours</td>
<td>Results are interpreted in conjunction with the plasma Osmolality</td>
</tr>
<tr>
<td>Osmolality Urine</td>
<td>Spot urine sample</td>
<td>Plain MSU container</td>
<td>N/A</td>
<td>400 – 1000 mOsm/Kg</td>
<td></td>
<td>Results must be interpreted only in the context of plasma Ca2+</td>
</tr>
<tr>
<td>Parathyroid Hormone (PTH)</td>
<td>Whole blood</td>
<td>EDTA (PINK cap)</td>
<td>4.9ml</td>
<td>10 – 65 pg/mL</td>
<td>72 hours</td>
<td>Results must be interpreted only in the context of plasma Ca2+</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>Serum</td>
<td>Plain (WHITE cap)</td>
<td>4.9mL</td>
<td>10.0 – 40.0 mg/L</td>
<td>24 hours</td>
<td>Therapeutic range ill-defined due to ‘tolerance’</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Serum</td>
<td>Plain (WHITE cap)</td>
<td>4.9mL</td>
<td>5.0 – 20.0 mg/L</td>
<td>24 hours</td>
<td>Severe toxicity likely if &gt; 40.0 mg/L</td>
</tr>
<tr>
<td>Phosphate</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>0.80 – 1.50 mmol/L</td>
<td>24 hours</td>
<td>Can be dramatically increased if sample is left on cells overnight.</td>
</tr>
<tr>
<td>Phosphate 24 Hour Urine</td>
<td>24 hour collection Urine</td>
<td>No Preservative</td>
<td>N/A</td>
<td>15 – 50 mmol/24hr</td>
<td>72 hours</td>
<td>Container available from Phlebotomy Department</td>
</tr>
<tr>
<td>Potassium</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>3.5 – 5.3 mmol/L</td>
<td>24 hours</td>
<td>Greatly increased if sample is left on cells overnight or refrigerated.</td>
</tr>
<tr>
<td>Potassium 24 Hour Urine</td>
<td>24 hour collection Urine</td>
<td>Plain</td>
<td>N/A</td>
<td>30 – 100 mmol/24hr (for person on average diet)</td>
<td>72 hours</td>
<td>Reference ranges are not available for this spot urine tests, results must be considered in conjunction with the age, sex and hydration status of the patient</td>
</tr>
<tr>
<td>Potassium Urine</td>
<td>Spot urine sample</td>
<td>Plain MSU container</td>
<td>N/A</td>
<td>N/A</td>
<td>24 hours</td>
<td>Reference ranges are not available for this spot urine tests, results must be considered in conjunction with the age, sex and hydration status of the patient</td>
</tr>
<tr>
<td>Test</td>
<td>Sample Required</td>
<td>Specimen Container</td>
<td>Minimum Volume</td>
<td>Reference Range/Unit of Measurement</td>
<td>TAT</td>
<td>Comment</td>
</tr>
<tr>
<td>------------------------</td>
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<td>--------------------</td>
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<td>---------------------------------------------------------------------------------------------------</td>
<td>-------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Prolactin</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>Male: 56 – 278 mIU/mL Female &lt; 50 yrs: 71 – 566 mIU/mL &gt;= 50 yrs 58 – 416 mIU/mL</td>
<td>72 hours</td>
<td>&lt; 50 years Pre-menopausal range quoted. &gt;50 years, Post-Menopausal range quoted.</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>See report (nmol/L)</td>
<td>72 hours</td>
<td>Note date of cycle in patient record.</td>
</tr>
<tr>
<td>Protein (Total)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>60 – 80 g/L</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Protein (TUP) 24 Hour Urine</td>
<td>24 hour Urine collection</td>
<td>Plain</td>
<td>N/A</td>
<td>0.05 – 0.140 g/24 hour</td>
<td>72 hours</td>
<td></td>
</tr>
<tr>
<td>Protein Creatinine Ratio</td>
<td>Spot urine sample</td>
<td>Plain MSU</td>
<td>N/A</td>
<td>3 – 14 mg/mmol</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Protein Electrophoresis</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>N/A</td>
<td>35 days</td>
<td></td>
</tr>
<tr>
<td>PSA (total)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>0 – 3.1 ng/mL</td>
<td>72 hours</td>
<td>Specimens for PSA should not be drawn immediately after digital rectal examination, prostatic massage or transrectal ultrasound. PSA sampling should not be carried out for at least 6 weeks after prostatic biopsy.</td>
</tr>
<tr>
<td>Test</td>
<td>Sample Required</td>
<td>Specimen Container</td>
<td>Minimum Volume</td>
<td>Reference Range/ Unit of Measurement</td>
<td>TAT</td>
<td>Comment</td>
</tr>
<tr>
<td>----------------------------------</td>
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<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>PSA (free) fPSA</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>Interpretative guidelines for % free PSA are based on a population of Caucasian men with a normal DRE exam. If fPSA is &gt;15% and &lt; 25% repeat blood tests and DRE within a year. If total PSA in the range 2.4 - 8.0 ng/mL and fPSA &gt; 25% usually indicates benign prostate hypertrophy, if fPSA &lt; 15% it is highly suggestive of carcinoma of the prostate</td>
<td>72 hours</td>
<td>Only carried out on those patient samples in which Total PSA was measured and found to be 2.4 - 8.0 ng/mL. Due to labile nature of fPSA please arrange for patient to attend Beaumont Hospital phlebotomy for this test.</td>
</tr>
<tr>
<td>Sodium 24 Hour Urine</td>
<td>24 hour Urine</td>
<td>Plain</td>
<td>N/A</td>
<td>80 – 250 mmol/24hr</td>
<td>72 hours</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>133 – 146 mmol/L</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Sodium Urine</td>
<td>Spot urine</td>
<td>Plain MSU container</td>
<td>N/A</td>
<td>N/A</td>
<td>24 hours</td>
<td>Reference ranges are not available for this spot urine tests, results must be considered in conjunction with the age, sex and hydration status of the patient</td>
</tr>
<tr>
<td>Tacrolimus (FK506)</td>
<td>Whole blood</td>
<td>EDTA (PINK cap)</td>
<td>2.6mL</td>
<td>5 – 20 ng/mL</td>
<td>24 hours</td>
<td>Trough level sample.</td>
</tr>
<tr>
<td>Test</td>
<td>Sample Required</td>
<td>Specimen Container</td>
<td>Minimum Volume</td>
<td>Reference Range/ Unit of Measurement</td>
<td>TAT</td>
<td>Comment</td>
</tr>
<tr>
<td>-------------------------------------------</td>
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<td>----------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Serum</td>
<td>Plain (WHITE cap)</td>
<td>4.9mL</td>
<td>Male: &gt;18 yr – 50: 8.64 – 29.0 &gt; 50 yr 6.68 – 25.7 .5 nmol/L Female: &gt;18 yr – 50: 0.29 – 1.67 &gt; 50 yrs 0.101 – 1.42 nmol/L</td>
<td>72 Hours</td>
<td>Reference Ranges apply to &gt; 18 years only. A gender specific interpretive comment will attach to all results if the patient is &lt; 18yrs. Reference ranges apply to samples drawn in the morning.</td>
</tr>
<tr>
<td>Theophylline</td>
<td>Serum</td>
<td>Plain (WHITE cap)</td>
<td>4.9mL</td>
<td>10.0 – 20.0 mg/L</td>
<td>24 hours</td>
<td>Lower levels ≥ 5.0mg/L may be effective. Concern level 14.0mg/L if age &lt; 3months. Severe toxicity likely if &gt; 60.0mg/L.</td>
</tr>
<tr>
<td>Transferrin Saturation</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>A Fasting Transferrin Saturation &gt; 55% in Males OR &gt; 50% in Females indicates Iron accumulation.</td>
<td>24 hours</td>
<td>If Transferrin Saturation &gt; 50% Please repeat on a morning fasting sample. REFER TO: BCSH GUIDELINES.</td>
</tr>
<tr>
<td>Thyroid Stimulating Hormone (TSH)</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>0.38 – 5.33mIU/mL</td>
<td>72 hours</td>
<td>Pregnant Females, 1st Trimester: 0.05 - 3.70 2nd Trimester: 0.31 - 4.35 3rd Trimester: 0.41 - 5.18</td>
</tr>
<tr>
<td>Total T3</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>1.0 - 3.0 nmol/L</td>
<td>72 hours</td>
<td></td>
</tr>
<tr>
<td>Urate 24 Hour Urine</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>1.49 - 4.46 mmol/24hr</td>
<td>72 hours</td>
<td></td>
</tr>
<tr>
<td>Urate/Uric Acid</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>140 – 420 μmol/L</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>2.5 – 8.5 mmol/L</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Urea 24 Hour Urine</td>
<td>Serum</td>
<td>Brown Cap</td>
<td>4.9mL</td>
<td>250 – 580 mmol/24hr</td>
<td>72 hours</td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>Sample Required</td>
<td>Specimen Container</td>
<td>Minimum Volume</td>
<td>Reference Range/Unit of Measurement</td>
<td>TAT</td>
<td>Comment</td>
</tr>
<tr>
<td>------------------------------</td>
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<td>-------------------------------------</td>
<td>-------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Valproic Acid</td>
<td>Serum</td>
<td>Plain (WHITE cap)</td>
<td>4.9mL</td>
<td>50 – 100 mg/L</td>
<td>24 hours</td>
<td>Therapeutic range ill-defined as toxic effects shows no clear relationship to plasma levels.</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Serum</td>
<td>Plain (WHITE cap)</td>
<td>4.9mL</td>
<td>Recommended Target: Sufficiency: &gt;50nmol/L Severe Deficiency: &lt;20 nmol/L</td>
<td>72 hours</td>
<td></td>
</tr>
<tr>
<td>Urinary Protein Electrophoresis (Bence Jones Protein)</td>
<td><strong>24 Hour Urine Collection</strong> for patients with diagnosed multiple myeloma. <strong>Early morning urine</strong> adequate for screening</td>
<td>Plain</td>
<td>N/A</td>
<td>Qualitative</td>
<td>35 days</td>
<td></td>
</tr>
</tbody>
</table>
3.20.1.1 Calculated / Derived Tests

<table>
<thead>
<tr>
<th>Calculated Parameter</th>
<th>Formula</th>
<th>Reference Range</th>
<th>Units</th>
<th>Important Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Calcium</td>
<td>$0.02 \times (40 - \text{Alb}) + \text{Ca}$</td>
<td>-</td>
<td>mmol/L</td>
<td>The calculation is unsuitable if the albumin result is &lt; 25g/L.</td>
</tr>
<tr>
<td>Globulin</td>
<td>Total protein - albumin</td>
<td>-</td>
<td>g/L</td>
<td></td>
</tr>
<tr>
<td>LDL (Low Density Lipoprotein) Cholesterol</td>
<td>Cholesterol – HDL – (triglyceride / 2.2) 2° prevention</td>
<td>&lt; 2.6 (NCEP, U.S.) &lt; 3.0 (Europe)</td>
<td>mmol/L</td>
<td>The calculation is unsuitable if the triglyceride level is &gt; 4.5mmol/l</td>
</tr>
<tr>
<td>Transferrin saturation (TfS)</td>
<td>(Iron / Transferrin) * 398</td>
<td>%</td>
<td></td>
<td>If transferrin saturation &gt; 50% please repeat on a morning fasting sample. Refer to: bcs guidelines. A fasting transferrin saturation &gt; 55% in males or &gt;50% in females,indicates iron accumulation.</td>
</tr>
<tr>
<td>Unconjugated Bilirubin</td>
<td>Total bilirubin – conjugated bilirubin</td>
<td>-</td>
<td>μmol/L</td>
<td></td>
</tr>
<tr>
<td>Non-HDL Cholesterol</td>
<td>Total Cholesterol – HDL Cholesterol.</td>
<td></td>
<td>mmol/L</td>
<td></td>
</tr>
</tbody>
</table>

3.20.2 Repertoire of Test Services – Toxicology

<table>
<thead>
<tr>
<th>Test</th>
<th>Sample Required</th>
<th>Specimen Container</th>
<th>Minimum Volume</th>
<th>Reference Range</th>
<th>TAT</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbiturates</td>
<td>Serum</td>
<td>Plain (WHITE cap)</td>
<td>7.5 mL</td>
<td>N/A</td>
<td>72 hours</td>
<td>Qualitative test only: positive / not detected</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>Serum</td>
<td>Plain (WHITE cap)</td>
<td>7.5 mL</td>
<td>N/A</td>
<td>72 hours</td>
<td>Qualitative test only: positive / not detected</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Plasma</td>
<td>Fluoride oxalate (YELLOW cap)</td>
<td>2.7 mL</td>
<td>Unit: mg %</td>
<td>24 hours</td>
<td>In suspected overdose, take sample more than 4 hours post ingestion.</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>Serum</td>
<td>Plain (WHITE cap)</td>
<td>7.5 mL</td>
<td>Units: mg/L</td>
<td>24 hours</td>
<td>In suspected overdose, take sample more than 4 hours post ingestion.</td>
</tr>
<tr>
<td>Salicylate</td>
<td>Serum</td>
<td>Plain (WHITE cap)</td>
<td>7.5 mL</td>
<td>Units: mg/L</td>
<td>24 hours</td>
<td>Concern level 280 mg/L if age &lt;5 years; Severe toxicity likely if level &gt;700 mg/L</td>
</tr>
<tr>
<td>Test</td>
<td>Sample Required</td>
<td>Specimen Container</td>
<td>Minimum Volume</td>
<td>Reference Range</td>
<td>TAT</td>
<td>Comment</td>
</tr>
<tr>
<td>--------------------------</td>
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<td>--------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>-------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Tricyclic Antidepressants</td>
<td>Serum</td>
<td>Plain (WHITE cap)</td>
<td>7.5 mL</td>
<td>N/A</td>
<td>24 hours</td>
<td>Qualitative test only: positive / not detected</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>Spot urine</td>
<td>Plain container</td>
<td>N/A</td>
<td>N/A</td>
<td>3 days</td>
<td>Qualitative test only: positive / not detected</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>Spot urine</td>
<td>Plain container</td>
<td>N/A</td>
<td>N/A</td>
<td>3 days</td>
<td>Qualitative test only: positive / not detected</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>Spot urine</td>
<td>Plain container</td>
<td>N/A</td>
<td>N/A</td>
<td>3 days</td>
<td>Qualitative test only: positive / not detected</td>
</tr>
<tr>
<td>Cannabis</td>
<td>Spot urine</td>
<td>Plain container</td>
<td>N/A</td>
<td>N/A</td>
<td>3 days</td>
<td>Qualitative test only: positive / not detected</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Spot urine</td>
<td>Plain container</td>
<td>N/A</td>
<td>N/A</td>
<td>3 days</td>
<td>Qualitative test only: positive / not detected</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Spot urine</td>
<td>Plain container</td>
<td>N/A</td>
<td>Units: mg%</td>
<td>3 days</td>
<td></td>
</tr>
<tr>
<td>Methadone</td>
<td>Spot urine</td>
<td>Plain container</td>
<td>N/A</td>
<td>N/A</td>
<td>3 days</td>
<td>Qualitative test only: positive / not detected</td>
</tr>
<tr>
<td>Opiates</td>
<td>Spot urine</td>
<td>Plain container</td>
<td>N/A</td>
<td>N/A</td>
<td>3 days</td>
<td>Qualitative test only: positive / not detected</td>
</tr>
<tr>
<td>Propoxyphene</td>
<td>Spot urine</td>
<td>Plain container</td>
<td>N/A</td>
<td>N/A</td>
<td>3 days</td>
<td>Qualitative test only: positive / not detected</td>
</tr>
</tbody>
</table>

NOTE: One full drug of abuse screen can be performed on a single urine sample
3.20.3 Catecholamines and Metabolites Reference Ranges:

3.20.3.1 Adult reference ranges:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noradrenaline</td>
<td>&lt; 0.900 umol/24hrs</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>&lt; 0.230 umol/24hrs</td>
</tr>
<tr>
<td>Dopamine</td>
<td>&lt; 3.300 umol/24hrs</td>
</tr>
<tr>
<td>Metanephrine</td>
<td>&lt; 1.80 umol/24 hrs</td>
</tr>
<tr>
<td>Normetanephrine</td>
<td>&lt; 2.80 umol/24 hrs</td>
</tr>
</tbody>
</table>

3.20.3.2 Paediatric Reference Ranges:
Units are mmol/mol Urinary Creatinine.

<table>
<thead>
<tr>
<th>Age Group (yrs)</th>
<th>Noradrenaline</th>
<th>Adrenaline</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>&lt; 0.43</td>
<td>&lt; 0.08</td>
<td>&lt; 1.95</td>
</tr>
<tr>
<td>1 – 3</td>
<td>&lt; 0.20</td>
<td>&lt; 0.08</td>
<td>&lt; 1.45</td>
</tr>
<tr>
<td>3 – 5</td>
<td>&lt; 0.19</td>
<td>&lt; 0.08</td>
<td>&lt; 0.95</td>
</tr>
<tr>
<td>5 – 8</td>
<td>&lt;0.18</td>
<td>&lt; 0.08</td>
<td>&lt; 0.85</td>
</tr>
<tr>
<td>8 – 11</td>
<td>&lt;0.17</td>
<td>&lt; 0.08</td>
<td>&lt; 0.75</td>
</tr>
<tr>
<td>&gt; 11</td>
<td>&lt;0.13</td>
<td>&lt; 0.08</td>
<td>&lt; 0.65</td>
</tr>
</tbody>
</table>

3.20.4 Endocrinology Reference Ranges

Where age and cycle reference ranges apply to females, 50 years has been agreed by the Endocrinologists as the age to apply a post-menopausal range.
3.21 IMMUNOLOGY

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.

3.21.1 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/Dr Khalib. 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.

3.21.2 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 below. Some immunology tests are carried out in the Protein chemistry and Haematology laboratories.

3.21.3 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 on-call, Dr. Keogan/Dr Khalib 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/Dr Khalib by a senior member of the clinical team who is familiar with the patient’s history.
3.21.4 Repertoire of Tests & Test Profiles

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.

<table>
<thead>
<tr>
<th>Test</th>
<th>Specimen</th>
<th>Minimum Volume</th>
<th>Method</th>
<th>Reference Range</th>
<th>TAT</th>
<th>Urgent Service</th>
<th>Comment</th>
<th>Frequency of Retesting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Adrenal Antibodies</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Indirect immunofluorescence</td>
<td>Negative</td>
<td>4 weeks</td>
<td></td>
<td></td>
<td>6 months</td>
</tr>
<tr>
<td>Anti-Beta2Glycoprotein 1</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>EliA (IMMUNOCAP)</td>
<td>&lt;7 U/ml</td>
<td>8 days</td>
<td>12 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Cardiolipin Antibodies (IgG and IgM)</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>EliA (IMMUNOCAP)</td>
<td>IgG: 0-10 GPLU/mL</td>
<td>8 days</td>
<td>12 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-CCP</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>EliA (IMMUNOCAP)</td>
<td>&lt; 3 U/ml</td>
<td>8 days</td>
<td></td>
<td></td>
<td>3 Months</td>
</tr>
<tr>
<td>Anti-Double-Stranded-DNA Antibodies</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>EliA (IMMUNOCAP) &amp; IIF by DNA crithidia</td>
<td>EliA:&lt;10 IU/mL IIF: Negative</td>
<td>On Request</td>
<td>&gt;3 weeks (unless plasma-apheresis/discussion)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-ENA (Extractable Nuclear Antigen) Antibodies – includes anti-Ro, La, RNP, Sm, Jo-1 &amp; Scl-70)</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>EliA with confirmation by EliA &amp; Immunoblot</td>
<td>Negative for all components</td>
<td>6-2-3 weeks</td>
<td>ENA Typing only performed on Equivocal and Positive ENA Screens</td>
<td></td>
<td>&gt;1 year</td>
</tr>
<tr>
<td>Anti-Endomysial (IgA) Antibodies</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Indirect Immunofluorescence</td>
<td>Negative</td>
<td>8 days</td>
<td></td>
<td></td>
<td>&gt;3 months</td>
</tr>
<tr>
<td>Anti-Endomysial (IgG) Antibodies</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Indirect Immunofluorescence</td>
<td>Negative</td>
<td>8 days</td>
<td></td>
<td>Only performed when IgA deficiency</td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>Specimen</td>
<td>Minimum Volume</td>
<td>Method</td>
<td>Reference Range</td>
<td>TAT</td>
<td>Urgent Service</td>
<td>Comment</td>
<td>Frequency of Retesting</td>
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</tr>
<tr>
<td>Anti-Gastric-Parietal Cell antibodies (Anti-GPC)</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Indirect Immunofluorescence</td>
<td>Negative</td>
<td>3-5 days</td>
<td></td>
<td></td>
<td>&gt;3 months</td>
</tr>
<tr>
<td>Anti-Glomerular Basement Membrane antibodies (Anti-GBM)</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>EliA (IMMUNOCAP)</td>
<td>Negative: &lt;7U/ml</td>
<td>1-3 days</td>
<td>On Request</td>
<td>As requested &amp; discussed</td>
<td></td>
</tr>
<tr>
<td>Anti-Histone Antibodies</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>ELISA</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td>Once Off</td>
</tr>
<tr>
<td>Anti-Intrinsic Factor Antibodies</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Indirect Immunofluorescence</td>
<td>Negative: &lt;6 U/ml Positive: &gt;/= 6 U/ml</td>
<td>8 days</td>
<td></td>
<td></td>
<td>&gt;6 months</td>
</tr>
<tr>
<td>Anti-Liver-Kidney Microsomal (LKM) Antibodies</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Indirect Immunofluorescence</td>
<td>Negative</td>
<td>3-5 days</td>
<td></td>
<td></td>
<td>&gt;1 month</td>
</tr>
<tr>
<td>Anti-Mitochondrial Antibody (including M2 subtyping)</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Indirect Immunofluorescence</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td>&gt;3 months</td>
</tr>
<tr>
<td>Anti-Myeloperoxidase antibodies (Anti-MPO)</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>EliA (IMMUNOCAP)</td>
<td>&lt;3.5IU/mL</td>
<td>2-4 days, or as required</td>
<td>On request</td>
<td>Follow-up of patients with known MPO-ANCA positive disease</td>
<td>3 Weeks, unless discussed</td>
</tr>
<tr>
<td>Anti-Neuronal Antibodies – Anti-Hu &amp; anti-Yo</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Indirect Immunofluorescence</td>
<td>Negative</td>
<td>15 days</td>
<td></td>
<td></td>
<td>&gt;6 months</td>
</tr>
<tr>
<td>Anti-Neutrophil Cytoplasm Antibodies (ANCA) (IIF)</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Indirect Immunofluorescence</td>
<td>Negative</td>
<td>3-5 days</td>
<td>On Request</td>
<td></td>
<td>3 Weeks, unless discussed</td>
</tr>
<tr>
<td>Test</td>
<td>Specimen</td>
<td>Minimum Volume</td>
<td>Method</td>
<td>Reference Range</td>
<td>TAT</td>
<td>Urgent Service</td>
<td>Comment</td>
<td>Frequency of Retesting</td>
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</tr>
<tr>
<td>Anti-Nuclear Factor</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Indirect Immunofluorescence</td>
<td>Negative. Weak positive (1:80 &amp; 1:100) are commonly seen particularly in healthy older women.</td>
<td>3-5 days</td>
<td></td>
<td>No more than 3 monthly</td>
<td></td>
</tr>
<tr>
<td>Anti-Nucleosome Antibodies</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Immunoblot</td>
<td>Normal value: Negative</td>
<td>4-6 weeks</td>
<td></td>
<td>Strong clinical suspicion of lupus with negative routine serology. Must discuss with Consultant Immunologist.</td>
<td>Once Off</td>
</tr>
<tr>
<td>Anti-PLA2R Antibodies</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>ELISA</td>
<td>Negative: &lt;14RU/mL Borderline: ≥14-&lt;20 RU/mL Weak Positive: 20 - 30 RU/mL Positive: &gt;30 RU/mL</td>
<td>3 Weeks</td>
<td></td>
<td>Discuss with clinical team</td>
<td></td>
</tr>
<tr>
<td>Anti-Pneumococcal antibodies</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>ELISA</td>
<td>Normal response to vaccination is a four fold increase in the level of titres. Units mg/L.</td>
<td>4-8 weeks</td>
<td></td>
<td>Should only be used to assess vaccine responses. Pre-vaccine, 1 month post, 3-6 months &amp; then annually for Pneumococcal. Pre &amp; Post vaccine for others.</td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>Specimen</td>
<td>Minimum Volume</td>
<td>Method</td>
<td>Reference Range</td>
<td>TAT</td>
<td>Urgent Service</td>
<td>Comment</td>
<td>Frequency of Retesting</td>
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</tr>
<tr>
<td>Anti-Proteinase 3 antibodies (Anti-PR3)</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>EliA (IMMUNOCAP)</td>
<td>&lt;2IU/mL</td>
<td>2-4 days, or as required</td>
<td>On request</td>
<td>Follow-up of patients with known PR3-ANCA positive disease.</td>
<td>3 Weeks, unless discussed</td>
</tr>
<tr>
<td>Anti-Ribosomal-P-Protein antibodies</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Immunoblot</td>
<td>Normal value: Negative</td>
<td>4-6 weeks</td>
<td>Strong clinical suspicion of lupus with negative routine serology. Must discuss with Consultant Immunologist.</td>
<td>Once Off</td>
<td></td>
</tr>
<tr>
<td>Anti-Scleroderma Antibodies</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Immunoblot</td>
<td>Negative</td>
<td>4-6 weeks</td>
<td></td>
<td></td>
<td>6 months but Positive ICS as requested</td>
</tr>
<tr>
<td>Anti-Skin Antibodies</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Indirect Immunofluorescence</td>
<td>Negative</td>
<td>8 days</td>
<td></td>
<td></td>
<td>&gt;3 months</td>
</tr>
<tr>
<td>Anti-Smooth Muscle Antibodies</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Indirect Immunofluorescence</td>
<td>Negative</td>
<td>3-5 days</td>
<td></td>
<td></td>
<td>&gt;3 months</td>
</tr>
<tr>
<td>Anti-Streptolysin-O Titre (ASOT)</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Nephelometry</td>
<td>&lt;200IU/ml</td>
<td>3-5 days</td>
<td></td>
<td></td>
<td>3 weeks</td>
</tr>
<tr>
<td>Anti-Thyroid Peroxidase Antibodies (anti-TPO)</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>EliA</td>
<td>Negative: &lt; 25 IU/ml Equivocal: 25-35 Positive: &gt; 35</td>
<td>8 days</td>
<td></td>
<td></td>
<td>&gt;6 months; if equivocal &gt;3 months</td>
</tr>
<tr>
<td>Anti-Tissue Transglutaminase Antibodies (anti-tTG)</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>EliA (IMMUNOCAP)</td>
<td>Negative: &lt; 4 U/ml Equivocal: 4-10 U/ml Positive: 10 U/ml</td>
<td>8 days</td>
<td></td>
<td></td>
<td>&gt;3 months</td>
</tr>
<tr>
<td>Test</td>
<td>Specimen</td>
<td>Minimum Volume</td>
<td>Method</td>
<td>Reference Range</td>
<td>TAT</td>
<td>Urgent Service</td>
<td>Comment</td>
<td>Frequency of Retesting</td>
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<td>------------------------</td>
</tr>
<tr>
<td>CH100 &amp; AP100</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Gel diffusion haemolysis</td>
<td>Normal</td>
<td>2-3 months</td>
<td></td>
<td>It is essential that serum is separated and frozen within 3 hours maximum after venepuncture</td>
<td>3 weeks</td>
</tr>
<tr>
<td>C1 Esterase Inhibitor (C1INH)</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Nephelometry</td>
<td>0.21-0.39g/L</td>
<td>4-6 weeks</td>
<td></td>
<td>Once off if normal. As required if low.</td>
<td></td>
</tr>
<tr>
<td>C1 Inhibitor Function</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>ELISA</td>
<td>&gt;68%</td>
<td>By arrangement</td>
<td></td>
<td>Discuss with Consultant Immunologist.</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Nephelometry</td>
<td>0.75 – 1.65g/L</td>
<td>1-5 days</td>
<td>On request</td>
<td>As Requested</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Nephelometry</td>
<td>0.14 – 0.54g/L</td>
<td>1-5 days</td>
<td>On request</td>
<td>As Requested</td>
<td></td>
</tr>
<tr>
<td>CTD Screen</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>EliA (IMMUNOCAP)</td>
<td>Negative</td>
<td>2-5 days</td>
<td></td>
<td>No more than 3 monthly</td>
<td></td>
</tr>
<tr>
<td>Direct Immunofluorescence (DIF) on Skin Biopsies</td>
<td>Fresh skin biopsy, transported on damp gauze to the laboratory</td>
<td>6 mls</td>
<td>Direct Immunofluorescence</td>
<td>1-2 weeks (urgent services available)</td>
<td>Unless special arrangements have been agreed specimen MUST reach the immunology laboratory by 4pm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>Specimen</td>
<td>Minimum Volume</td>
<td>Method</td>
<td>Reference Range</td>
<td>TAT</td>
<td>Urgent Service</td>
<td>Comment</td>
<td>Frequency of Retesting</td>
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</tr>
<tr>
<td>IgG Subclasses</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Nephelometry</td>
<td>IgG 6.0-16.0 g/L</td>
<td>8 weeks</td>
<td></td>
<td>Note: These are adult specific reference ranges</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IgG1 3.2-10.2 g/L</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IgG2 1.2-6.6 g/L</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IgG3 0.2-1.9 g/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannose Binding Lectin (MBL)</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>ELISA</td>
<td>0.55 – 4.00 mg/L</td>
<td>2-3 months</td>
<td></td>
<td>For specific sample handling requirements see Section 2.4.34</td>
<td>Once off</td>
</tr>
<tr>
<td>Mast Cell Tryptase</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>FEIA (IMMUNOCAP)</td>
<td>2-14 μg/L (Anti-mortem specimens only)</td>
<td>1 month</td>
<td></td>
<td>As requested/disclosed</td>
<td>As requested/disclosed</td>
</tr>
<tr>
<td>Myositis Screen</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Immunoblot, correlated with ANF apperance</td>
<td>Negative</td>
<td>4-6 weeks</td>
<td></td>
<td>Once off</td>
<td></td>
</tr>
<tr>
<td>Query Test</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Consultant, SPR or Chief Medical Scientist will select appropriate tests</td>
<td>As appropriate</td>
<td>As per assay</td>
<td>Full clinical details &amp; bleep number required</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>Nephelometry</td>
<td>&lt;20 IU/mL</td>
<td>3-6 days</td>
<td></td>
<td></td>
<td>&gt;3 Months</td>
</tr>
<tr>
<td>Test</td>
<td>Specimen</td>
<td>Minimum Volume</td>
<td>Method</td>
<td>Reference Range</td>
<td>TAT</td>
<td>Urgent Service</td>
<td>Comment</td>
<td>Frequency of Retesting</td>
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</tr>
<tr>
<td>Specific IgE</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>FEIA (IMMUNOCAP)</td>
<td>&lt;0.35</td>
<td>Class 0 Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.35-0.7</td>
<td>Class 1 Weakly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.7-3.5</td>
<td>Class 2 Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.5-17.5</td>
<td>Class 3 Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17.5-52.5</td>
<td>Class 4 Strongly</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>52.5-100</td>
<td>Class 5 Strongly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;100</td>
<td>Class 6 Strongly</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15 days</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21 days for sIgE to Drugs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 year for same allergens</td>
</tr>
<tr>
<td>Specific IgGs</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>FEIA (IMMUNOCAP)</td>
<td>&lt;40 mgA/l</td>
<td>Class 0 Negative</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>sIgG Aspergillus</td>
<td></td>
<td></td>
<td></td>
<td>&lt;22 mgA/l</td>
<td>Class 1 Weakly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sIgG M. Faeni</td>
<td></td>
<td></td>
<td></td>
<td>&lt;30 mgA/l</td>
<td>Class 2 Positive</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>sIgG Budgie</td>
<td></td>
<td></td>
<td></td>
<td>&lt;38 mgA/l</td>
<td>Class 3 Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sIgG Pigeon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Class 4 Strongly</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Class 5 Strongly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IgE</td>
<td>Serum, White tube</td>
<td>6 mls</td>
<td>FEIA (IMMUNOCAP)</td>
<td>Range is age related.</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>Adult reference range 0-100 kU/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15 days</td>
<td></td>
<td></td>
<td></td>
<td>1 year</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. If you are uncertain of how best to investigate the patient, you are welcome to contact the Chief Medical Scientist, the Specialist Registrar or Dr. Keogan/Dr Khalib, 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.
### 3.21.4.1 Current available Specific IgE Allergens

<table>
<thead>
<tr>
<th>ALLERGENS AVAILABLE</th>
<th>ALLERGENS AVAILABLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACARUS SIRO (Flour mite)</td>
<td>CRAB</td>
</tr>
<tr>
<td>ALMOND</td>
<td>DOG DANDER</td>
</tr>
<tr>
<td>ALTERNARIA ALTERNATA</td>
<td>ECZEMA PANEL*</td>
</tr>
<tr>
<td>AMOxicilloyl</td>
<td>EGG</td>
</tr>
<tr>
<td>AMPICILLIN</td>
<td>EGG WHITE</td>
</tr>
<tr>
<td>ANIMAL PANEL*</td>
<td>FISH MIX</td>
</tr>
<tr>
<td>APPLE</td>
<td>FRUIT MIX*</td>
</tr>
<tr>
<td>Arah2 (Peanut component)</td>
<td>GRAPE</td>
</tr>
<tr>
<td>ASPERGILLUS</td>
<td>GRASS MIX*</td>
</tr>
<tr>
<td>ASTHMA PANEL*</td>
<td>HAKE</td>
</tr>
<tr>
<td>BANANA</td>
<td>HAZELNUT</td>
</tr>
<tr>
<td>BARLEY</td>
<td>HORSE</td>
</tr>
<tr>
<td>BEE</td>
<td>HOUSE DUST MITE</td>
</tr>
<tr>
<td>BIRCH</td>
<td>KIWI</td>
</tr>
<tr>
<td>BRAZILNUT</td>
<td>LATEX</td>
</tr>
<tr>
<td>CAGEBIRD FEATHER MIX*</td>
<td>LENTIL</td>
</tr>
<tr>
<td>CANDIDA</td>
<td>LOBSTER</td>
</tr>
<tr>
<td>CASHEW NUT</td>
<td>MACKEREL</td>
</tr>
<tr>
<td></td>
<td>MAIZE</td>
</tr>
<tr>
<td>CAT DANDER</td>
<td>MILK</td>
</tr>
<tr>
<td>CEFAclor</td>
<td>MORPHINE</td>
</tr>
<tr>
<td>CEREAL MIX*</td>
<td>MUSHROOM</td>
</tr>
<tr>
<td>CHEESE</td>
<td>MUSSEL</td>
</tr>
<tr>
<td>CHICKEN</td>
<td>NUT MIX*</td>
</tr>
<tr>
<td>CHICKPEA</td>
<td>OATS</td>
</tr>
<tr>
<td></td>
<td>OMEGA-5 GLIADIN</td>
</tr>
<tr>
<td>CHLORHEXIDINE</td>
<td>ORANGE</td>
</tr>
<tr>
<td>CITRUS FRUIT MIX*</td>
<td>OYSTER</td>
</tr>
<tr>
<td>CLADOSPORIUM HERBARUM</td>
<td>PEA</td>
</tr>
<tr>
<td>COCONUT</td>
<td>PEACH</td>
</tr>
<tr>
<td>COD</td>
<td>PEANUT</td>
</tr>
<tr>
<td>Cor a9 (Hazelnut component)</td>
<td>PECAN</td>
</tr>
<tr>
<td>Cor a14 (Hazelnut component)</td>
<td>PENICILLIN PANEL*</td>
</tr>
</tbody>
</table>

*Details of Panel & Mix contents are given overleaf*
<table>
<thead>
<tr>
<th>ALLERGENS</th>
<th>Panel &amp; Mix Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANIMAL PANEL</td>
<td>Cat, Dog</td>
</tr>
<tr>
<td>ASTHMA PANEL</td>
<td>HDM, Aspergillus fumigatus, Cat</td>
</tr>
<tr>
<td>CAGEBIRD FEATHER MIX</td>
<td>Budgerigar, Canary bird, Parakeet, Parrot &amp; Finch feathers</td>
</tr>
<tr>
<td>CEREAL MIX**</td>
<td>Wheat, Rye, Barley, Rice</td>
</tr>
<tr>
<td>CITRUS FRUIT MIX</td>
<td>Orange, Lemon, Grapefruit, Mandarin</td>
</tr>
<tr>
<td>ECZEMA PANEL</td>
<td>HDM, Egg, Milk, Wheat</td>
</tr>
<tr>
<td>FISH MIX**</td>
<td>Fish, Shrimp, Blue mussel, Tuna, Salmon</td>
</tr>
<tr>
<td>FRUIT MIX</td>
<td>Kiwi, Melon, Banana, Peach, Pineapple</td>
</tr>
<tr>
<td>GRASS MIX</td>
<td>Cock's-foot or orchard grass, Meadow fescue, Ryegrass, Timothy-grass, Common</td>
</tr>
<tr>
<td></td>
<td>Meadow-grass (Dactylis glomerata, Festuca elatior, Lolium perenne, Phleum</td>
</tr>
<tr>
<td></td>
<td>pratense, Poa pratensis)</td>
</tr>
<tr>
<td>NUT MIX**</td>
<td>Peanut, Hazel nut, Brazil nut, Almond, Coconut</td>
</tr>
<tr>
<td>PEANUT</td>
<td>Peanut &amp; Arah2</td>
</tr>
<tr>
<td>PENICILLIN PANEL</td>
<td>Penicillin G, Penicillin V, Amoxicillin, Ampicillin, Cefaclor</td>
</tr>
<tr>
<td>POLLEN PANEL</td>
<td>Trees, Grass</td>
</tr>
<tr>
<td>RHINITIS PANEL</td>
<td>HDM, Cat, Trees, Grass</td>
</tr>
<tr>
<td>SHELLFISH PANEL</td>
<td>Lobster, Crab, Shrimp, Mussel</td>
</tr>
<tr>
<td>SPICE MIX</td>
<td>Caraway, Mace, Cardamom, Clove; Basil, Fennel seed, Ginger, Anise</td>
</tr>
<tr>
<td>TREE MIX</td>
<td>Maple, Silver Birch, Common Hazel, white oak, plane tree (Acer negundo, Betula</td>
</tr>
<tr>
<td></td>
<td>verrucosa, Corylus avellana, Quercus alba, Platanus acerifolia)</td>
</tr>
</tbody>
</table>

**For nut mix, fish mix & cereal mix, if we get a positive result, we would automatically do the individual allergens included in the relevant mix.

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.
### 3.21.4.2 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.

<table>
<thead>
<tr>
<th>Profile</th>
<th>Tests Included</th>
<th>Indication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Renal Failure</td>
<td>CTD ANCA ANCA</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>
<tr>
<td>Screen ARF SCR</td>
<td>CTD ANCA GBM C3/C4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory Arthritis</td>
<td>RF C3/C4</td>
<td>Isolated inflammatory arthritis, in the absence of systemic features.</td>
<td>ANCA should be added if urinalysis is abnormal.</td>
</tr>
<tr>
<td>Antibodies INFL ABS</td>
<td>ANF ANF ANF ANF</td>
<td>Suspected chronic liver disease.</td>
<td>If MITO pos, M2 subtyping will be performed, on the first occasion only.</td>
</tr>
<tr>
<td>Liver Autoantibodies</td>
<td>Anti-Smooth Muscle Anti-Mitochondrial Anti-LKM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIV ABS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vasculitis Screen VAS SCR</td>
<td>CTD ANCA RF C3/C4</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>
<tr>
<td>Asthma sIgE RASTA</td>
<td>sIgE House dust mite sIgE Aspergillus sIgE Cat</td>
<td>Allergic asthma.</td>
<td></td>
</tr>
<tr>
<td>Rhinitis sIgE RASTR</td>
<td>sIgE House dust mite sIgE Cat sIgE Trees sIgE Grass</td>
<td>Perennial Rhinitis, thought to be allergic.</td>
<td></td>
</tr>
<tr>
<td>Food sIgE RASTF</td>
<td>The Food sIgE panel has been discontinued due to low yield. However all individual sIgEs for milk, wheat, mixed nuts, sesame, soya, mixed fish and egg, are still available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eczema RSTECZE</td>
<td>sIgE House Dust Mite sIgE Milk sIgE Egg sIgE Wheat</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>Shellfish RSTSHELL</td>
<td>sIgE Lobster sIgE Crab sIgE Shrimp sIgE Mussel</td>
<td>Suspected allergy to shellfish. Negative result does not rule out shellfish allergy. If this is suspected clinically referral to a Clinical Immunologist is advised.</td>
<td></td>
</tr>
</tbody>
</table>
### Profile Tests Included

<table>
<thead>
<tr>
<th>Profile</th>
<th>Tests Included</th>
<th>Indication</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollen</td>
<td>RSTPOLL IgE – Mixed grass IgE – Mixed trees</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coeliac Screen</td>
<td>COEL SCR Anti-tTG Anti-EmA (if tTG is Equivocal or Positive)</td>
<td>Suspected Coeliac Disease. Malabsorption. Anaemia. Gastrointestinal symptoms.</td>
<td>Anti-AGA has been discontinued.</td>
</tr>
</tbody>
</table>

### 3.21.4.3 Immunological Tests performed in other Laboratories in Beaumont Hospital

<table>
<thead>
<tr>
<th>Test</th>
<th>Specimen</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoglobulins</td>
<td>Serum Gel</td>
<td>Proteins (809) 2305</td>
</tr>
<tr>
<td>C Reactive Protein</td>
<td>Heparin</td>
<td>Clin Chem (809) 2668</td>
</tr>
<tr>
<td>Protein electrophoresis</td>
<td>Serum Gel</td>
<td>Proteins (809) 2305</td>
</tr>
<tr>
<td>Urine electrophoresis (Bence Jones Protein)</td>
<td>24 hour urine collection</td>
<td>Proteins (809) 2305</td>
</tr>
<tr>
<td>β2 Microglobulin</td>
<td>Serum Gel</td>
<td>Proteins (809) 2305</td>
</tr>
<tr>
<td>Cryoglobulins</td>
<td>Contact for details</td>
<td>Proteins (809) 2305</td>
</tr>
<tr>
<td></td>
<td>Contact laboratory</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte subsets</td>
<td>EDTA</td>
<td>Haematology (809) 2763</td>
</tr>
</tbody>
</table>

NOTE: Instruct patient to fast for 8 hrs prior to phlebotomy
### 3.22 Microbiology

#### 3.22.1 Repertoire of Test Services

<table>
<thead>
<tr>
<th>Test</th>
<th>Target Pathogens</th>
<th>Specimen</th>
<th>Minimum Volume</th>
<th>Reference Range</th>
<th>TAT</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscopy</td>
<td>N/A</td>
<td>Approved screw-capped (Sarstedt) container</td>
<td>10 mls</td>
<td>N/A</td>
<td>Within 24 hours of receipt</td>
<td></td>
</tr>
<tr>
<td>Culture</td>
<td>Urinary pathogens</td>
<td>Approved screw-capped (Sarstedt) container</td>
<td>10 mls</td>
<td>N/A</td>
<td>6 days</td>
<td></td>
</tr>
<tr>
<td>Pregnancy test</td>
<td>N/A</td>
<td>Approved screw-capped (Sarstedt) container</td>
<td>10 mls</td>
<td>N/A</td>
<td>Same day</td>
<td></td>
</tr>
<tr>
<td>Legionella antigen</td>
<td>Legionella antigen</td>
<td>Approved screw-capped (Sarstedt) container</td>
<td>10 mls</td>
<td>N/A</td>
<td>Same day</td>
<td></td>
</tr>
<tr>
<td>S. pneumoniae or Streptococcus pneumoniae or pneumococcal antigen</td>
<td>Approved screw-capped (Sarstedt) container</td>
<td>10 mls</td>
<td>N/A</td>
<td>Same day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB culture</td>
<td>Mycobacterium spp</td>
<td>Approved screw-capped (Sarstedt) container</td>
<td>10 mls</td>
<td>N/A</td>
<td>8 weeks</td>
<td>3 consecutive EMUs needed – For diagnosis of disseminated or urinary tract mycobacterial infection only</td>
</tr>
<tr>
<td><strong>Faeces</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteric pathogens</td>
<td>Enteric pathogens</td>
<td>Approved screw-capped (Sarstedt) container</td>
<td>1-2g</td>
<td>N/A</td>
<td>5 days</td>
<td>Perfomed only on specimens which take the shape of the</td>
</tr>
<tr>
<td>Test</td>
<td>Target Pathogens</td>
<td>Specimen</td>
<td>Minimum Volume</td>
<td>Reference Range</td>
<td>TAT</td>
<td>Comment</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------------------------------</td>
<td>--------------------------------------------------</td>
<td>----------------</td>
<td>-----------------</td>
<td>------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>C. difficile</td>
<td>C. difficile</td>
<td>Approved yellow screw-capped (Sarstedt) container</td>
<td>1-2mls</td>
<td>N/A</td>
<td>2 days</td>
<td>Performed only on specimens which take the shape of the container and &gt;2 years of age.</td>
</tr>
<tr>
<td>Rota/adeno virus</td>
<td>Rota/adeno virus</td>
<td>Approved yellow screw-capped (Sarstedt) container</td>
<td>1-2g</td>
<td>N/A</td>
<td>4 days</td>
<td>Performed routinely on children &lt;2 years</td>
</tr>
<tr>
<td>Ova/parasites</td>
<td>Ova &amp; Parasites</td>
<td>Approved yellow screw-capped (Sarstedt) container</td>
<td>1-2g</td>
<td>N/A</td>
<td>5 days</td>
<td>Clinical/travel details essential</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em> antigen</td>
<td><em>Helicobacter pylori</em></td>
<td>Approved yellow screw-capped (Sarstedt) container</td>
<td>1-2g</td>
<td>N/A</td>
<td>4 days</td>
<td></td>
</tr>
<tr>
<td>Sputum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Routine culture</td>
<td>Respiratory pathogens</td>
<td>Approved yellow screw-capped (Sarstedt) container</td>
<td>As available</td>
<td>N/A</td>
<td>6 days</td>
<td>Salivary samples are unsuitable</td>
</tr>
<tr>
<td>TB</td>
<td><em>Mycobacterium</em> spp.</td>
<td>Approved yellow screw-capped (Sarstedt) container</td>
<td>As available</td>
<td>N/A</td>
<td>65 daysweeks</td>
<td>3 consecutive morning samples</td>
</tr>
<tr>
<td>Skin Scrapplings/Nail Clippings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscopy</td>
<td>Fungal Elements</td>
<td>Screw capped container (as above) or possible Dermapak</td>
<td>As much as possible</td>
<td>N/A</td>
<td>7 days</td>
<td>Swabs are not an appropriate specimen for fungal culture. Hair must contain root</td>
</tr>
<tr>
<td>Culture</td>
<td>Dermatophytes, moulds &amp; Yeasts</td>
<td>Screw capped container (as above) or possible Dermapak</td>
<td>As much as possible</td>
<td>N/A</td>
<td>28 days</td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>Target Pathogens</td>
<td>Specimen</td>
<td>Minimum Volume</td>
<td>Reference Range</td>
<td>TAT</td>
<td>Comment</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------------------------------</td>
<td>---------------------------</td>
<td>----------------</td>
<td>-----------------</td>
<td>-----------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>SWABS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRSA Screen</td>
<td>MRSA</td>
<td>Charcoal Transswab</td>
<td>N/A</td>
<td>N/A</td>
<td>4 days</td>
<td>MRSA- Only from Nasal, Groin &amp; Wound sites</td>
</tr>
<tr>
<td>Non uro-genital (e.g. wound, eye, ear, nasal, throat)</td>
<td>Pathogens appropriate to site</td>
<td>Charcoal Transswab</td>
<td>N/A</td>
<td>N/A</td>
<td>6 days</td>
<td>Relevant clinical details essential, e.g. surgery, post-partum</td>
</tr>
<tr>
<td>Penile/vulval</td>
<td>Non-STI pathogens</td>
<td>Charcoal Transswab</td>
<td>N/A</td>
<td>N/A</td>
<td>4 days</td>
<td></td>
</tr>
<tr>
<td>HVS –microscopy</td>
<td>Bacterial vaginosis</td>
<td>Charcoal Transswab</td>
<td>N/A</td>
<td>N/A</td>
<td>6 days</td>
<td>Within 24 hours of receipt</td>
</tr>
<tr>
<td>HVS –Culture</td>
<td>Bacterial vaginosis</td>
<td>Charcoal Transswab</td>
<td>N/A</td>
<td>N/A</td>
<td>48-72hrs</td>
<td></td>
</tr>
</tbody>
</table>

1. 25mls = half-filled MSU jar. It is important not to overfill the container.
2. Ova & parasites only performed when specifically requested and with relevant clinical details eg. Foreign travel.
3. Microscopy is prioritised over culture if insufficient sample is received.

**Suspected STI Specimen Requirements**

<table>
<thead>
<tr>
<th>MALE</th>
<th>Specimen</th>
<th>Test</th>
<th>Minimum Volume</th>
<th>Reference Range</th>
<th>TAT</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swab - urethral</td>
<td>Charcoal Transswab or GenProbe Collection Device</td>
<td>N. gonorrhoeae</td>
<td>25 mls</td>
<td>N/A</td>
<td>4 days</td>
<td>Clinical details essential, e.g. urethral discharge/ ?STI</td>
</tr>
<tr>
<td>- rectal</td>
<td>Charcoal Transswab or GenProbe Collection Device</td>
<td>N. gonorrhoeae</td>
<td>N/A</td>
<td>N/A</td>
<td>4 days</td>
<td>Clinical details essential</td>
</tr>
<tr>
<td>- pharyngeal</td>
<td>Charcoal Transswab or GenProbe Collection Device</td>
<td>N. gonorrhoeae</td>
<td>N/A</td>
<td>N/A</td>
<td>4 days</td>
<td>Clinical details essential</td>
</tr>
</tbody>
</table>

**FEMALE**

<table>
<thead>
<tr>
<th>Swab</th>
<th>Specimen</th>
<th>Test</th>
<th>Minimum Volume</th>
<th>Reference Range</th>
<th>TAT</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>endocervical</td>
<td>Charcoal Transswab or GenProbe Collection Device</td>
<td>N. gonorrhoeae</td>
<td>N/A</td>
<td>N/A</td>
<td>4 days</td>
<td>Clinical details essential</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>GenProbe Collection Device</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>5 days</td>
<td>Clinical details essential</td>
</tr>
<tr>
<td>Test</td>
<td>Target Pathogens</td>
<td>Specimen</td>
<td>Minimum Volume</td>
<td>Reference Range</td>
<td>TAT</td>
<td>Comment</td>
</tr>
<tr>
<td>------</td>
<td>------------------</td>
<td>----------</td>
<td>----------------</td>
<td>----------------</td>
<td>-----</td>
<td>---------</td>
</tr>
<tr>
<td>- cervical</td>
<td><em>N. gonorrhoeae</em></td>
<td>Charcoal Transwab or GenProbe Collection Device</td>
<td>N/A</td>
<td>N/A</td>
<td>4 days</td>
<td>Clinical details essential</td>
</tr>
<tr>
<td>- urethral</td>
<td><em>N. gonorrhoeae</em></td>
<td>Charcoal Transwab or GenProbe Collection Device</td>
<td>N/A</td>
<td>N/A</td>
<td>4 days</td>
<td>Clinical details essential</td>
</tr>
<tr>
<td>- HVS</td>
<td>Bacterial vaginosis, Candida and non-STI pathogens</td>
<td>Charcoal Transwab</td>
<td>N/A</td>
<td>N/A</td>
<td>4 days</td>
<td>Clinical details essential</td>
</tr>
</tbody>
</table>

1. Specimens received in Aptima Collection Devices will be sent to the NVRL for processing. The GenProbe (Aptima) collection devices (urine containers and swabs) are supplied by the NVRL.
2. In cases where *Neisseria gonorrhoeae* (GC) is suspected, clinical details of ?STI or ‘DISCHARGE’ must be provided on the request form. If not, samples will not be cultured for GC.

### 3.22.2 General Notes

- Beaumont Hospital Microbiology Laboratory does not provide a referral service for tests carried out in other centres.
- If the test you require is not on our User Guide list, we may be able to provide you with information as to possible referral centres.
- If we receive a request for a test not carried out in Beaumont, the specimen will be rejected with the ‘test not performed in this laboratory’ comment.

### 3.22.3 Key Factors Affecting Turn Around Times:

The main reason for extended turn-around-times in Microbiology is in follow up of positive specimens. Microbial isolation and identification can be extensive in some instances, occasionally requiring referral of an isolate to a Reference Laboratory for typing or confirmation.
3.22.4 Samples sent to External Laboratories e.g., NVRL for analysis

<table>
<thead>
<tr>
<th>Test</th>
<th>Sample Required</th>
<th>Turnaround Time (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucella</td>
<td>S/CB</td>
<td>5</td>
</tr>
<tr>
<td><em>Chlamydia trachomatis</em></td>
<td>US/CS/U/ES</td>
<td>5</td>
</tr>
<tr>
<td>Cytomegalovirus (CMV)</td>
<td>S/CB/U</td>
<td>3-21</td>
</tr>
<tr>
<td>Enteroviruses</td>
<td>ST/TS</td>
<td>14</td>
</tr>
<tr>
<td>Epstein Barr Virus (EBV)</td>
<td>S/CB</td>
<td>3</td>
</tr>
<tr>
<td>Hepatitis A Virus</td>
<td>S/CB</td>
<td>3</td>
</tr>
<tr>
<td>Hepatitis B Virus</td>
<td>S/CB</td>
<td>3</td>
</tr>
<tr>
<td>Hepatitis C Virus</td>
<td>S/CB</td>
<td>3-5</td>
</tr>
<tr>
<td>Herpes simplex Virus (HSV)</td>
<td>S/CB</td>
<td>5</td>
</tr>
<tr>
<td>Human Immunodeficiency Virus 1,2</td>
<td>S/CB</td>
<td>3</td>
</tr>
<tr>
<td>(HIV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza</td>
<td>TS/NPA</td>
<td></td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>S/CB</td>
<td>5</td>
</tr>
<tr>
<td>Measles Virus</td>
<td>S/CB</td>
<td>5</td>
</tr>
<tr>
<td>Mumps Virus</td>
<td>S/CB</td>
<td>5</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td>S/CB</td>
<td>5</td>
</tr>
<tr>
<td>Norovirus</td>
<td>ST</td>
<td>3</td>
</tr>
<tr>
<td>Parainfluenza Virus</td>
<td>NPA/MW</td>
<td>2-14</td>
</tr>
<tr>
<td>Parvovirus B19</td>
<td>S/CB</td>
<td>5</td>
</tr>
<tr>
<td>Q Fever (Coxiella burnetti)</td>
<td>S/CB</td>
<td>5</td>
</tr>
<tr>
<td>Respiratory Syncytial Virus</td>
<td>NPA/SP</td>
<td>2-14</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>TS</td>
<td>14</td>
</tr>
<tr>
<td>Rubella</td>
<td>S/CB</td>
<td>3</td>
</tr>
<tr>
<td>Syphilis</td>
<td>S/CB</td>
<td>3</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>S/CB</td>
<td>3</td>
</tr>
<tr>
<td>Varicella Zoster Virus (VZV)</td>
<td>S/CB</td>
<td>2</td>
</tr>
</tbody>
</table>

S= Serum  U= Urine  CB=Clotted Blood
CS= Cervical Swab  TS= Throat Swab  US= Urethral Swab
NPA= Nasopharyngeal Aspirate  ES= Eye Swab
SP= Sputum  MW= Mouth washings  ST= Stool

3.22.4.1 Notes on Samples Sent to the NVRL

- Turnaround times given are reliant on samples going directly to NVRL from the GP surgery.
- For tests where clotted blood is required, one 10ml vial is the specimen of choice.
- No records of results of NVRL samples are kept in the Microbiology Department. For results or enquiries, the NVRL can be contacted on 01 716 1354. Address: UCD National Virus Reference Laboratory, University College Dublin, Belfield, Dublin 4.
• Request forms can be printed on-line via the NVRL web site (www.nvrl.ie) – at ‘How do you send samples?’ prompt

### 3.22.5 Abbreviations Used on Microbiology Reports

- CRE: Carbapenem resistant *Enterobacteriaceae*
- ESBL: Extended spectrum Beta-lactamase producing *Enterobacteriaceae*
- MRSA: Meticillin resistant *Staphylococcus aureus*
- VRE: Vancomycin resistant enterococci

### 3.22.6 Time Limits for Requesting Additional Tests:

The normal limit of acceptability for culture for most microbiology specimens is 48hrs, once the specimen is in an appropriate transport medium.

Fungal culture specimens (nail clippings and skin scrapings) have a longer ‘shelf life’, though any delay increases the risk of contamination of the primary specimen, thus possibly compromising the ability to culture a pathogen
3.23 HISTOPATHOLOGY/CYTOPATHOLOGY/NEUROPATHOLOGY

The Histopathology Department provides an extensive Histopathology service, including supporting the symptomatic breast service, urology and gastrointestinal units. The department provides a diagnostic Renal Pathology service in addition to supporting the renal transplant service, including an Out of Hours service. Electron Microscopy, Cytopathology and an Autopsy service are also provided by the Histopathology laboratory. The Non-Gynae Cytopathology service includes provision of assistance and support for the Fine Needle Aspirate and endoscopic ultra sound services.

The Neuropathology section provides a diagnostic service for Neurosurgery and Neurology (including paediatric neurology and paediatric neurosurgery). A rapid intra-operative service is provided for the diagnosis of intracranial and spinal lesions including brain tumour. A range of investigations are available for the interpretation of muscle and nerve biopsies including molecular screening of common mitochondrial disorders. In addition Neuropathology is the national centre for the CJD Surveillance Unit. A Neuropathology autopsy service is also available and provides pathologic diagnosis in a variety of conditions including dementia and other neurodegenerative disorders. A CSF cytology service is also provided.

Other diagnostic services are provided on a consultative basis and include CSF analysis for 14.3.3 protein and mitochondrial genetic studies.

3.23.1 Frozen Sections

A frozen section service is offered between 09.00 – 17.00. Twenty Four hours notice should be given to the laboratory, prior to a frozen section. Frozen sections outside usual working hours may be provided by prior arrangement with the Consultant Pathologist.

Specimens from patients with TB, HIV or Hepatitis B or C infection should not be sent for frozen section. If such a suspicion is present, the medical staff concerned must inform laboratory personnel in order to safeguard the laboratory staff from risk of infection.

In addition, if the laboratory inadvertently processes such specimens, a decontamination procedure of the equipment required for frozen sections must be carried out. Decontamination of this equipment takes 12 hours. During this time no further frozen sections can be performed.

Frozen section reports are delivered to theatre, usually via the intercom. A written report is available following subsequent routine processing of the specimen.
3.23.2 Other Urgent Specimens

Other urgent specimens are dealt with on an individual basis. The laboratory should be contacted directly with these requests in order to ensure that they are handled appropriately.

3.23.3 Reports

Printed reports are sent to the Clinical Consultant, source (wards / OPD) or requesting GP. Reports are available by phoning the Histopathology Office at 2632/2636 or the Neuropathology Office at 2631 or the Renal Pathology Office at 2008. Reports are not available in the laboratory. Unauthorized reports and any issues of clinical concern can be discussed with the registrar or consultant involved in the case.

3.23.4 Specimen Requirements For Histopathology

The following is a guideline on the requirements of the various specimen types and the appropriate manner in which they should be delivered to the laboratory. This ensures the integrity of the specimen for laboratory investigations.

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Fixative Required</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen for Frozen Section.</td>
<td>Send fresh to the laboratory - immediately.</td>
<td>24 hours notice of Frozen sections should be given where possible. Contact the Histopathology Lab Ext 2353. Details supplied with the specimen must include a bleep number or theatre intercom to deliver report to.</td>
</tr>
<tr>
<td>Renal biopsies</td>
<td>Send in saline (Dublin Hospitals)</td>
<td>Please inform Renal/EM Office Ext. 2008 of specimen. The Main Lab can be contacted @ 2353.</td>
</tr>
<tr>
<td></td>
<td>Send in Formalin/Zeus (Regional Centres)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(full details in section 3.23.9)</td>
<td></td>
</tr>
<tr>
<td>Lymph nodes (for lymphoma diagnostics)</td>
<td>Send fresh to the laboratory - immediately.</td>
<td>Please supply all relevant clinical details.</td>
</tr>
<tr>
<td>Solid Tumours (Colon, Breast, Lung etc.)</td>
<td>Send fresh to the laboratory - immediately.</td>
<td>Please supply all relevant clinical details.</td>
</tr>
<tr>
<td>Liver biopsies</td>
<td>Where possible, send two specimens – one in 10% Neutral Buffered Formalin and one wrapped in saline moistened gauze.</td>
<td>Please supply relevant clinical details.</td>
</tr>
</tbody>
</table>
### Tissue Type | Fixative Required | Comment
---|---|---
All other tissue | Send in 10% Neutral Buffered Formalin. | An adequate volume of formalin in a specimen container of suitable size is essential for proper fixation. The volume of formalin used should be at least twice the volume of the tissue to be fixed. Small specimens should be placed in biohazard bags.

### 3.23.5 Requirement for External Centres

The responsibility for sending slides/blocks/material lies with the external centre (Sender). External centres may be send slides/blocks/material to Pathology for review/conferences etc. Ensure that packaging and transportation comply with current UN legislation.

Address the package to:
- Neuropathology,
- Beaumont Hospital,
- Dublin 9
- Include the Consignee address and telephone number.

### 3.23.6 Factors Affecting Fresh/Unfixed Tissue Specimens

The techniques that are performed on fresh tissue are affected by the length of time that the tissue is removed from the patient before it is received for analysis. Therefore it is imperative that all tissue samples required to be sent fresh should be done so immediately. Fresh samples should be sent during normal working hours and the department must be informed in advance if a fresh sample is to arrive out of hours.

NOTE: Specimens from patients with TB, HIV or Hepatitis B or C infection should not be sent “fresh”. If such a suspicion is present, the medical staff concerned must inform laboratory personnel in order to safeguard the laboratory staff from risk of infection.

The following may be obtained from the Histopathology laboratory.
- Specimen containers – various sizes.
- 10% Neutral Buffered Formalin (in polycubes with taps/5lt containers).
- Pre-filled 60ml 10% Neutral Buffered Formalin containers.
- Histopathology/ Cytopathology/ Neuropathology / Renal Request Cards
- Slides and slide containers with fixative for Fine Needle Aspirates (FNAs).
- EM fixative.
- Liquid nitrogen for the Dermatology clinics.
SAFETY: Formalin is a potent eye and nasal irritant and can cause respiratory distress and allergic dermatitis. Gloves, goggles and aprons should be used when dealing with formalin. Contact the Histopathology Laboratory for any additional information that may be required and if a formalin spillage should occur.

Liquid nitrogen can cause cold burns and is dangerous to use in confined spaces as it is an asphyxiant. It can also shatter receptacles that are unsuitable for its storage. Subsequently it will only be given to Beaumont Hospital personnel and transferred into a suitable receptacle. Information on safety on any of the above may be obtained from Histopathology on request @ ext. 2353

3.23.7 Turn Around Time for Results

The turn around time of specimens for Histopathology will vary depending on the nature of the specimen and the complexity of the investigations required. The following is an outline of estimated turn around time for different specimen types from time of receipt in the laboratory:

- Biopsies – 4-6 working days (on average)
- Resections – 5-10 working days (on average)
- Renal Biopsies – 4-6 weeks for Electron Microscopy
  – 2-3 weeks for Light Microscopy
  – 6-8 days for Immunofluorescence
- Post Mortem Cases – 3-4 months

This is only a guideline and the complexity of a case and the requirement for further investigations may lengthen the turn around time. Results can be obtained from the Histopathology office, ext. 2636/2632/3150/3919. The Consultant/NCHDs can be contacted to discuss individual patients.
### 3.23.8 Cytopathology Specimen Requirements

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Specimen requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchial brushings</td>
<td>Place material in a sterile container labelled with patient and specimen details, including the time of specimen collection.</td>
</tr>
<tr>
<td>Sputum</td>
<td>Take a deeply coughed early morning specimen into a sterile container labelled with patient and specimen details.</td>
</tr>
<tr>
<td>Fluids (Pleural, Ascitic etc.)</td>
<td>Place material in a sterile container labelled with patient and specimen details, including the time of specimen collection. At least 20 mls of fluid is required for diagnosis.</td>
</tr>
<tr>
<td>Urine</td>
<td>Total voided specimen is required for cytology. The first morning specimen is not suitable. Place in a container labelled with patient and specimen details.</td>
</tr>
<tr>
<td>Fine Needle-Apical Cytology</td>
<td>Laboratory personnel are available to help in the preparation of FNA samples. Contact the laboratory to arrange this service (Ext. 2640/2353). Smears received from clinics made from FNA material must be labelled clearly with patient name and at least one other form of ID (MRN / DOB) with accompanying request form. Pencil must always be used when labelling slides. Pathologists will perform FNA’s on request. Contact Histopathology office at ext. 2636/2632/3150/3919 Please book in advance</td>
</tr>
<tr>
<td>Cerebrospinal Fluid for Cytology</td>
<td>Specimen must be collected in a sterile container labelled with patient and specimen details and delivered to the Neuropathology laboratory.</td>
</tr>
<tr>
<td>Flow Cytometry</td>
<td>Cytometry placed directly into RPMI are viable for up to 18Hrs. (Contact Cytology on Ext. 2640)</td>
</tr>
</tbody>
</table>

**Items that can be obtained from the Cytopathology laboratory**

- Slides
- Slide holders
- Spray fixative
- Coplin jars of alcohol (Fixing FNA smears)
- Cervical cytology request forms
- ThinPrep kits for cervical smears (Hospital Clinics only)
- Biohazard bags
**TURN AROUND TIMES FOR CYTOLOGY SAMPLES**

Non-Gynae Cytology Samples – 3-4 Days

**3.23.9 Specimen Requirements for Renal Pathology**

The Laboratory should be notified in advance when a renal biopsy is to be taken.

Contact the Renal Pathology Secretary or if she is not available the Medical Scientists in the Renal Pathology/EM/ Histopatholgy Laboratories:

**DETAILS REQUIRED FOR RENAL BIOPSIES**

The following *minimum* information must be supplied LEGIBLY:

On the body of the specimen container:

A Renal Biopsy Request Form must be filled in *(use a ballpoint pen please to make details legible on all copies of the form)* and sent with each biopsy:

- Name of patient
- Date of birth
- Medical record number
- Address of patient
- Name of Consultant
- Source (Ward Name/OPD/Hospital)
- Date sample taken
- Relevant clinical details
- Please give *as much clinical information on the form* as possible, as this will be required by the Renal Pathologist when considering differential diagnoses.
- If using addressograph labels please attach one to both flimsies and to the backing card – these copies are sent with each portion of the biopsy to the three laboratories involved in the investigation.
- *Do not* attach labels, use date stamps or write in the portion marked for “Laboratory use” as this area is used by Beaumont Scientific staff for recording the gross description of the biopsy. If your despatch procedures require that stamps or bar codes be attached please use the reverse (blank side) of the form’s card copy.
3.23.10 Renal Pathology Requirements for External Centres

- The Renal Pathology Department should be notified in advance
- The responsibility for sending specimens rests with the external centre.
- The **minimum details** required are as set out above, including the use of the Renal Biopsy request form. Supplies of the Request Form can be obtained by contacting the Renal laboratory on 01-8092634.
- Packaging and transportation should comply with current UN legislation and the Transport of Dangerous Goods Act.
- The specimen should be dispatched so as to arrive at Beaumont Hospital no later than 16.30.

Packages should be addressed to:
  
  Dr. Tony Dorman,  
  Renal Pathology,  
  Histopathology Department,  
  Beaumont Hospital,  
  Dublin 9

NB Beaumont Hospital does not supply containers or fixative solutions for renal biopsies to external centres.

**FOR REFERRING HOSPITALS IN THE DUBLIN AREA**, if the sample can be transported to Beaumont Hospital within a couple of hours of excision, then place all of the tissue in normal saline in a 60 ml specimen jar or a universal container at least half full of liquid.

**FOR REFERRALS FROM REGIONAL CENTRES**, tissue can be examined and divided in the Histopathology Laboratory of the hospital prior to dispatch. Fresh tissue for immunofluorescence (0.3-0.4 cm of cortical tissue) should be placed in a transport medium suitable for preserving antigenic activity such as the Tissue Fixative available from Zeus Scientific Ltd. For best results, tissue should not spend any longer than 5 days in Zeus Tissue Fixative.

A small piece about 0.1-0.2 cm in length should be cut from the cortical part of a core and placed in 3% Glutaraldehyde (cacodylate buffered) if it is available from your laboratory. A piece can be taken for EM from the Formalin fixed tissue on arrival at Beaumont Hospital Histopathology Department if your laboratory does not carry a stock of glutaraldehyde.

The remainder of the tissue should be placed in Formalin.
3.23.11 Urgent Renal Biopsies for Rapid Processing

If a renal biopsy result is required urgently, i.e. the day of biopsy, then rapid processing can be requested:

- You must contact Dr. Tony Dorman to discuss the request, and when the request has been agreed, the Histopathology Laboratory should also be informed.
- The tissue must arrive in the Histopathology Laboratory by 12.30 pm at the latest. The tissue processor is then run for this single biopsy, and cannot be used until the process is completed. The surgical and biopsy specimens from that day’s cut-up must be processed daily to maintain continuity of service to all other clinical specialities, so the processor must be available for use again at 5pm.

3.23.12 Electron Microscopy

The Electron Microscopy (EM) Laboratory was initially set up to serve diagnostic Renal Pathology, but its remit has expanded to include the Neuropathology Department and a small amount of Surgical Pathology work.

The Laboratory is equipped with a JEOL 1200 EX Transmission Electron Microscope and an AMT Advantage HRL 1 megapixel Digital Camera system. Samples of solid organs, tumours and biopsies are batched and processed automatically once a week.

The EM Laboratory is not equipped or staffed to deal with Virological EM requests, and due to low frequency of request does not accept nasal brushings for analysis of immotile cilia – this is a highly specialised investigation and requires familiarity which cannot be gained in this hospital. Nasal Brushings should be sent to the EM Laboratory from where they will be referred to UCD for analysis.
### 3.23.13 Specimen Requirements for Neuropathology

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Means of Delivery to Neuropathology</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen for urgent frozen section</td>
<td>Send fresh. Hand deliver immediately.</td>
<td>The Neuropathology consultation form must include a bleep number or intercom number to deliver the report.</td>
</tr>
<tr>
<td>Muscle Biopsy</td>
<td>Send on gauze that is barely dampened in saline. Do not fix in formalin. Hand deliver immediately.</td>
<td>Must be received during normal working hours unless previously arranged.</td>
</tr>
<tr>
<td>Nerve Biopsy</td>
<td>Send on gauze that is barely dampened in saline. Do not fix in formalin. Hand deliver immediately.</td>
<td>Must be received during normal working hours unless previously arranged.</td>
</tr>
<tr>
<td>Hippocampus &amp; Amygdala</td>
<td>Send fresh. Hand deliver immediately to the laboratory.</td>
<td></td>
</tr>
<tr>
<td>Temporal Lobe (Epilepsy)</td>
<td>Send fresh. Hand deliver immediately to the laboratory.</td>
<td></td>
</tr>
<tr>
<td>Temporal Artery</td>
<td>Send in 10% Neutral Buffered Formalin.</td>
<td>Send Immediately/ASAP</td>
</tr>
<tr>
<td>Skin Biopsy (CADASIL)</td>
<td>Send on gauze that is barely dampened in saline. Do not fix in formalin. Hand deliver immediately.</td>
<td>Must be received during normal working hours unless previously arranged.</td>
</tr>
<tr>
<td>Laminectomy/Disc</td>
<td>Send in 10% Neutral Buffered Formalin.</td>
<td></td>
</tr>
<tr>
<td>Tumour fluid for cytology</td>
<td>Hand delivery immediately.</td>
<td>Must be received during normal working hours.</td>
</tr>
<tr>
<td>CSF for cytology</td>
<td>Hand delivery immediately.</td>
<td>Must be received during normal working hours.</td>
</tr>
<tr>
<td>CSF for 14.3.3 Protein</td>
<td>Hand deliver immediately in a biohazard bag. See Section 3.23.10 for requirements from external centres.</td>
<td>Must be received during normal working hours unless previously arranged. CJD Questionnaire must accompany specimen.</td>
</tr>
<tr>
<td>Tissue Type</td>
<td>Means of Delivery to Neuropathology</td>
<td>Comment</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>----------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Blood for Mitochondrial Disease Analysis</td>
<td>10mls in EDTA tubes</td>
<td>Must be received during normal working hours. Contact Rachel Howley 2706</td>
</tr>
<tr>
<td>Blood for Genetic Analysis</td>
<td>10mls in EDTA tubes</td>
<td>Must be received during normal working hours. Contact Rachel Howley 2706</td>
</tr>
<tr>
<td>Autopsy &amp; Biopsy tissue (e.g./ Brain / Tonsil) for Prion Protein Analysis</td>
<td>Hand delivery immediately.</td>
<td>Must be received during normal working hours. Contact Rachel Howley 2706</td>
</tr>
<tr>
<td>All other tissue</td>
<td>Sent in 10% neutral buffered formalin indicating volume.</td>
<td>Must be received during normal working hours.</td>
</tr>
</tbody>
</table>

**Requirements for External Centres**

The responsibility for sending specimens lies with the external centre (Sender). Specimens must be pre-booked with the Neuropathology department (Tel. 8092633) in advance to enable the department to make arrangements should the sample arrive after hours. Ensure that packaging and transportation comply with current UN legislation.

Address the package to:
Neuropathology,
Beaumont Hospital,
Dublin 9

Include the Consignee address and telephone number. Record that the sample is an 'Urgent sample for Neuropathology'.
Confirm by contacting the Neuropathology department when the sample has been collected.

**Results**

Muscle Biopsies: Laboratory tests on muscle biopsies are performed on a weekly basis due to the complexity of the techniques involved. Results are generally available in the Neuropathology office on the Friday or Monday following receipt of the sample.

CSF Samples for 14.3.3 Protein Analysis: There is an approximate turn around of 10 days from receipt of the sample to results.

Blood and Tissues for Mitochondrial DNA Analysis: Results are posted out to the relevant consultant within six weeks of receiving the sample.
Nerve Biopsies: Results are available 3–4 weeks from specimen receipt.

Post Mortem Brains: Results are available 4-6 weeks from specimen receipt.

REQUIREMENTS / FACTORS AFFECTING MUSCLE BIOPSIES

Requirements

All investigations are performed on unfixed frozen tissue. Samples must be delivered to the lab on gauze that is barely dampened with saline as excess causes swelling and separation of fibres. This makes interpretation difficult. A muscle having grade 3/5 on MRC strength scale is best. A fatty muscle (‘end-stage’ biopsy) may have insufficient fibres for diagnosis.

The department must be informed in advance if a sample is being delivered after hours. Ensure a requisition form is properly completed to include clinical details.

Specimen Size

An open biopsy is preferable to a needle biopsy especially if mitochondrial DNA (mtDNA) and protein analysis be required. A biopsy of at least 1.5 x 1x 1cm is ideal. This allows extra samples to be banked in case it is necessary to forward any to an external centre for further studies. Biopsies less than 0.5cu cm are insufficient for this purpose.

CSF SAMPLES FOR 14.3.3 PROTEIN ANALYSIS

Requirements

The sample should be sent to the Neuropathology lab immediately after aspiration for freezing as suboptimal sample storage may give unpredictable results. All samples must be logged in with the Neuropathology Lab prior to sending. All samples must accompany a completed questionnaire (LF-NCJD-CSF), copies of which are available from the Nueropathology Laboratory (Ext. 2633).

The sample volume should be between 2-5mls and be clear and colourless. The sample should have a low white cell count. Samples with a high white cell count i.e. greater than 20 are unsuitable as patients with CJD rarely have increased number of CSF white cells. Please rethink the diagnosis if CSF white cell count is high. Blood samples are not suitable for analysis. Blood stained samples will increase the chance of a false positive result as 14.3.3 protein is found in red blood cells.

Safety Precautions

TREAT ALL CSFs FOR 14-3-3 protein AS POTENTIALLY INFECTIOUS. [see www.cjd.ed.ac.uk]
In the event of accidental leakage of the sample please contact the Neuropathology laboratory. There is no immediate hazard to health unless the sample is ingested or injected into the body. Disposable gloves must be worn before attempting to handle the material.

**REQUIREMENTS / FACTORS AFFECTING BLOOD / TISSUE FOR MITOCHONDRIAL ANALYSIS**

**Requirements**

LF-NEU-mtDNA Referral Form must accompany the sample. Approximately 10mls blood (in EDTA tubes) or fresh unfixed muscle tissue (see section 3.23.13 Muscle Biopsy) is required for the extraction of mitochondrial DNA (mtDNA). All mtDNA mutation analysis can be performed on blood specimens, however, mtDNA rearrangements (deletions / duplications / depletions) are preferably performed on muscle tissue (see table below)

**Specimen Size**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Mitochondrial Disease</th>
<th>Blood</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHON</td>
<td>Leber’s Hereditary Optic Neuropathy</td>
<td>***</td>
<td>Not Necessary</td>
</tr>
<tr>
<td>Pearsons</td>
<td>Pearson’s Syndrome</td>
<td>***</td>
<td>Not Necessary</td>
</tr>
<tr>
<td>KSS</td>
<td>Kearns Sayre Syndrome</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>CPEO</td>
<td>Chronic Progressive External Opthalmoplegia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depletion</td>
<td>mtDNA Depletion</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>MELAS</td>
<td>Mitochondrial Encephalopathy with Lactic Acidosis and Stroke-like Episodes</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>MIDD</td>
<td>Maternally Inherited Diabetes Mellitus</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>MERRF</td>
<td>Myoclonic Epilepsy with Ragged Red Fibres</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>MILS</td>
<td>Maternally Inherited Leigs Syndrome</td>
<td>***</td>
<td>Not Necessary</td>
</tr>
<tr>
<td>NARP</td>
<td>Neurogenic weakness, Ataxia and Retinitis Pigmentosa</td>
<td>***</td>
<td>Not Necessary</td>
</tr>
<tr>
<td>POLG</td>
<td>Alper’s Syndrome, Epilepsy, PEO, Myopathy</td>
<td>***</td>
<td>Not Necessary</td>
</tr>
</tbody>
</table>

*** = preferable sample type; * = analysis can be performed

**Factors affecting test performance / interpretation of results**

- Sample Type is very important (see table above)
- Clinical and family history must accompany each sample.
- Blood MUST be stored in EDTA tubes. Specimens sent in any other tubes can not be analysed.
- Blood samples of patients older than 30 years will not be screened for MELAS / mtDNA rearrangements.
### 3.23.14 Molecular Pathology Tests and Requirements:

<table>
<thead>
<tr>
<th>Molecular Test performed</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p19q Array CGH</td>
<td>10x5micron sections of requested block on unbaked glass slides.</td>
</tr>
<tr>
<td>MGMT methylation analysis</td>
<td>10x5micron sections of requested block on unbaked glass slides.</td>
</tr>
<tr>
<td>BRAF Fusion qPCR</td>
<td>5x5micron sections of requested block on unbaked glass slides.</td>
</tr>
<tr>
<td>IDH 1&amp;2 pyrosequencing analysis</td>
<td>10x5micron sections of requested block on unbaked glass slides.</td>
</tr>
</tbody>
</table>

From an external referral centre the samples must arrive with adequate paperwork outlining patient details i.e. demographics, diagnosis and the test requested.

**MOLECULAR PATHOLOGY TURNAROUND TIMES**

The turn around time of specimens for a molecular test will vary depending on the nature of the specimen and the complexity of the investigations required. The following is an outline of estimated turn around time from time of receipt in the laboratory:

- 4-6 weeks

This is only a guideline and the complexity of a case and the requirement for further investigations may change this.

### 3.23.15 Autopsy Services (Post Mortems)

The Histopathology and Neuropathology Department provide an autopsy service. Autopsies may be performed at the request of the clinical staff responsible for the care of the patient or under the direction of the Coroner.

Written consent from the next of kin on the appropriate post-mortem examination consent form is required for non-Coroners cases (ie "Hospital" or "House" consent cases) before an autopsy is performed. (LAB 358B6)

In Coroners cases, including query CJD cases, the Coroner Autopsy Post Mortem Examination Form (LAB 357B) detailing the nature of the procedure and giving the name and number of a family member must be completed.
3.24 NHISSOT

3.24.1 Introduction

The National Histocompatibility and Immunogenetics Service for Solid Organ Transplantation (NHISSOT) provides a nationwide transplant immunology service for solid organ transplantation, including HLA typing and crossmatching donors and recipients for solid organ transplants, HLA antibody screening for post transplant monitoring and HLA typing for disease association.

3.24.2 How to Order Tests

The department offers a national service. As renal patients can move between different dialysis centres around the country, accurate labelling of patients’ samples is very important. The standard required by the H&I department is name and date of birth on all specimens received and date the sample was taken. Failure to supply the required details will result in specimen rejection.

1. For patients to be HLA typed and HLA antibody screened a request form must accompany the specimens. Specimens can only be processed if both specimen and form are identified with the patient’s name and date of birth. These details must be legible on both request form and bottle, and must correspond with each other. For Beaumont Hospital patients, the information is automatically generated on the specimen label by the Beaumont Hospital Information System (BHIS) but a request form must accompany the specimens. The request form must be fully completed including all patients’ details, sample date, blood transfusion history, pregnancies, previous transplant(s) and the Consultant’s name and hospital to which the report should be returned. For all patients, the request/consent forms must be signed by the patient (or their guardian) and the medical person ordering the test.

   It is the responsibility of the requesting clinician to ensure that the patient has read and understood the permission statement on the request form and that this is initialled by the patient. For more information on the permission statement please contact the laboratory.

2. Patients for antibody screening only (HLA antibodies) must meet the same standards of identification. An HLA antibody Screening request form must be completed and can be faxed to the H&I Department. This form is then checked against the specimens sent and faxed back to the referring dialysis centre as confirmation of receipt of samples.
Request forms for HLA typing and HLA antibody screening are available from the department and can be supplied to dialysis centres around the country by phoning or emailing: crossmatch@beaumont.ie.

### 3.24.3 Repertoire of Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Specimen Container</th>
<th>Minimum Volume</th>
</tr>
</thead>
</table>
| HLA typing of patients for solid organ transplantation               | Citrated Blood     | Paeds: 5ml
|                                                                      |                    | Adult: 10ml      |
| HLA antibody screening                                              | Clotted Blood      | Paeds: 3ml
|                                                                      |                    | Adult: 10ml      |
| Urgent requests:                                                     |                    |                  |
| De-sens, AMR                                                         | Clotted Blood      | 10ml             |
| HLA-B27                                                             | Citrated Blood     | 10ml             |
| HLA-B57                                                             | Citrated Blood     | 10ml             |
| Transplant Crossmatching for Deceased Donors                        | Citrated Blood     | 10ml             |
| bloods required from the potential recipient(s)                     | Clotted Blood      | 10ml             |
| Living donor work-up:                                               |                    |                  |
| Potential Donors                                                    | 1<sup>st</sup>     |                  |
|                                                                     | Citrated Blood     | 10ml             |
|                                                                     | EDTA               | 7.5ml            |
|                                                                     | 2<sup>nd</sup>     |                  |
|                                                                     | Citrated Blood     | 10ml             |
|                                                                     | EDTA               | 7.5ml            |
|                                                                     | EDTA               | 7.5ml            |
|                                                                     | Citrated Blood     | 10ml             |
|                                                                     | Clotted Blood      | 7.5ml            |
|                                                                     | 2<sup>nd</sup>     |                  |
|                                                                     | Citrated Blood     | 50ml             |
|                                                                     | Clotted Blood      | 10ml             |
|                                                                     | 3<sup>rd</sup>     |                  |
|                                                                     | Citrated Blood     | 10ml             |
|                                                                     | Clotted Blood      | 10ml             |
|                                                                     | Full house potential donor |        |
|                                                                     | Citrated Blood     | 40ml             |
|                                                                     | Clotted Blood      | 10ml             |
|                                                                     | 3<sup>rd</sup>     |                  |
|                                                                     | Citrated Blood     | 40ml             |
|                                                                     | Clotted Blood      | 10ml             |
|                                                                     | 3<sup>rd</sup> and final | 7 days of the proposed transplant date | |
|                                                                     | Citrated Blood     | 40ml             |
|                                                                     | Clotted Blood      | 10ml             |
| Autocrossmatching                                                    |                    |                  |
| CDC Autocrossmatching Only                                          | Clotted Blood      | 10ml             |
| Flow Autocrossmatching                                              | Clotted Blood      | 10ml             |
|                                                                     | Clotted Blood      | 40ml             |
| Post transplant monitoring: See section on post-transplant Monitoring | Clotted Blood      | 10ml             |
| Post transplant monitoring – Urgent antibody screening request for query graft rejection: See section on post-transplant Monitoring | Clotted Blood      | 10ml             |
| HLA typing for disease association                                   | Citrated Blood     | 10ml             |
| HLA typing for partners                                              | Citrated Blood     | 10ml             |
| ABO blood grouping                                                   | EDTA               | 7.5ml            |
3.24.4 HLA Typing of Patients for Solid Organ Transplantation

All potential recipients are routinely typed by low to medium resolution molecular techniques and by serology for HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ and HLA-DP. These techniques use commercial sera and probes and primer sets selected according to the current standards of EFI.

Note 1: Specimens for HLA typing received into the H&I Department are separated and the DNA stored. The patient is blood grouped and a Specimen Received Report is then issued to the referring unit/consultant. When the transplant evaluation pack for a patient is received in the Transplant Office, HLA typing will be carried out on that patient and a report issued before the patient’s appointment at the Transplant Clinic. Additional examinations may be requested at anytime once the DNA is stored.

Exceptions to this protocol are paediatric patients, cardiothoracic patients and liver patients.

**SEROLOGICAL (COMPLEMENT DEPENDENT CYTOTOXICITY) TYPING**

Patient’s lymphocytes are isolated from citrated blood by using commercial immunomagnetic beads for HLA typing by serology. The lymphocytes can then be ‘tissue typed’ using commercial tissue typing trays.

**MOLECULAR (DNA) TYPING**

The development of PCR (Polymerase chain reaction) has led to a higher resolution of HLA typing. Patient’s DNA is isolated from citrated blood and typed by molecular means using two techniques:

1. **PCR-SSP (sequence specific primers)** – these SSP primers consist of allele and group specific primers that are designed to anneal to specific sequences characteristic of a given allele or group of alleles. If SSP anneal to each of the complementary strands of a target DNA sample, PCR amplification can occur. Amplified products of DNA are visualised by gel electrophoresis

2. **PCR-SSO (sequence specific oligonucleotides)** – After PCR amplification the amplicons are denatured to form single stranded DNA which are added to a microsphere or chip containing specific SSO probes. The amplicons then hybridise to those probes that contain a complementary target sequence. The amplicon-probe complex is then visualised using a colourmetric reaction or fluorescence and analysed.

Anomalous results by molecular techniques are referred for high resolution SSP or DNA sequencing to the National Histocompatibility and Immunogenetics Reference Laboratory (NHIRL), Irish Blood Transfusion Service (IBTS),
National Blood Centre, James’s Street, Dublin 8, or Anthony Nolan, 2 Heathgate Place, 75-87 Agincourt Road, London, NW3 2NU, United Kingdom

Note 2: Patients are HLA typed on two separate occasions to ensure there are no discrepancies between samples.

Note 3: Molecular techniques are generally regarded as being more accurate than serological techniques. However, one limitation is that molecular techniques may not distinguish between null alleles (alleles which are not expressed) and expressed alleles of some HLA antigens. Serological techniques may confirm presence of null alleles.

**3.24.5 Antibody Screening**

The antibody screening and sample request policies are consistent with the current standards issued by EFI.

All patients for solid organ transplantation are screened for HLA antibodies using various screening techniques.

Note 1: Specimens for Antibody Screening for the transplant pool work-up are separated and the serum stored. A Specimen Received Report is then issued to the referring unit/consultant. When the transplant evaluation pack for a patient is received in the Transplant Office, antibody screening will be scheduled for the patient and a report issued before the patient’s appointment at the Transplant Clinic. Additional examinations may be requested at anytime once the serum is stored.

Exceptions to this protocol are cardiothoracic patients and patients already on the waiting list.

**SCREENING**

**Luminex Single Antigen:** These techniques use microbeads coated with purified HLA antigens for antibody detection. Up to 100 different coloured beads may be combined in one suspension for a single test. Following incubation of the serum with the beads, bound antibodies are labelled with a fluorescent conjugate. The Luminex flow analyser detects the fluorescent emission from the beads, and the amount of fluorescence indicates the amount of antibody present.

**Lambda Cell Tray™ (LCT):** These commercial trays are used to detect the presence of HLA antibodies by CDC (complement dependent cytotoxicity). Each well on the tray contains HLA typed cells that have been frozen. Patient’s serum is incubated with the cells and on addition of complement any antibody present will cause the cells to break down (lysis). Any antibody present can then be characterised.
ANTIBODY ANALYSIS AND IDENTIFICATION

Once the results from the antibody screens are available, the antibody profile of each patient is then analysed and any unacceptable antibodies are identified.

These unacceptable antibodies are entered onto the patient’s profile in the H&I database.

On creating a potential recipient list during donor processing, any potential recipient that has an antibody to the transplant organ will be rejected. This reduces a patient’s disappointment on being called for transplant and then being rejected due to a positive crossmatch.

COMMENT

CDC screening using LCT trays is insensitive and only detects complement fixing antibodies. When present, these antibodies will cause hyperacute rejection if directed against the donor. However, a variety of non-HLA specific antibodies can interfere with these assays giving false positive results.

Luminex techniques are more sensitive and detect both complement fixing and non-complement fixing antibodies.

SPECIMEN REQUIREMENTS FOR ANTIBODY SCREENING

HLA antibody screening samples required from renal/pancreatic patients who are on the transplant waiting list:

- Patients on the transplant waiting list are screened for HLA antibodies routinely every 3 months
- The department requires an HLA antibody screening sample from these patients every 90 days
- CAPD and pre-emptive patients can have the samples taken by their local GP and send them directly to the department by post—see page 91 for transport requirements
- All patients post transfusion (blood products or platelets) require a clotted sample 14 days post transfusion or as soon as possible thereafter. It is vital we receive these samples to determine if donor specific HLA antibodies have developed

Note: The routine 3 monthly samples are essential for screening and crossmatching patients on the waiting list. If we do not have a sample less than 90 days old, the patient will not be listed until one is received and analysed

HLA antibody screening samples required from renal/pancreatic patients who are not on the transplant waiting list:
• All patients post transfusion require a clotted sample 14 days post transfusion or as soon as possible thereafter. It is **vital** we receive these samples

**Cardiothoracic patients on either the lung or heart waiting list**
• Patients identified as positive for HLA antibodies – **Sample every month**
• Patients with no identified HLA antibodies – **Sample every 3 months**

**Post transfusion samples from Cardiothoracic patients on the waiting lists**

Because time constraints in cardiothoracic transplants often preclude getting a day of transplant sample, the following schedule applies to ensure that a sample within an acceptable time frame is available for crossmatch

• **Weeks 2-4** following transfusion, we require a sample every week

**For antibody negative patients we require a sample every 3 months**
**For antibody positive patients we require a sample every month**

**Cardiothoracic patients on LVADs/BiVADs or Novalungs**
A sample every month is required unless otherwise notified

### 3.24.6 Solid Organ Transplant Pools Work-Up

**RENAI/PANCREATIC PATIENTS**

Once a patient has been seen at the clinic by the Consultant Transplant Surgeon, a transplant pool request authorisation card will be completed by the transplant co-ordinators with all the patient’s details and signed by the Surgeon. On receiving a transplant pool request authorisation card, all the patient’s details and relevant information including tissue typing report forms, blood group and antibody screening results are placed in the patient’s transplant folder. A transplant pool card will also be completed for each patient. All patients on the waiting list have their own transplant folder and transplant pool card. All information regarding the patient is contained in these folders and cards.

At this time patients will appear on the ‘NHISSOT workup list’ on the monthly transplant waiting lists distributed.
Patients are then ‘activated’ when all the documentation and immunological work has been completed and checked. For patients to be activated they must satisfy the following criteria:

- All relevant information must be available including information on pregnancies, transfusions, previous transplants and transplants performed overseas.
- HLA typed twice by serological and molecular techniques.
- Blood group typed twice.
- All relevant archived sera samples and current samples on patient are screened by Luminex (as appropriate).

Note: When all the immunological work has been completed the patient and their consultant nephrologist will receive a letter from the H&I department informing them that they are active on the transplant list.

**CARDIOTHORACIC PATIENTS**

The Cardiothoracic Transplant Co-ordinators in the Mater Hospital inform the H&I Department of patients going on the cardiothoracic transplant waiting lists. Assessment proceeds over several weeks. During this time the patient is HLA typed and screened for HLA antibodies. A report is issued indicating whether the patient will need a prospective crossmatch or not, if listed.

All the patient’s details and relevant information including tissue typing report forms, blood group and antibody screening results are placed in the patient’s transplant folder. A transplant pool card will also be completed for each patient. All patients on the waiting list have their own transplant folder and transplant pool card.

Additional samples will be requested on patients listed for lung transplant for auto crossmatching. These bloods will be requested from the patients centre when the laboratory is informed of the patient going on the cardiothoracic transplant pool.

**LIVER PATIENTS**

Patients awaiting liver transplantation are HLA-B typed and ABO blood grouped. Once transplanted, repeat specimens must be sent for confirmatory HLA-B type. The patient has a full HLA type done (HLA-A, -B, -DR) if the donor is a full match at the B locus. A report is then issued indicating the risk of GVHD (Graft Versus Host Disease), which is influenced by the matchgrade. This report is faxed to the Liver Transplant Co-ordinators usually within 24 hours of the transplant taking place.
3.24.7 Deceased Donor Work-up and Potential Recipient List Generation

The ODTI Transplant Co-ordinator (Organ Donation and Transplant Ireland) contacts the H&I Department and informs them that a potential donor has been identified and organise bloods to be taken and sent to the laboratory.

On receiving bloods, the Medical Scientist on duty will:

- HLA type the potential donor by serology and molecular techniques.
- Send a sample to the blood transfusion department for ABO blood grouping.
- Run a match programme that identifies suitable recipients
- A potential recipient list is prepared according to agreed criteria, to include clinically urgent patients, paediatric patients, acceptable mismatched patients, significantly sensitised patients, best HLA-matched patients, potentially suitable patients with rare tissue types (low matchability) and patients waiting for the longest period.
- Review all antibody screening results for each recipient on the potential recipient list.
- Once the Medical Scientist is satisfied that all the potential recipients on the list are immunologically suitable, the list is sent to the Renal Transplant Co-ordinator on duty.

3.24.8 Acceptable Mismatch Programme

The acceptable mismatch programme exploits Single Antigen technology to define ‘windows’ or HLA antigens to which patients are not sensitised. All patients with a Pgen > 94% are evaluated for windows, and when a donor organ becomes available which is suitable, they will receive additional priority on the potential recipient list.

3.24.9 Matchability Scores

The H&I Department has a database of over 1,000 HLA types of previous deceased donors from our population. We use this database of donor HLA types to calculate the chance of a patient getting a good match from our donor population.

This data is expressed as a percentage of the population and is made available to the referring clinicians on the monthly transplant waiting lists. It can be used to accurately discuss the likelihood of a patient getting a very good match, or how unlikely this is, depending on the patient’s HLA type.

The scores from over 2,500 patients analysed to date range from below 0.01 to 16% i.e. ranging from 1 in every 10,000 donors to 16 in every 100 donors being
a close genetic HLA match. To use the matchability score, you need to know the patient’s blood group. If the blood group is B or AB, there are very few donors of these blood groups, and therefore, waiting for a close match is inadvisable.

The ODT (Organisation for donation and transplantation in the UK) define a favourable match as:

- 000, 100, 200, 010, 110, 210 (HLA -A, -B, -DR) – Figures represent donor mismatched antigens
- These grafts show a definite survival advantage in most large studies. Additionally, for patients likely to require another transplant in the future, the degree of sensitisation after a well matched graft is usually less than that seen after a poorly matched graft.
- Any DR mismatch negates any advantage of matching at the A or B locus.
- We still list up to 1 DR mismatch as ‘reasonably matched’ in an effort to reduce future sensitisation.

### DEFINING MATCHABILITY

For patients of blood groups A and O:

<table>
<thead>
<tr>
<th>Score</th>
<th>Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% or under</td>
<td>Low</td>
</tr>
<tr>
<td>5.1-7.9%</td>
<td>Medium</td>
</tr>
<tr>
<td>8% and over</td>
<td>High</td>
</tr>
</tbody>
</table>

3.24.10 **Living Donor Work-Up**

The number of people suffering from end stage renal failure (ESRF) is rapidly rising and a kidney transplant is the treatment of choice for many patients. Approximately 150-200 renal transplants are performed in Ireland each year, the majority of which are from deceased donors. Deceased donors are those who have been declared brain stem dead.

A Living donor programme has been established in Beaumont Hospital. A kidney from a living donor is the optimal form of transplant. The kidney usually starts working immediately after the operation and the graft survival for a kidney from a living donor is superior to that from a deceased donor. Since family members have a similar genetic makeup the chance of a recipient receiving a well matched kidney is increased. This offers a particular advantage to children and patients who have made HLA antibodies.

**What is Living Donation?**

Living donation takes place when a living person donates an organ (or part of an organ) for transplantation to another person. Donation of an organ or part of an organ is only considered after thorough evaluation when the donor is healthy,
where the loss of the organ or part of an organ is not deemed to place their longterm health at undue risk, and where the donor understands the process and freely consents to donation.

**WHAT MAKES A DONOR SUITABLE?**

- **Compatible blood group**
  The donor and recipient blood groups are compatible.

- **Compatible tissue type**
  HLA antigens determine a person’s tissue type. Half of these are inherited from the mother and half from the father therefore; blood relatives are more likely to have a similar tissue type. A brother and sister have a one in four chance of having an identical type. People who are genetically unrelated can also be donors. Any potential donors are tissue typed to ensure that their HLA type is compatible (suitable) for the potential recipient.
  We look at six different sets of HLA antigens for matching (HLA-A, -B, -C, -DR, -DQ, -DP) and the best compatibility between donor and recipient is a six loci match.

- **Antibody screening**
  All potential recipients for living donation are screened for antibodies so any unacceptable antigens that are on the potential kidney are identified and the potential donors can be eliminated at the first stage of living donor work-up, if these antibodies pose a risk to the graft.

- **The Virtual Crossmatch**
  A virtual crossmatch is a technique whereby a potential donor’s type at all six loci is compared to the relevant patient’s HLA antibody profile. If the patient does not have any HLA antibodies to the potential donor’s HLA type the virtual crossmatch is considered negative and the potential donor may proceed into the work-up process. If the patient has circulating or historic HLA antibodies to the potential donor’s HLA type the next stage before reporting is a Risk Assessment.

- **The Risk Assessment**
  Potential donors for living donation are risk assessed. This means a detailed analysis of any antibody incompatibility between the recipient and the potential donor and assigning a level of risk that the transplant, if it were to proceed, would be at higher risk of suffering immune mediated injury. A donor/recipient combination that has no antibody incompatibilities is assigned as ‘standard risk’.
The Crossmatch

A crossmatch is carried out to ensure that the recipient has no antibodies directed against the potential donor kidney. Cells, obtained from a blood sample from the potential donor, are incubated with recipient’s serum and if there are any antibodies directed against any antigen on the kidney they may cause a positive crossmatch which is not always a bar to transplantation. This result will be assessed in conjunction with the patients antibody screening results to determine the immunological risk involved.

SUMMARY OF STAGES FOR LIVING DONOR WORK-UP

Note: Families willing to donate must contact the Transplant Co-ordinators to arrange a suitable time for samples to be taken. Any samples received into the laboratory will not be processed without prior contact with the Transplant Co-ordinators

- **First live donor work-up – virtual crossmatch**
  We require:
  10ml citrated sample and 7.5ml EDTA samples from the potential donors for blood group and tissue typing.

- **Second live donor work-up**
  We require:
  - **2a Workup:** 10ml citrated and 7.5ml EDTA from the potential donor
  - **2b Workup:** 50ml citrated and 7.5ml EDTA from the potential donor
  40ml citrated and 10ml clotted from the recipient for Auto Flowcrossmatch
  This stage of the work-up takes place no more than two months before the planned date of transplant. A repeat blood group and tissue type is carried out and for 2b workup, a crossmatch using the potential donor’s cells and recipient’s sera and autocrossmatch is carried out.

- **Third and final live donor work-up**
  We require:
  - 50ml citrated and 10ml clotted from the potential donor
  - 40 ml citrated samples from the recipient for auto crossmatching
  NOTE: Samples for auto antibody crossmatches should reach the laboratory within 24 hours
  - 10ml clotted sample from the recipient within 7 days of Transplant date
  - Donor and recipient samples taken together on same day if possible
  - A final crossmatch is carried out.

  This final stage of the work-up takes place no more than one week pre-transplant.
Risk Assessments
Donor/Recipient pairs have a final risk assessment post 3rd and final crossmatch. Using antibody screening data, sensitisation history and crossmatch results the immunological risk is assigned by the Consultant Immunologist.

REPORTING

Reports for the first and second work-up are issued to the Transplant Coordinator and the final work-up sent to the Consultant Surgeon and Transplant Coordinator.

Note: Results cannot be transmitted directly to the potential recipient’s Nephrologists or dialysis centre.

3.24.11 Crossmatching for Solid Organ Transplantation

The purpose of the crossmatch assay is to detect any recipient antibody which is reactive against the HLA antigens of a potential donor. Lymphocytes (white blood cells), extracted from donor lymph nodes/spleen are used for these assays. In the case of living donors peripheral blood lymphocytes are used.

The crossmatch assays are generally performed prior to the transplantation in order to ensure that there is no detectable antibody mediated sensitisation of the recipient to the donor. Multiple sera of a potential recipient including a recent serum (within 3 months) and ‘historic’ samples, if necessary, are tested against the donor lymphocytes be. Since there are multiple methods which can be used and each has its unique clinical application, a narrative interpretation of the assays for the clinician is provided. The Consultant Immunologist and the Medical Scientist on duty are available on a 24 hour basis for telephone consultation relating to H&I.

The main techniques of crossmatch routinely used in the laboratory are flow cytometry and to a lesser extent complement dependent cytotoxicity.

COMPLEMENT DEPENDENT CYTOTOXIC (CDC) CROSSMATCH
This is the standard crossmatch, based on complement-mediated cytotoxicity (cell death). The crossmatch is performed using donor T cells and donor B cells to detect recipient class I and/or class II antibodies directed against the donor antigens. In this assay patients’ sera is incubated with donor cells. Complement is then added and any antibody present in the patient’s serum directed against the donor HLA antigens will cause lysis (break down) of the cells resulting in a positive crossmatch. No antibodies present against the donor antigens, the cells remain intact and the crossmatch is negative.

In order to categorise the immunoglobulin class of any antibody reacting with the donor cells the serum is treated with DTT (Dithiothreitol) to destroy all IgM
antibodies rendering crossmatches negative if IgM is the only specific antibody presents.

**IgM positive crossmatches (CDC crossmatch only)**
These are particularly complicated given that autoreactive antibodies, sensitisation episodes and medical history are all involved in their interpretation. These IgM antibodies are often not a contraindication to successful transplantation. An interpretive report will be issued verbally and in writing indicating whether these antibodies are deemed clinically relevant or not.

**FLOW CYTOMETRY CROSSMATCHING**

The flow crossmatch is a more sensitive technique than cytotoxic crossmatch assays. It can detect HLA class I and/or class II donor specific IgG antibodies. It can also detect non-complement fixing antibodies, which can go undetected in the cytotoxic crossmatch, and very low titre (strength) antibodies. In this assay patient’s serum is incubated with donor lymphocytes. The cells are then stained with fluorescent dyes that detect human IgG antibodies, T cells and B cells. The cells are then analysed by a fluorescence activated cell sorter for the presence of donor specific antibodies.

Flow cytometry crossmatching is poorly standardised between laboratories and great care must be used when interpreting published data. In Beaumont, we have clinically validated our technique and cut-offs and in our hands, a positive flow cytometry cut-off indicates a 90% positive predictive value for acute humoral rejection

However a positive crossmatch is not necessarily a bar to transplant. A patient’s sensitisation history and antibody screening profile is also taken into account and additional screening/crossmatching may be requested before proceeding with the transplant if time allows.

**VIRTUAL CROSSMATCHING**

A certain cohort of patients are suitable for transplant without a prospective crossmatch. This is a distinct advantage for DCD (Donors following Cardiac Death) and in meeting theatre time slots for both DCD and DBD (Donors following Brain Death) donors.

Renal and Cardiothoracic patients who fulfil certain criteria are suitable for consideration for virtual crossmatch in discussion with the transplant team.

Note: If the patient has had transfusion/pregnancies or has a failing transplant they are not suitable for virtual crossmatch.

**All patients transplanted using virtual crossmatching require a flow crossmatch retrospectively in accordance with EFI standards**
INTERPRETATION OF CROSSMATCH RESULTS

Transplanting an organ into a patient who has circulating complement-fixing antibodies to donor HLA antigens would result in hyperacute rejection and immediate loss of the organ. Transplantation where donor specific non-complement-fixing antibodies are present is associated with acute rejection and a high risk of graft loss. The crossmatch prior to transplantation will detect any donor specific antibodies and thus prevent hyperacute rejection, and greatly reduce acute rejection.

The standard cell preparations used for crossmatching are as follows:
- Mixed T&B cell preparations.
- T cell preparation.
- B cell preparation.

The antigens found on the different cell types are as follows:
- T cells - HLA Class I antigens, autoantigens and various T cell markers.
- B cells – HLA Class I and HLA Class II antigens, autoantigens and various B cell markers.

Therefore, HLA Class I antibodies should be T and B cell positive and HLA Class II antibodies should be B cell positive only.

B cells are also positive for HLA class I antigens and indeed express class I antigens more strongly than T cells. Hence they are more sensitive than T cells in detecting low levels of HLA Class I antibodies. This can complicate interpretation of B cell positive crossmatches.

The crossmatch uses a selection of both current and historical sera in the crossmatch for the following reasons:
- Historical antibodies are important because they indicate sensitisation (exposure) of the patient to the donor antigens and hence the presence of memory T and B cells which can lead to rapid immunological responses in a patient if challenged with the same antigen again.
- Current antibodies are important because if they are directed against HLA antigens present on the graft they can cause hyperacute rejection of the organ, or an acute rejection.
- A day of transplant (DoTX) sample is required for crossmatch in certain situations e.g. recent sensitisation, graft in situ, failed graft within 12 months or borderline donor specific reactivity against donor HLA antigen.

Please note:
- It must be stressed that all crossmatch interpretation should be done in consultation with the H&I staff and the Consultant Immunologist or designated Senior Medical Scientist.
• All positive crossmatches are discussed by the Medical Scientist on duty with the Consultant Immunologist or designated Senior Medical Scientist

• For positive crossmatches, the Consultant Immunologist/ designated Senior Medical Scientist may advise that from an immunological point of view, it is reasonable to proceed with the transplant, taking the sensitisation history and antibody screening profile of the patient into account, and/or may request additional screening/crossmatching before proceeding with the transplant.

• Occasionally, particularly in cardiothoracic transplantation, in discussion with the Transplant Surgeon, it may be appropriate to transplant with a positive crossmatch using antibody removal techniques or other forms of augmented immunosuppression.

3.24.12 Autocrossmatching

This assay involves a crossmatch of the recipient’s lymphocytes with autologous (own) serum, and is done to identify any self-reactive antibodies which may have developed due to an autoimmune disorder, viral infection, SLE, connective tissue diseases and/or certain medications.

There are a number of variants on the autocrossmatch assay which can help determine the nature of the antibody. Knowledge of the presence and type of autoantibody can be extremely helpful in interpreting positive crossmatches and ensuring that patients do not miss out on potentially suitable organs due to false positive crossmatches.

• NOTE: Samples for autocrossmatches should reach the laboratory within 24 hours

• Please contact the Laboratory to book in the samples for autocrossmatch

Note: It is now routine for the laboratory to request bloods for auto flow crossmatch on patients when they are first listed for lung transplant. As cardiothoracic crossmatching is done prospectively any additional information that can be collected before the event such as the presence of auto reactivity in a person’s flow cytometry crossmatch can be vital to interpretation of results and in clarifying the level of risk in a transplant (risk stratification)

3.24.13 Post-Transplant Monitoring

RENEAL/PANCREATIC PATIENTS

A sample for post transplant antibody screening is requested on all solid organ transplants. This enables the detection of any HLA antibodies that develop during rejection episodes and is essential for evaluation and
crossmatching, should the patient require another graft at some time in the future

Specimen Requirements
- Clotted sample weekly for the first month post transplant.
- Clotted sample monthly for the next 2 months
- Clotted sample at 6, 9 and 12 months post transplant
- Clotted sample should then be sent on each subsequent anniversary of the transplant
- Clotted sample should be sent when clinically indicated e.g. at biopsy, when concerned re graft function, change to immunosuppressive regimen etc

Post transplant antibody screening schedule
Please contact the laboratory for the post transplant schedule, either by email at posttransplant@beaumont.ie or by phone.

Note: Where antibody mediated rejection is suspected clinically, the patient should be discussed with the Antibody Screening Senior Medical Scientist/Consultant Immunologist and samples ordered using the mnemonic PTXAB, or if outside Beaumont Hospital the term URGENT should be used. 
Note: Please email posttransplant@beaumont.ie when screening is clinically indicated. Please include any clinical indicators such as creatinine levels and a contact number for urgent results if at all possible. Samples received without accompanying email will be stored unless otherwise indicated.

CARDIOTHORACIC PATIENTS

FOR ALL PATIENTS
- Clotted sample weekly for the first month
- Clotted sample monthly for the next two months
- Clotted sample -should then be sent at 6, 9 and 12 months post transplant
- Clotted sample should then be sent on each subsequent anniversary of the transplant

Clotted sample should be sent each time the patient is biopsied, or concerned about graft function or immunosuppression is altered. Routinely these samples will be tested as follows:

Post transplant antibody screening schedule
Please contact the laboratory for the post transplant schedule, either by email at posttransplant@beaumont.ie or by phone.

The Consultant Immunologist is notified of any new or increased positivity and a post transplant antibody monitoring report is sent to the Cardiothoracic Consultant.
LIVER PATIENTS

Graft versus host disease (GvHD) results from the reaction of donor immunocompetent cells against tissues of the immunosuppressed host, usually donor lymphocytes v recipient tissue. This can pose significant risks to liver transplant patients. Donors homozygous at all HLA loci carry a higher risk for GVHD. Diagnosis of patients with suspected GVHD can be confirmed by the demonstration of substantial donor lymphocyte chimerism. If GVHD is suspected the Consultant Immunologist should be contacted immediately.

3.24.14 Patients for Disease Association

Some patients suffer from diseases which have been known to be associated with certain HLA antigens.

We HLA type patients for disease association, in particular B27, from Beaumont Hospital patients only and for GP patients in our catchment area. All disease association typing from outside hospitals is carried out in the NHIRL, IBTS, National Blood Centre, James’s Street, Dublin 8.

3.24.15 Patients for HLA-B57 Typing

Patients who express a specific allele of HLA-B57 (HLA-B*57:01) are at risk of a life-threatening reaction if exposed to abacavir, a useful antiretroviral drug. We HLA type patients who are on or will require antiretroviral treatment for HLA-B57 only. Patients who do not express HLA-B57 are reported as negative. Patients found to be HLA-B57 positive on low resolution are reflex tested for high-resolution HLA-B57 typing to confirm if the patient is HLA-B*57:01.

3.24.16 HLA Typing for Partners of Recipients

A baby has HLA antigens of which half come from the mother and half from the father. During pregnancy or birth the baby’s cells can cross the placenta into the mother’s blood and exposes the mother to the father’s HLA antigens.

Occasionally this can induce an immune response and the mother subsequently can develop HLA antibodies. These antibodies do not harm the baby or the mother, and only become clinically relevant if the mother subsequently requires a transplant.

HLA typing the partner is helpful to identify the antigens the mother has been exposed to or those which may develop in time. This can aid antibody identification and help to build up an antibody profile on a patient.
3.24.17  **ABO blood group typing**

The Beaumont Hospital Blood Transfusion Department carries out all donor and recipient blood groups and issues a printed report to the H&I Department.

3.24.18  **Pgen**

**PGEN - GENERATED OR CALCULATED PRA**

Using our database of donor HLA types, it is possible to calculate how many donors are unsuitable due to the presence of HLA antibodies and we refer to this as generated PRA – Pgen.

The Pgen value is not used to guide immunosuppressive therapy but as an indicator of how difficult it is to find a compatible graft.

3.24.19  **Out of Hours services (On-Call)**

The H&I department provides an out-of-hours service for solid organ transplantation. This service is available at all times including nights, weekends, holidays such as Christmas etc.

The services available are:
- HLA typing and crossmatching all potential donors for solid organ transplantation.
- Urgent antibody screening for cardiothoracic patients.
- Urgent antibody screening for post transplant rejection episodes.

**Note:**
- All requests for urgent antibody screening **out of hours must be** done in consultation with the Medical Scientist on-call
- For clinical advice **out-of-hours**, the Consultant Immunologist on-call can be contacted through the switch board.

During normal working hours urgent requests must be discussed with a Senior Medical Scientist or e-mailed to one of following e-mail addresses:

- posttransplant@beaumont.ie
- transplantlab@beaumont.ie

3.24.20  **Data Protection Act and freedom of information**

The H&I Department keeps patient data on its computer system and on a back-up paper system. All data is stored in compliance with data protection legislation to protect patients’ confidentiality.

The data held can include some or all of the following, where relevant:
- Name.
• Hospital chart numbers.
• Date of birth.
• Address.
• Phone number(s).
• Email address.
• Dates of dialysis.
• Type of dialysis.
• Dates of transfusions/transplants.
• Dates of sera samples received.
• Antibody screening information and results.
• HLA type.
• Molecular DNA typing information.
• Blood group
• Number of pregnancies.
• Virology results on potential recipients.
• Related donor information, where patients have been transplanted.
• Related family information, where a family study has been performed.
• Partner’s HLA type where applicable.

3.24.21 Reports and expected Turn Around Times (TAT)

The following reports are issued regularly by the department:
1. HLA typing and screening report (NHISSOT patient Report)
2. Cardiothoracic patient reports.
   This includes the HLA type, blood group, antibody screening results, generated PRA and crossmatch recommendations. Generated and issued to the Cardiothoracic Transplant Co-ordinators. Post transplant antibody screening reports are issued routinely to the Post Cardiothoracic Transplant Co-ordinators following receipt of screening samples.
3. Potential recipient listing.
   Issued to the Renal Transplant Co-ordinators and/or Surgical Consultant/registrar following work-up on a deceased donor.
   Issued to the Consultant Transplant Surgeon following donor/recipient crossmatch.
5. Cardiothoracic crossmatch report.
   Issued to the Consultant Transplant Surgeon following donor/recipient crossmatch.
6. Post transplant matching reports for liver recipients.
   This includes the recipient’s HLA type, blood group and donor details including HLA type, mismatch and risk of GVHD. Issued to the Liver Transplant Co-ordinators following transplantation.
7. Living donor reports for renal transplantation.
• 1\textsuperscript{st} workup - all family members HLA typed and blood group. Potential donors identified.
• 2\textsuperscript{nd} workup - Immunological compatibility confirmed.
• 3\textsuperscript{rd} work-up - Final crossmatch report.

\textbf{Issued to the Transplant Co-ordinators only.}

8. \textbf{Renal/pancreatic transplant pool(s) listings.}
   • Generated monthly

9. \textbf{Renal/pancreatic transplant service statistics}
   • Generated and issued monthly to the relevant Consultants and dialysis centres

\textbf{10. Antibody analysis and matchability report}
   Issued to all centres and the Transplant Co-ordinators on request

\textbf{11. Requests for antibody screening samples}
   Issued weekly for:
   • Renal/pancreatic patients.
   • Cardiothoracic patients.

\textbf{12. Post transplant antibody screening monitoring reports.}
   Reports are issued upon physicians request for testing or in clinically indicated cases

The following table lists the turn-around-times for H&I reports:

<table>
<thead>
<tr>
<th>TESTS</th>
<th>TURN AROUND TIMES</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA typing for Solid Organ Transplants</td>
<td>3 weeks – \textit{Urgent service available}</td>
</tr>
<tr>
<td>HLA Antibody Screening</td>
<td>2-4 weeks – \textit{Urgent service available}</td>
</tr>
<tr>
<td>\textit{HLA Antibody Screening HLA typing requests for emergency transplantation}</td>
<td>\textit{Same day service if requested}</td>
</tr>
<tr>
<td>Transplant pool work-up</td>
<td>2-6 weeks</td>
</tr>
<tr>
<td>Deceased donor work-up</td>
<td>6 hours</td>
</tr>
<tr>
<td>Potential donor recipient list</td>
<td>8 hours</td>
</tr>
<tr>
<td>Crossmatching for renal transplants</td>
<td>6 hours</td>
</tr>
<tr>
<td>Crossmatching for pancreatic/cardiothoracic transplants</td>
<td>6.5 hours for standard crossmatch (up to 4 patients)</td>
</tr>
<tr>
<td>Living donor work-up</td>
<td>1\textsuperscript{st} work-up 4 weeks</td>
</tr>
<tr>
<td></td>
<td>Risk Assessment 3-4 weeks</td>
</tr>
<tr>
<td></td>
<td>2\textsuperscript{nd} work-up 2 weeks</td>
</tr>
<tr>
<td></td>
<td>Final work-up 2 days</td>
</tr>
<tr>
<td>Autocrossmatching</td>
<td>2-3 days</td>
</tr>
<tr>
<td>Post Transplant Monitoring – Non-Urgent</td>
<td>Routinely: 2 weeks, unless contact is made from the referring clinician/centre to get a quicker report, in which case the sample will be set on the next screen to be run. Further testing/typing: 3 weeks</td>
</tr>
</tbody>
</table>
**TESTS**

*Post Transplant monitoring - Urgent antibody screening request for query graft rejection*

<table>
<thead>
<tr>
<th><strong>TURN AROUND TIMES</strong></th>
<th>For discussion with Antibody Screening Senior/Chief Medical Scientist and the referring clinician as to the level of urgency. Same day service available if required, otherwise the sample is set on the next screen to be run</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA typing for disease association</td>
<td>4 weeks</td>
</tr>
<tr>
<td>HLA typing for BMT/HSCT</td>
<td>2 weeks (unless awaiting further potential donors from overseas)</td>
</tr>
<tr>
<td>HLA typing for B57</td>
<td>4 weeks</td>
</tr>
<tr>
<td>HLA typing for partners</td>
<td>3 weeks</td>
</tr>
<tr>
<td>ABO Blood Grouping</td>
<td>2-3 hours</td>
</tr>
</tbody>
</table>

**3.24.21.1 Abbreviations Used on H&I Reports and Printouts**

**DIALYSIS CENTRES**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>Antrim Area Hospital</td>
</tr>
<tr>
<td>BE</td>
<td>Beacon Clinic Sandyford, Dublin</td>
</tr>
<tr>
<td>BD</td>
<td>Beacon Clinic Drogheda, Dublin</td>
</tr>
<tr>
<td>BT</td>
<td>Beacon Clinic Tallaght, Dublin</td>
</tr>
<tr>
<td>BF</td>
<td>Belfast City Hospital</td>
</tr>
<tr>
<td>BH</td>
<td>Beaumont Hospital, Dublin</td>
</tr>
<tr>
<td>CA</td>
<td>Cavan General Hospital</td>
</tr>
<tr>
<td>CB</td>
<td>Mayo General Hospital, Castlebar</td>
</tr>
<tr>
<td>CO</td>
<td>Cork University Hospital</td>
</tr>
<tr>
<td>CR</td>
<td>Our Lady’s Hospital for Sick Children, Crumlin, Dublin</td>
</tr>
<tr>
<td>EU</td>
<td>Patients dialysing in hospitals overseas within the EU</td>
</tr>
<tr>
<td>FR</td>
<td>Fresenius Limerick</td>
</tr>
<tr>
<td>GA</td>
<td>Merlin Park Hospital, Galway</td>
</tr>
<tr>
<td>GW</td>
<td>Wellstone Clinic, Galway</td>
</tr>
<tr>
<td>JA</td>
<td>St. James’s Hospital, Dublin</td>
</tr>
<tr>
<td>KK</td>
<td>Wellstone Clinic, Kilkenny</td>
</tr>
<tr>
<td>LE</td>
<td>Letterkenny General Hospital</td>
</tr>
<tr>
<td>LI</td>
<td>Limerick University Hospital</td>
</tr>
<tr>
<td>MA</td>
<td>Mater Misericordiae University Hospital, Dublin</td>
</tr>
<tr>
<td>NC</td>
<td>Northern Cross Clinic, Dublin</td>
</tr>
<tr>
<td>NE</td>
<td>Daisy Hill Hospital, Newry</td>
</tr>
<tr>
<td>OM</td>
<td>Omagh General Hospital</td>
</tr>
<tr>
<td>OS</td>
<td>Patients dialysing overseas – outside the EU zone</td>
</tr>
<tr>
<td>SL</td>
<td>Sligo General Hospital</td>
</tr>
<tr>
<td>SV</td>
<td>St. Vincent’s University Hospital, Dublin</td>
</tr>
<tr>
<td>TA</td>
<td>Tallaght Hospital (AMANCH), Dublin</td>
</tr>
<tr>
<td>TE</td>
<td>Children’s University Hospital, Temple Street, Dublin</td>
</tr>
<tr>
<td>TR</td>
<td>University Hospital Kerry</td>
</tr>
</tbody>
</table>
TU  Tullamore General Hospital
UK  Patients dialysing in hospitals within the United Kingdom
WA  University Hospital Waterford

**RENALE/PANCREATIC TRANSPLANT POOL PRINTOUT ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Age in years</td>
</tr>
<tr>
<td>Blood G</td>
<td>Blood Group</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>Compno</td>
<td>H&amp;I computer number</td>
</tr>
<tr>
<td>Cyto Due Date</td>
<td>Date a sample for antibody screening is required</td>
</tr>
<tr>
<td>Days Sample</td>
<td>Number of days sample is due. Minus number indicates the number of days the sample is outstanding.</td>
</tr>
<tr>
<td>Dial Cen</td>
<td>Dialysis centre</td>
</tr>
<tr>
<td>Dial</td>
<td>Dialysis type: P = CAPD/CCPD H =Haemodialysis</td>
</tr>
<tr>
<td>KG</td>
<td>Weight in kilos</td>
</tr>
<tr>
<td>Match %</td>
<td>Matchability score</td>
</tr>
<tr>
<td>PGen</td>
<td>Generated PRA</td>
</tr>
<tr>
<td>Prev Tx</td>
<td>Previous transplant(s): Number is printed</td>
</tr>
<tr>
<td>Ref Hosp</td>
<td>Referring Hospital</td>
</tr>
<tr>
<td>Urgent</td>
<td>First available crossmatch negative ABO compatible kidney (Highest urgency)</td>
</tr>
<tr>
<td>Wait</td>
<td>Length of time on transplant pool in months</td>
</tr>
</tbody>
</table>

**CROSSMATCH CODES – POTENTIAL DONOR OFFER LIST**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DoTx</td>
<td>Day of transplant sample required</td>
</tr>
<tr>
<td>Std</td>
<td>Standard – sample(s) available in the laboratory and suitable for crossmatch</td>
</tr>
<tr>
<td>VXM</td>
<td>Suitable for virtual crossmatch</td>
</tr>
</tbody>
</table>