Beaumont Hospital Clinical Directorate of Laboratory Medicine

Guide to Use of Laboratory Services by External Users

Document Number: LP-GEN-0014 **Revision**: 13 **Active Date:** 19th Feb 2024
Authorised By: Prof. P. Thornton , Clinical Director of Laboratory Medicine

DEPARTMENTAL INFORMATION

See next page for detailed table of contents.

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

Beaumont Hospital Blood Transfusion Department is fully licensed by the Health Products Regulatory Authority (HPRA). The Blood Transfusion Department is also accredited to ISO 15189 by the Irish National Accreditation Board (INAB). It incorporates the Blood Transfusion Laboratory, the Haemovigilance Office and Traceability functions.

The department supplies blood components and blood products on site to patients and red blood cells to off-site Beaumont Hospital patients in St Joseph's Raheny. It also supplies red cells to St Francis Hospice (Raheny & Blanchardstown) and to St Luke's Radiation and Oncology Centre on Beaumont Hospital campus.

NOTE: Comprehensive policies and procedures are available on the Blood Transfusion intranet page.

Related documents: available on hospital intranet

BTD-HVO-041:	Indications for Blood components and blood products.
BTD-HVO-026	Patient Identification
BTD-HVO-002	Pre-Transfuison Type & Screen Sampling
BTD-HVO-007	prescription of Blood Components and Products
BTD-HVO-039	Ordering Blood components and products from the Blood Bank
BTD-HVI-001:	Quick Reference Blood Transfusion Product Administration Guidelines
BTD-HVO-009:	Administration.
BTD-HVO-008	Care and monitoring of Transfusion recipents
BTD-HVO-011	Fateing/Disposal of Blood Components and Products
BTD-HVI-007	Guidelines for use of CMV and irradiated Blood Components
BTD-HVI-008:	Maximum Surgical Blood Ordering Schedule
BTD-HVO-042:	Emergency/Massive transfusion protocol (MTP) MTP Poster
BTD-F-0283:	MTP Poster
BTD-HVO-040:	Blood Track for Users.
BTD-HVO-031:	Transport of Blood Components and products from the Blood Transfusion Department
BTD-HVO-015:	Serious Adverse Reactions (Clinical Areas)
BTD-HVO-016	Serious Adverse Events (Clinical Areas)
BTD-HVO-002	Haemovigilance Management of Serious Adverse Events (Including Reporting to the NHO)
BTD-HVI-005	Non Blood Transfusion staff required training

2.2 SERVICE PROVISION HOURS AND CONTACT DETAILS:

- Routine hours: 8am to 5pm Monday to Friday, 9am to 1pm Saturday. Ext: 2705
- Emergency out of hours: 5pm to 8am Monday to Friday, 1pm Saturday to Monday 8am. Bleep: 252

Contact details:

- Consultant Haematologist: Dr Philip Murphy
  Email: philipmurphy@beaumont.ie
- Chief Medical Scientist: Janice O Shaughnessy. Ext: 2705
  Email: janiceoshaughnessy@beaumont.ie
- Haemovigilance. Ext: 2034
  Email: haemovigilance@beaumont.ie

Training:

Blood Transfusion training is mandatory in order to partake in any aspect of transfusion. Details of staff specific training can be found on BORIS and the hospital Intranet Blood Transfusion page. (See BTD-HVI-005)

2.3 BLOOD PRODUCTS/ COMPONENTS AVAILABLE

- Red Blood Cells
- Platelets
- Frozen Plasma
- Fibrinogen
- Flexbumin 20% and Albumin 5%
- Factor Concentrates (human & recombinant) e.g. factor VIIa, factor VIII, factor VIII/ human von Willebrand factor, factor IX, Prothrombin Complex Concentrate, PCC.
- Other Products on request

See BTD-HVO-041 Indications for Blood components and blood products

2.4 TURNAROUND TIMES

On receipt, specimens are date and time stamped, barcoded, initialled and logged into the LIS by the receiving Scientist. Turnaround Times are defined as the length of time taken from receipt of the sample to release of the report /product. This may be a manual report, electronic report visible on the LIS or verbal report via phone call. Turnaround time for products requested by users will be reflected by clinical need. If the patient has an antibody, turnaround time will vary from

above depending on the serological investigations required to identify the antibody.

2.5 PATIENT IDENTIFICATION

All patients admitted to Beaumont Hospital require a patient identification band applied to their dominant wrist (and ankle where patients are admitted to theatre.)

The ID Band is placed on the patient's wrist or ankle (or both) by the admitting Registered Nurse.
In the event of removal of an ID Band, it is the responsibility of the person who removed it to ensure that the patient is re-identified and the ID Band is repositioned on the patient as per PPCC-NCAR-080

2.5.1 *Positive Patient Identification (PPID)*

Ask the patient to state, without prompting, his/her full name and date-of-birth and verify these details with the patient's ID band. 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 any of the information does not correspond, the attending nurse must be contacted to clarify and amend the details before any blood transfusion transactions occur.

PPID must be carried out by an appropriately trained member of Beaumont Hospital staff:

- before a Beaumont Hospital ID Band is placed on a patient's wrist or ankle.

- before taking a blood specimen for Type and Screen testing (manual / Blood Track™ PDA Device). This procedure is described in the SOP BTD-HVO-004 Pre-transfusion (Type & Screen) Sampling.

- at the start of the Pre-transfusion Checking Procedure, (manual/ Blood Track™ PDA Device). This procedure is described in the SOP BTD-HVO-009 Administration of Blood Components and Products.

- after a transfusion is stopped due to a suspected Serious Adverse Reaction or suspected Serious Adverse Event, in order to determine that the correct blood has been given.

2.5.2 *Unidentified Patient*

A list of unique identification codes will be maintained by the managers and supervisors in the emergency department designed for use in correlating positive identification for blood transfusion purposes, of an unidentified patient. These are designed for use in an emergency/life threatening situation for an unidentified patient(s) either single or multiple but not in the context of a Major Incident. Where details are unknown, a unique computer generated ID is entered as a first name, 'Trauma' is entered as the surname and the pseudo DOB is 01/01/1901. If any patient details are known these should be entered at the time of registration as per ED-SOP-1.

2.6 BEAUMONT HOSPITAL MAJOR EMERGENCY PLAN

In the event of a Major Incident where there are multiple unidentified casualties, pre assigned hospital records will be used.

On being advised that the Beaumont Major Incident Plan is in operation, patients will initially be assigned Patient numbers (history numbers) and pseudonyms on admission into the Emergency Department using the Major Emergency Patient Identification Pack (MEPIP). This contains a pre-printed ID Bracelet and a Type & Screen (TS) request Form as per PPCC-ED-11.

The PDA device, where possible, should be used to label TS sample and for transfusion purposes, scanning the 2D barcode on patients BH MAJOR EMERG ID band.

2.7 PATIENT INFORMATION / CONSENT:

Signed consent for transfusion is not a legal requirement in the UK and Ireland.

However, the patient should be informed of the reason for transfusion and associated risks / benefits. A record of this discussion should be documented in the patients' medical notes. The blood transfusion prescription (BTD-HVF-018) question on consent should also be answered by the prescribing Doctor. Patient Information leaflets are available in all wards and on the Hospital intranet site and on the HSE website in various languages. Patient's decision to refuse transfusion should also be documented in the medical notes.

2.8 TYPE AND SCREEN SAMPLE:

- A Type and Screen determines the patients' blood group (Type) and if they have any antibodies in their plasma (Screen).
- A T/S is required if a patient needs: Red Cells, Platelets, Plasma or granulocytes.

- Once processed a T/S is valid for 3 days (*expiring at midnight on the 3rd day)*.
- Check if patient already has an in-date T/S to save time and avoid unnecessary phlebotomy.
- In emergencies, T/S should be hand delivered to avoid delays.
- You should only order a T/S if your patient is likely to require transfusion within the next 3-6 days (please refer to the Maximum Surgical Blood Ordering Schedule for pre-operative patients and the GAIN Guidelines for Red Cell Transfusion for haemoglobin transfusion triggers. All guidelines are available on the Hospital intranet under Blood Transfusion Department.

2.8.1 ***Extending a TS***

A T/S is valid for:

1. 3 Days (expiring at midnight of the 3rd day) - if the patient has been transfused or pregnant in the last 3 months

2. 6 days - Once a Doctor has confirmed that a patient has not had a transfusion or pregnancy within the last 90 days, they can phone the Blood Transfusion Laboratory with this information whereby the TS can be extended.

2.8.2 ***Pre-Transfusion Type and Screen Sampling***

The Blood Transfusion Dept. has strict sample acceptance criteria for Type & Screen samples. A fully completed request form (BTD-F-0001) must accompany the specimen. An addressograph label, PIPE label, or handwritten details are acceptable on the form provided the details are accurate and correlate exactly with details on specimen. No changes are permitted to the three main patient identifiers i.e, Patient name, History number and DOB either on the sample or the TS form once received in the BTD.

Type & Screen sampling must be performed as one continuous, uninterrupted procedure at the patient's bedside by a fully trained member of staff. (BSH 2017). The Patient must be wearing a Beaumont Hospital ID band or St Francis Hospice ID band. *Please do not take a TS sample if patient is not wearing an ID band or if ID band details are incorrect.*

- TS samples should be taken using electronic blood track system. Manually taken samples are accepted if this system is unavailable.
- For both methods, order a TS on PIPE and attach label to Type & Screen card.

- Ask patient to state their name and date of birth. Verify these details against patient wristband and the PIPE label on TS card.
- If patient is unable to confirm their identity, verify details with Healthcare record and ID band.
- Draw the sample.

Before leaving the patient bedside:

- If using electronic PDA device to take sample, follow on screen prompts in "collect sample" module and attach the 2 printed PDA labels, one on sample tube and one on TS card. This label acts as an electronic signature of the person taking the sample.

For manually taken samples,

- handwrite ***all*** details on the specimen tube and complete TS card
- Complete the top right specimen taker section of the T/S form.

* If specimen taker is a medical doctor, please answer the questions on form relating to special requirements, transfusion history and reason for transfusion.

The TS requests from GPs are not tested in the BTD as they do not satisfy patient identification criteria as detailed in ISO15189.

2.9 ELECTRONIC RESULTS:

TS:

***Received*:** Specimen "RECEIVED" – Specimen in lab but not processed. Routine specimens are processed between 08:00-17:00 hrs Monday-Friday, Saturday between 09:00-12:00. For urgent specimens contact medical scientist on call, bleep 252. Urgent specimens do not include correction of non-symptomatic anaemia's or elective surgical procedure

Accepted: No Antibody(s) detected. During routine hours red cells will take approximately 20 mins to cross-match for a patient. If a patient has had a red cell transfusion or been pregnant within the last 3 months a TS specimen is valid for 3 days, expiring at midnight on the third day. If a patient has not had a red cell transfusion or pregnancy within the last 3 months a Type & Screen is valid for 6 days

***See Note*:** T&S ACCEPTED-"No Antibody(s) detected. If transfusion is required please take 2nd T&s for ABO Group verification. During routine hours Red Cells will take approximately 20 mins to crossmatch for patient.

Rejected: Reason for rejection will be stated in a comment.

***Antibody*:** Antibody(s) detected. Please give 24 hrs notice prior to transfusion or surgery. If a patient has had a red cell transfusion or been pregnant within the last 3 months a TS specimen is valid for 3 days, expiring at midnight on the third day. If a patient has not had a red cell transfusion or not been pregnant within the last 3 months a Type & Screen is valid for 6 days.

***AHGXM*:** A serological crossmatch is required on this specimen. Please give notice prior to transfusion or surgery

Resulting of Other Tests	
Mnemonic	**Comment**
DIGG	DAT Positive, IgG only detected on patient red cells.
C3D	DAT Positive, C3d only detected on patient red cells.
IGCD	DAT Positive, IgG & C3d detected on patient red cells.
NSEHTRXN	No serological evidence of a haemolytic transfusion reaction. However, this cannot rule out non-serological reactions. Further report to follow.
POSHTR	Serological evidence of a haemolytic transfusion reaction. Haematology Team have been informed. Further report to follow.
FINALR	Transfusion reaction investigation report sent to patient's consultant and placed in patients chart, please contact haemovigilance officers for a copy of this report.
PLTRXN	Suspected transfusion reaction to platelets, under investigation. Final report will be available from Hospital Blood Bank/Haemovigilance.
LDPBGR	Patient Blood Group Report sent to Renal Consultant via Internal Post

2.10 SECOND SAMPLE REQUEST

A second sample is only required when the patient is for red cell transfusion, is not group O and has no other historical group on file, however group O compatible red cells can be issued without delay. This applies exclusively to 1st time non-blood group O patients (i.e. group A, B and AB) requiring a transfusion. A second sample is required for group verification and must be taken independently of 1st TS sample. Duplicate sample taken at the same time will be rejected.

2.11 CROSSMATCH

A valid TS is required for red blood cell crossmatch, a historical TS is required for platelets and plasma.

There are two types of red cell crossmatch performed in the lab:

Electronic crossmatch/Issue: Electronic cross-match is the selection and issue of red cell units where compatibility is determined by the laboratory information systems (LIMS) without serological testing of donor cells against patient plasma. This allows for the immediate issue of blood and applies to patients who have a valid TS and have no history of antibodies and no blood group anomalies.

Serological crossmatch is carried out when patients has or had antibodies/blood group anomalies: red cells may take from 60 minutes up to 24 hours. The indirect antiglobulin test is used to detect ABO and non ABO antibody incompatibility between donor red cell and patient plasma.

2.12 Ordering and Prescribing Blood Components and Products

Decision to transfuse:

The decision to transfuse should be based on the clinical assessment of the patient and individual needs. Promotion of a single unit transfusion policy is recommended by National Institute of Health and Care Excellence (NICE) 2015 in adult non bleeding patients – "Don't give two without review"

The clinical assessment of the patient should include an evaluation of risk factors when determining whether to transfuse, for example, risk of transfusion-associated circulatory overload (TACO) in vulnerable patient groups e.g. low body weight, patients > 70 years of age, pre-existing conditions, such as cardiac failure or renal impairment (British Society for Haematology 2017)

The prescribing Doctor is responsible for checking the patient's previous transfusion history and any special requirements, such as CMV-/ Irradiated. They are also responsible for checking whether the patient has a valid T/S prior to transfusion – the results of which are available on the PIPE system. A T/S is valid for 3 days expiring at midnight on the 3rd day, the blood bank should be contacted if the T/S is to be extended to 6 days, following confirmation that the patient has not had a transfusion or pregnancy in the last 90 days.

All blood products should be ordered electronically on PIPE and followed up with a phone order to the Hospital Blood Bank. Once PIPE label prints it must be placed on the prescription prior to verbal order

Ensure:

- Prescription is filled out completely. Details when ordering should include all patient details (*name, patient/history number and location*), blood product and quantity required, Doctors full name, IMC and contact number and the name of person placing order.
- Consent: it is a general ethical principle that valid consent is obtained from a patient before they receive a blood transfusion. Verbal consent suffices

and can be documented in chart. Patient information leaflets are available on each ward.

- Special requirements are considered. Pre-meds for patients with history of previous transfusion reactions, fluid overload etc.
- Maximum Surgical Blood Ordering Schedule is referred to: This is a guide to blood ordering for routine/elective surgical procedures.
- Risk of Transfusion Associated Circulatory Overload (TACO) has been assessed: patient weight, cardiac history, fluid balance, etc.
- Once prescription is completed, an electronic request from the clinical area must be followed up with a verbal request by phoning the Blood Bank.

St Francis Hospice:

- Symptoms suggestive of anaemia noted and Palliative care Outcome Score (POS) recorded for dyspnoea and fatigue in patient's healthcare record.
- Once prescription is completed, email the request for red cells to bloodbank@Beaumont.ie. This must be followed up with a verbal request by phoning the Blood Bank.

2.13 Requesting of Blood Products with Special Requirements

See guidelines for use of CMV- and/or Irradiated blood components on hospital intranet refer to BTD-HVI-007

2.14 Maximum Surgical Blood Ordering Schedule

The Maximum Surgical Blood Order Schedule (M.S.B.O.S) should be adhered to when taking TS samples and ordering Blood for surgical procedures. The M.S.B.O.S is available for review as a Clinical Policy on Hospital intranet. Each member of staff has a professional responsibility to avoid over exposure of patients to blood / blood products. Over ordering of blood / blood products and unnecessary TS sampling should be avoided in order to prevent wastage and over phlebotomising patients. Blood / Blood Products are extremely costly and are frequently in short supply. Where a patient has allo-antibodies, please give the Hospital Blood Bank 24 hours' notice prior to surgery. (Refer to BTD-HVI-008).

The MSBOS is a guide only and should never replace expert clinical judgement.

2.15 BloodTrack

Bloodtrack is used to monitor and record all transactions in the relation to movement of Red Cells, Platelets and Granulocytes. It allows a full electronic audit trail of these components. Users of the BloodTrack System must be trained in such prior to use.

Blood and platelets are processed for electronic move out / move in to fridges / platelet agitators respectively via the Blood Track system. All other Blood Products are signed out manually via the Blood and Blood Products register. If Blood Track is non-functional all products must be manually signed out of the Blood and Blood Products register. (Refer to BTD-HVO-040 on hospital intranet).

BloodTrack is also used for specimen collection in Beaumont Hospital.

2.16 EMERGENCY ISSUE OF BLOOD COMPONENTS AND PRODUCTS

If no TS or testing is incomplete and patient is unable to wait for compatible red blood cells, the lab can issue uncrossmatched RCCs immediately once requested by clinician.

- O NEG uncrossmatched units are available in the Blood Transfusion Issue fridge for use in emergencies.
- Ensure a Type and Screen specimen is taken before the O NEG uncrossmatched units are transfused.
- O NEG uncrossmatched units are not antigen negative specific to antibodies that a patient may have and therefore may result in incompatible units being transfused.
- O NEG stock is limited. Once these stocks are depleted it may be necessary to switch to O POS if patient is
  - Male
  - Female > 55 years

2.17 MASSIVE TRANSFUSION PROTOCOL

A successful Massive Transfusion Protocol can provide a timely, coordinated, delivery of blood products to the bleeding patient refer to BTD-HVO-042 Emergency/Massive transfusion protocol (MTP) and MTP Poster BTD-F-0283

- Accurate patient identification is of paramount importance. Every patient must wear an identification wristband (beware of external wristbands!).
- The Serious Hazards of Transfusion (SHOT) report suggests that the risk of error may be particularly high in an emergency situation. It is important that a correctly labelled TS specimen is taken before any blood products are administered. Use ED TRAUMA numbers if required and PDA device to ensure safe transfusion.
- One person should be nominated to liase with the BTD ensuring clear communication and to avoid confusion and unnecessary phone calls.
- When the MTP has ceased, communicate this to the Blood Bank.
- Traceability of blood products is a legal requirement – ensure traceability has been recorded / maintained for all blood products transfused or discarded in the event.

- Early administration of Tranexamic acid (Crash2 study)
- The use of rapid infusers / blood warmers are recommended for RCCs and fluids in major haermorrhage to avoid hypothermia which can exacerbate the clinical situation.

Bleeding in patients on anticoagulant therapy. **Discuss with Consultant Haematologist**. (Refer to Q pulse document PPCC-HAEM-24)

A&E / Pharmacy:

- Dabigatran- specific reversal agent Praxbind (Idarucizumab)
- Direct Factor-Xa Inhibitors (Rivaroxaban, Apixaban) – Ondexxya (Andexanet Alpha).

Bloodbank:

- In life threatening bleeds the products available from the BTD are PCC, rFactor VIIa - under the direction of the haemotology team.

2.18 Collection from the Blood Transfusion Laboratory

Only collect the Components/Products from the Hospital Blood Bank when required, as Components/Products must not be stored outside strictly controlled storage conditions or in areas such as ward refrigerators

Theatre are the only department with a remote temperature controlled fridge to store crossmatched red cells only.

Blood components and products are transported within Beaumont Hospital and St Lukes Radiation Oncology Centre by trained porters and healthcare assistants. Blue cabs transfer to St. Francis Hospice sites & St. Joseph's Hospital Raheny refer to BTD-HVO-031

2.19 Blood / Blood Product Administration

Bloodtrack PDAs are used to transfuse Red cells and platelets. The product barcodes and patient ID band are scanned on commencement of transfusion of Red cells and Platelets to a patient. Any discrepancies will be highlighted on PDA device and the transfusion cannot proceed. Contact the Blood Transfusion Dept for any alerts / alarms.

The manual method is used for all other blood products or can used for blood and platelets if bloodtrack is unavailable or undergoing upgrade. This requires 2 fully trained staff members checking and signing transfusion documentation independent of each other.

**See quick reference blood transfusion product administration guidelines on hospital Intranet refer to BTD-HVI-001 and BTD-HVO-009 Blood Administration.

2.20 TRACEABILITY

It is a legal requirement to maintain 100% traceability of all blood and blood products. If Bloodtrack is used, traceability is electronically updated. Following commencement of transfusion the labels generated should be placed in the patient transfusion record.
For manual transfusions, the middle pink portion of the compatibility label must contain the signatures of both administrators and the date and time of transfusion. This section is then removed and placed in the designated traceability box in the clinical area and / or returned to the blood bank as soon as possible for fating of the product. . The lower portion of the compatibility label is also signed by both administrators, including the date and time. This lower portion is then placed in the patient's medical record. The patient transfusion record must also be signed and updated by both staff members with record of the transfusion.

2.21 MANAGEMENT OF TRANSFUSION REACTIONS

Serious adverse Events & Reactions
It is mandatory to report all serious errors, incidences and reactions related to transfusion to Haemovigilance / Blood Transfusion Department. This data is then reviewed by the Consultant Haematologist and Hospital Transfusion Committee and may be submitted to the NHO (National Haemovigilance Office) in collaboration with the HPRA (Health Products Regulatory Authority) for further review.

Please refer to Transfusion reaction information on the Hospital intranet site refer to BTD-HVF-023 Transfusion Reaction Notification Form.

Also: BTD-HVO-015 Serious Adverse Reactions (Clinical Areas) –Mandatory Reporting including Rapid Alert Procedure), BTD-HVO-016*:* Serious Adverse Events (Clinical Areas) –Mandatory Reporting including Rapid Alert Procedure), Guidelines on the investigation and management of acute transfusion reactions, British Society of Haematology, 2023 (BSH website).

2.22 CLINICAL ADVICE

A Consultant Haematologist with Administrative Charge for the Blood Transfusion Department is in place. This Consultant Haematologist provides an extensive advisory service and clinical advice. Examples include indications for platelet transfusion, management of massive transfusion and the appropriate use of blood products.

Issues relating to Haemovigilance policies and protocols are referred to the Haemovigilance officers. Examples include sample labelling and management of transfusion reactions.

Comments or suggestions relating to the service should be addressed to the Chief Medical Scientist

Further information on all aspects of Blood Transfusion including blood product information, administration guidelines, relevant policies and procedures, forms etc are all available on the Beaumont Blood Transfusion Intranet page. Please email haemovigilance@beaumont.ie with any questions or queries.

2.23 BLOOD TRANSFUSION

2.23.1 *Test Repertoire and Blood Components/Products Available*

Test	**Sample Type**	**Minimum Volume**	**TAT**	**Comment**	**Mnemonic**
Type & Screen	4.9ml EDTA Specimen bottle labelled: "EDTA - FOR BLOOD BANK" (Blue top tube)	2.5ml	**Routine:** Same day if received before cut off time of 17:00 during routine hours. **Emergency:** 1 hour	TS specimens should be taken using the Blood Track™ PDA devices. When the Blood Track™ PDA device is not available the patient details must be handwritten on the specimen bottle. Unavoidable delays in the provision of results can occur when a patient has a positive antibody screen and/ or when a specimen is referred to the IBTS.	TS
Direct Antiglobulin Test	EDTA Specimen (2.7 ml)	2.5ml	**Routine:** Same day if received before cut off time of 17:00 during routine hours. **Emergency:** 1 hour	A PIPE label placed over the manufacturer's label on the specimen bottle. .	DAT
Transfusion Reaction Investigation	4.9ml EDTA Specimen + 7.5ml clotted serum specimen	2.5ml	Depends on the complexity of investigation.	Should be sent to the hospital Blood Bank as soon as possible after taking the specimens.	TRX

Test	**Sample Type**	**Minimum Volume**	**TAT**	**Comment**	**Mnemonic**
ABO Antibody titration	4.9ml EDTA Specimen.	4.0ml	**Routine:** 24 hours from time of receipt during routine hours **Urgent:** Same day if received before 1400.	This test is requested only under the instruction of the Nephrology team.	TITR
Cold Agglutinins	2 X 4.9ml EDTA specimen bottle.	4.9ml.	5 working days from sample receipt by NHSBT As per Specimen User Manual IBTS	PDA should be used or handwritten using 3 patient identifiers Referred to IBTS	COLDAGGS
ABO Rh D grouping for Living donor and H&I	4.9ml EDTA Specimen.	2.5ml.	**Routine:** Same day if received before cut off time of 17:00 during routine hours. **Emergency:** 1 hour		NA
RC	N/a	N/a	With valid TS: 15 mins to 3hours * From receipt of TS: up to 4 hours* Emergency • Uncrossmatched RC: <10 mins • Crossmatched RC: 60 min from receipt of TS*		BBRC

Test	Sample Type	Minimum Volume	TAT	Comment	Mnemonic
Platelets	N/a	N/a	15mins to 12hours ** The blood bank strives to maintain 2 stock platelets at all times for emergency issue. However this depends on demand and supply. Non-emergency orders should be placed prior to 14:00 hrs during routine working hours and will be available from 16:30 hrs the same day and before 22:00 hrs for approx. 11am next day delivery.		BBPLT
Frozen Plasma	N/a	N/a	40min to 3 hours		BBPLASMA
Fibrinogen Concentrate and Albumin	N/a	N/a	15min to 60mins		BBDER
PCC and factor concentrates	N/a	N/a	15 mins to 60 mins Discussion with Haematology Medical Team required		BBOCT BBDER

*Turnaround Time provided the patient has no Antibodies or blood group discrepancies. **Availability is dependent on national supply

*Specimens referred to the IBTS for antibody investigation, serological crossmatch or/and Cold Agglutinins, the results of these tests are not covered by the scope of Beaumont Hospital Blood Bank Department ISO15189 accreditati

2.23.2 ***Specialised Tests Referred to the IBTS (Irish Blood Transfusion Service)***

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

* In these circumstances, requests should be confirmed by the patient's Consultant and the IBTS medical personnel prior to referring a specimen.
Dispatch forms and specimens to the Haematology Secretary, Pathology Laboratories, Haematology Secretary Lower Ground Floor

2.24 HAEMATOLOGY

2.24.1 *Thrombophilia Screening*

Beaumont Hospital's specialist coagulation laboratory has a test repertoire which includes **Inherited Thrombophilia Screens, Lupus Anticoagulant, Clotting Factor Assays and von Willebrand Screens**. In the majority of cases, patients do not meet the clinical criteria for testing.

When there is a clinical indication for testing, specialist interpretation is required so that patients can be counselled appropriately, and the ability to interpret these tests correctly requires knowledge of the clinical scenario.

The Hematology Department has introduced a demand management protocol to limit access to these specialised tests.

From 1St May 2023 onwards General Practitioners will no longer be able to request the following tests directly:

- **Inherited Thrombophilia Screen** (Antithrombin, Protein C, Protein S, Factor V Leiden Mutation and Prothrombin Gene Mutation)
- **Lupus Anticoagulant**
- **Clotting Factor Assays** (Factors II, V, VII, VIII, IX, X, XI and XII)
- **von Willebrand Screen** (vWF:Ag and Factor VIII)

Testing will be reserved for specific patients who are deemed to meet the clinical criteria after being reviewed by the Haematology team.

If you believe your patient meets the criteria for testing, they should be referred to the relevant specialist, e.g.:

- If you suspect your patient has a bleeding disorder or an inherited thrombophilia, they can be referred to Dr Karl Ewins' Specialist Coagulation Clinic (Department of Haematology).
- If the patient has a family member with a known bleeding disorder who already attends the National Coagulation Centre (NCC) in St James's Hospital, they should be referred directly to the NCC for family screening.
- Please note that Antiphospholipid Syndrome is <u>not</u> an inherited disorder, so family screening is not required.
- Please note that recurrent miscarriage is <u>not</u> an indication for inherited thrombophilia testing and such requests will not be processed.

If you believe your patient warrants <u>urgent</u> investigation, please contact the Haematology Registrar on consults via Beaumont Hospital switch 01-8093000.

The current national thrombophilia laboratory testing guideline can be accessed at:
https://www.hse.ie/eng/about/who/cspd/ncps/pathology/resources/national%20laboratory%20handbook.html

<u>WHAT SAMPLES ARE REQUIRED?</u>

- Complete the Request Form with the Patient's name, DOB, unique Number, Ward/Location and Thrombophilia Screen (TPSC) requested
- Include patient relevant clinical information on the form
- Include anticoagulant status.
- Date and sign
- Take FOUR 2.9 mL Tri-sodium citrate 9NC (green) samples and
- Take ONE 2.6 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.
- **If a patient is on anticoagulation at the time of testing, certain assays within the Thrombophilia profile may be rejected, see above.**

2.24.2 *Referral of TPSC samples from an External Hospital*

<u>If sending Separated Samples:</u>

Plasma samples must be separated by a double centrifugation procedure according to the following instruction in order to prepare platelet poor plasma (plt< 10 $x10^9$/L).

- Check all samples for clots and adequate volume prior to centrifugation.
- Centrifuge samples for 10 mins at 3,000 g at RT°C.
- Pool plasma from all samples and centrifuge for a second time.
- Aliquot approximately 1 ml of plasma into a minimum of seven 1.5mL Micro Tube PCR-PT
- Frozen samples must be sent in appropriate frozen transport containers. All samples must remain in these containers.

2.24.3 *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.

2.24.4 *Blood Film Examination*

All FBCs are screened to see if a blood film will be of benefit to the patient/clinician. If the FBC results and instrument flags obtained meet the criteria required for blood film examination as set down by the Haematology Consultants, a film will be examined irrespective of whether one was ordered or not.
All FBCs with results/flags which do not meet these criteria will not have a blood film added on unless clinical details are provided. The following comment will be added in such instances 'Due to the volume of FBC and blood film requests received form GP practices, blood film requests will no longer be processed unless they meet the laboratory criteria for blood film examination as set down by the Haematology Consultant.'

2.24.5 *Haematology Molecular Tests*

- **All requests** for Haematology Molecular testing will not be processed unless accompanied by the fully completed request form, TPSC Request form HAEMC-LF-023 or Haemochromatosis Genetic Screening request form HAEMC-LF-077.
- **Changes to genetic consent for all haematology molecular tests (HFE, Fator V Leiden and PT mutation)**. From the 04/07/2022, the laboratory will no longer take receipt or store the form containing patient genetic consent. It is the responsibility of the ordering clinician to obtain and file a copy of genetic consent in the patient's record.

- A new form HAEMP-LF-003 "*Haematology Genetic Consent Form"* is available on the Beaumont Hospital Internet/Intranet site and must be printed and kept in the patient's record by the ordering clinician.

If this form is received in the lab it will be disposed and not returned to the sender.

2.24.5.1 Haemochromatosis Testing

Due to the significant increase in orders for Haemochromatosis HFE genotypes, the Haematology Department is introducing a demand management protocol to

address unnecessary ordering of this test. From the 04/07/2022 all HFE test requests **must** fulfill the following criteria:

- Testing of adult siblings (brothers and sisters) and adult offspring of **p.C282Y homozygotes** is recommended owing to increased risk of p.C282Y homozygosity and related increased morbidity. **Please indicate on the form if there is a First Degree Relative (SIBLING/PARENT) with p.C282Y homozygosity. Stating both relationship and genotype.**

- The results of iron studies: Iron, Ferritin and Transferrin Saturation (%) are available and meet certain criteria. In particular, the Transferrin Saturation should be raised (> 45%).

- **It is required to confirm elevated Transferrin Saturation on two occasions before HFE genetic diagnosis testing.**

- **Note: if Transferrin Saturation <45% and/or no first degree relative with p.C282Y homozygosity, the sample will not be processed.**

- Samples must be sent to the laboratory with the updated form *HAEMC-LF-077 "Haemochromatosis Genetic Screening Request Form" Version 3.* **If any form other than this version is sent to the laboratory, the samples will be rejected and not processed.**

Note: these guidelines are formed in line with Best Practice Guidelines "EMQN best practice guidelines for the molecular genetic diagnosis of hereditary haemochromatosis (HH) European Journal of Human Genetics (2016) 24, 479–495"

2.24.6 *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

2.25 CHEMICAL PATHOLOGY

See section 3.3 below

2.26 IMMUNOLOGY

2.26.1 *Rheumatoid Factor*

INDICATIONS

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

INTERPRETATION OF RESULTS

In October 2020 we changed the method for RF from Nephelometry to Immunoturbidimetry with updated reference ranges as outlined in the table below.

Assay	Old Reference Range	New Reference Range
RF	<20 IU/mL	<14 IU/mL

Negative rheumatoid factor, <14 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, 14-70 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.

2.26.2 *Anti-Cyclic Citrullinated Peptide antibodies (CCP)*

INDICATIONS

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

INTERPRETATION OF RESULTS

If result is <7 U/ml = Negative CCP

Anti-CCP has a sensitivity of 80% for detection of Rheumatoid arthritis (RA). It appears less sensitive for detection of extra-articular disease. If RA is strongly suspected, RF should be measured, as at least 10% of RA patients are negative for CCP, but positive for RF.

If result is 7 – 10 U/ml = Equivocal

If patient has evidence of an inflammatory arthropathy, suggest referral to Consultant Rheumatologist.

If result is >/= 11 U/ml = Positive CCP

Anti-CCP antibodies appear to be relatively specific for rheumatoid disease (Specificity 96%). Suggest referral to Consultant Rheumatologist

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.

2.26.3 *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.

Clinical indication	**Suggestions**	**Comments**
Connective tissue disease screen Excluding connective tissue disease in patients with low clinical suspicion	CTD screen C3 C4	Positive CTD screen samples will be tested for ENA and DNA
Scleroderma	CTD screen ENA (for anti-Scl-70)	If scleroderma suspected, consider rheumatology opinion, regardless of results

Clinical indication	Suggestions	Comments
Myositis	CTD screen ENA (for anti-Jo1)	If negative Jo-1 but clinical suspicion high with a raised CK, please discuss with laboratory (details as below)
Raynaud's with suspicion of connective tissue disease	CTD screen, C3, C4 HEP2 ANF	HEP2ANF: ANF will be tested by indirect Immunofluorescence on HEP 2 cells
Monitoring lupus	DNA, C3, C4	
Patients with negative CTD screen but strong clinical suspicion	Liaise with lab : ANF by immunofluorescence is available if clinically indicated	immunologylab@beaumont.ie Lab extension 2635 Clinical team bleep 797
Autoimmune liver disease	Liver antibodies panel	ANF component is tested by immunofluorescence on HEP 2 cells

In most cases, a positive CTD screen result would also yield a positive result for anti-DNA and/or anti-ENA. The lab will automatically test for anti-DNA and anti-ENA on all equivocal & positive CTD screen results.

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

If CTD screen is negative, connective tissue disease is unlikely.

If CTD screen is positive or equivocal, follow on testing for anti-ENA and anti-DNA will be done.

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

INDICATIONS

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

ANF (also known as ANA for anti-nuclear antibody) 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.*

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).

2.26.5 *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.

2.26.6 *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 1:160 or greater
- Clinical suspicion of SLE/CTDs with ANF of 1:160/1:320
- 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.

2.26.7 *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.

2.26.8 *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.

2.26.9 *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.

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

Urgent service and plasmapherisis monitoring available.

INDICATIONS

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

In December 2022, the Immunology Department updated our ANCA testing algorithm in line with the current International Consensus for ANCA testing. As per the consensus, we now carrying out anti-MPO and anti-PR3 antibody tests as the first line test when ANCAs are requested.

There was no change in the requesting pathway. The test code for requesting the test remains the same as before i.e. ANCA. However the sample is processed for the more specific anti-MPO and anti-PR3 immunoassays as the first line tests instead of ANCA by Indirect Immunofluorescence (IIF). Any new positives for anti-MPO and anti-PR3 will also be tested by IIF.

ANCA by IIF is still be available in the laboratory and can still be specifically requested if required for individual patients. The test code for requesting ANCA by IIF is IIFANCA.

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 (includingTB)
- 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 anti-nuclear 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**. However with the current testing algorithm, this scenario will be less frequently encountered as anti-nuclear factor does not interfere with the specific MPO and PR3 immunoassays.

INTERPRETATION OF RESULTS

Negative MPO & PR3: Active vasculitis is highly unlikely in patients with negative MPO-ANCA and PR3-ANCA.

Weak MPO – first testing (MPO 3.5 to 10 IU/mL): Weakly positive MPO-ANCA is frequently not associated with vasculitis and may be seen in a variety of inflammatory conditions, including infection. However, patient should be thoroughly reassessed, including urinalysis, to exclude a vasculitis. Weakly positive MPO-ANCA may be associated with renal-limited disease. ANCA by immunofluorescence to follow.

Weak PR3 –first testing (PR3 2 to 10 IU/mL): Weakly positive PR3-ANCA may NOT be associated with vasculitis, however patient should be thoroughly reassessed, including urinalysis, to exclude evidence of a vasculitis. Weakly positive PR3 may be associated with limited disease. ANCA by immunofluorescence to follow.

Positive MPO – first testing (>10 IU/mL): Positive MPO-ANCA is suggestive of vasculitis, which may be renal limited. Occasionally MPO-ANCA may be seen in other inflammatory conditions. ANCA by immunofluorescence to follow.

Positive PR3 – first testing (>10 IU/mL): Significantly positive PR3-ANCA is suggestive of vasculitis, particularly Granulomatosis with polyangitis (GPA). However false positives do occur- hence the result is not entirely specific. ANCA by immunofluorescence to follow.

IIFANCA: ANCA is positive in over 90% of patients with generalised Granulomatosis with polyangitis (GPA) or microscopic polyarteritis (MPA). If result is positive / equivocal / obscured, please refer to the results for anti-MPO and anti-PR3. If ANCA is negative these conditions are less likely, however it does not completely exclude them. For positive / equivocal / obscured ANCA with a negative anti-MPO and PR3 results, then this is not usually associated with systemic vasculitis. Non specific ANCA positivity may be found in other inflammatory conditions including intercurrent infection.

Monitoring disease activity – Serial Measurement of Anti-MPO or Anti-PR3:

Serial measurement of anti-MPO or anti-PR3 can be helpful in monitoring response to treatment or disease activity in patients who have ANCA associated vasculitis, the frequency of testing should take into account of the half life of IgG which is 3 weeks. Therefore 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.

2.26.11 ***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.

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.

In June 2023, received a Field Safety Notice regarding the EliA anti-GBM assay that we use from the reagent manufacturer. There has been reports of false positive anti-GBM results with this EliA method. Investigations by the manufacturer suggest that the false positive results were due to cross reactive antibodies i.e. not due to antibody to the GBM antigen. The Field Safety Notice we received is specific to the EliA anti-GBM assay. For any positive anti-GBM results, we will continue to contact the clinical team as we currently do and will issue a preliminary result. We have identified an external laboratory abroad for secondary confirmatory testing for positive results. Should you have a patient with previously positive anti-GBM results that did not fit their clinical context and you wish to organise repeat testing done, please contact us. The Immunology

Clinical Team is also available should you wish to discuss a specific patient's anti-GBM result (Bleep 797 or via email immunologydepartment@beaumont.ie). Should you have any further queries, please do not hesitate to contact the laboratory on (01) 8092635.

2.26.12 *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 (>40GPLU/mL) IgG or IgM anti-cardiolipin
- Lupus anticoagulant
- Anti- Beta 2 glycoprotein 1 antibody

While many patients with APS will have abnormal results in both tests, approximately 10% of patients are positive for lupus anticoagulant only with normal anti-cardiolipin antibodies. Therefore when APS is suspected **both** anti-cardiolipin and lupus anticoagulant should be routinely requested. When clinical suspicion of APS is high, β2Glycoprotein 1 should also be requested. Lupus anticoagulant test is offered by the Haematology (Coagulation) laboratory, please refer to the relevant section with regards to test / specimen requirements.

INTERPRETATION OF RESULTS

Negative IgG and IgM anti-cardiolipin antibodies: Antiphospholipid syndrome (APS) unlikely, however lupus anticoagulant testing could be considered if APS strongly suspected.

Weak positive (IgG and/or IgM anti-cardiolipin >=10 <=40 GPLU/mL or MPLU/mL): Weakly positive Anti-Cardiolipin Antibodies which do not fulfill the laboratory criteria for antiphospholipid syndrome. A moderate or high titre Anticardiolipin IgG and/or IgM antibody of ≥40 GPL units or MPL units is considered to be positive in this institution. To fulfill the laboratory criteria for antiphospholipid syndrome, a patient must have persistent positivity of one or more antiphospholipid antibodies on 2 or more occasions, at least 12 weeks apart.

Positive (IgG and/or IgM anti-cardiolipin >=40 GPLU/mL or MPLU/mL): Positive Anticardiolipin IgG and/or IgM antibodies. A moderate or high titre Anticardiolipin IgG and/or IgM antibody of ≥40 GPL units or MPL units is considered to be positive in this institution. To fulfill the laboratory criteria for antiphospholipid syndrome, a patient must have persistent positivity of one or more antiphospholipid antibodies on 2 or more occasions, at least 12 weeks apart. Both clinical criteria (e.g. thrombosis or pregnancy morbidity) and laboratory criteria need to be fulfilled to make a diagnosis of APS.

2.26.13 ***Antibodies to Beta 2 Glycoprotein 1***

INDICATIONS

Suspected Antiphospholipid syndrome
See Section 2.4.121.1.1

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

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

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

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

INTERPRETATION OF RESULTS

Negative: Antiphospholipid syndrome (APS) unlikely, however lupus anticoagulant testing could be considered if APS strongly suspected.

Weak Positive Anti B2 glycoprotein IgG Antibodies >=10 & <40: Weakly positive Anti-β2–glycoprotein I IgG antibody which does not fulfill the laboratory criteria for antiphospholipid syndrome. An Anti-β2–glycoprotein I IgG antibody titre > the 99th centile is considered to be positive (i.e. ≥40 U/mL in this institution). To fulfill the laboratory criteria for antiphospholipid syndrome (APS), a patient must have persistent positivity of one or more antiphospholipid antibodies on 2 or more occasions, at least 12 weeks apart. Both clinical criteria

(e.g. thrombosis or pregnancy morbidity) and laboratory criteria need to be fulfilled to make a diagnosis of APS.

Positive Anti B2 glycoprotein IgG Antibodies >=40: Positive Anti-β2–glycoprotein I IgG antibody. An Anti-β2–glycoprotein I IgG antibody titre > the 99th centile is considered to be positive (i.e. ≥40 U/mL in this institution). To fulfill the laboratory criteria for antiphospholipid syndrome (APS), a patient must have persistent positivity of one or more antiphospholipid antibodies on 2 or more occasions, at least 12 weeks apart. Both clinical criteria (e.g. thrombosis or pregnancy morbidity) and laboratory criteria need to be fulfilled to make a diagnosis of APS.

Weak Positive Anti B2 glycoprotein IgM Antibodies >=10 & <17: Weakly positive Anti-β2–glycoprotein I IgM antibody which does not fulfill the laboratory criteria for antiphospholipid syndrome. An Anti-β2–glycoprotein I IgM antibody titre > the 99th centile is considered to be positive (i.e. ≥17 U/mL in this institution). To fulfill the laboratory criteria for antiphospholipid syndrome (APS), a patient must have persistent positivity of one or more antiphospholipid antibodies on 2 or more occasions, at least 12 weeks apart. Both clinical criteria (e.g. thrombosis or pregnancy morbidity) and laboratory criteria need to be fulfilled to make a diagnosis of APS.

Positive Anti B2 glycoprotein IgM Antibodies >=17: Positive Anti-β2–glycoprotein I IgM antibody. An Anti-β2–glycoprotein I IgM antibody titre >the 99th centile is considered to be positive (i.e. ≥17 U/mL in this institution). To fulfill the laboratory criteria for antiphospholipid syndrome (APS), a patient must have persistent positivity of one or more antiphospholipid antibodies on 2 or more occasions, at least 12 weeks apart. Both clinical criteria (e.g. thrombosis or pregnancy morbidity) and laboratory criteria need to be fulfilled to make a diagnosis of APS.

2.26.14 *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.

2.26.15 *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.

2.26.16 *Anti-Liver Cytosol 1 (LC1) Antibodies*

Note: When IIF results demonstrate an anti-LC1 antibody, the specificity of this result is confirmed by an immunoblotting system.

INTERPRETATION OF RESULTS

Negative: No serological evidence of autoimmune hepatitis.

Positive IIF, Positive Immunoblot: 'Positivity for anti-LC1 antibody is suggestive of autoimmune hepatitis. Please correlate with clinical features, virology, other serology markers and histology features'

2.26.17 *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.

Note: Where M2 antibodies are detected by Immunoblot they are reported with appropriate interpretative comments.

2.26.18 *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.

2.26.19 *Anti-Intrinsic Factor Antibodies*

INDICATIONS

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

INTERPRETATION OF RESULTS

In September 2020 we changed the method for anti-intrinsic factor antibodies from ELISA to EliA. The Intrinsic Factor assay will continue to be reported qualitatively with the introduction of an equivocal range in addition to negative & positive. Interpretative comments will be added on all reports.

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.

Equivocal: The clinical significance of intrinsic factor results that fall in the equivocal range is uncertain. Correlation with clinical history and B12 level is advised.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.

2.26.20 *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 October 2020 we changed the method for anti-TPO antibodies from EliA to Immunoassay. Both assays use the same units but the Immunoassay 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:

	Negative result	**Equivocal Result**	**Positive Result**
OLD EliA ASSAY IU/mL	<25	25-35	>35
CURRENT Immunoassay ASSAY IU/mL	<=34		>34

Negative (Anti-TPO <= 34 IU/mL): Autoimmune thyroid disease unlikely.

Positive (Anti-TPO > 34 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.

2.26.21 *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.

2.26.22 *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 deficiency is excluded by using the background reading on the EliA or measurement of total IgA. Total IgA is measured on all children with a negative TTG. In cases of IgA deficiency, IgG EMA testing or other IgG serological testing is performed. Anti-tTG has a high sensitivity for untreated coeliac disease, while the anti-endomysial antibody is more specific. Sequential testing offers optimal diagnostic utility.

Please refer to NCPP Serological Testing for Coeliac Disease Guideline from National Laboratory Handbook for further guidance.

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.

2.26.23 *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 and autoimmune disease. You may wish to arrange for a Clinical Immunology appointment for further assessment.

2.26.24 *Anti-Neuronal Antibodies*

INDICATIONS

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

A screening indirect immunofluorescence assay (IIF) is performed, with a follow up confirmatory Immunoblot. The presence of an ANF renders the IIF test difficult to interpret. ANF positive specimens are also run on the Immunoblot. Not all antibodies available on the Immunoblot have concurrent specific Immunofluorescent staining patterns. Both IIF and Immunoblot results must be interpreted in the clinical context. If you are concerned about some of the more recently described antibodies please discuss the case with Senior Laboratory Staff or Prof. Keogan/Dr Khalib.

INTERPRETATION OF RESULTS

Negative Neuronal Antibodies: Negative results do not exclude a paraneoplastic syndrome. Correlation with other clinical findings is advised.

Positive Anti-Hu: Also known as Type I anti-neuronal nuclear antibody (ANNA-1) is associated with Cerebellar ataxia, paraneoplastic encephalomyelitis and sensory neuropathy. It has been reported in patients with Small cell lung carcinoma and neuroblastoma. Correlation with other clinical findings is advised.

Positive Anti-Yo: Also known as Anti-Purkinje cell antibody is associated with Paraneoplastic Cerebellar Degeneration. It has been reported in patients with Breast and Ovarian carcinoma. Correlation with other clinical findings is advised.

Positive Anti-Ri: Also known as Type II anti-neuronal nuclear antibody (ANNA-2) is associated with Cerebellar degeneration and paraneoplastic opsoclonus myoclonus ataxia (POMA). It has been reported in patients with neuroblastoma (children) and fallopian, breast and small cell lung carcinoma (adults). Correlation with other clinical findings is advised.

Positive Anti-Amphiphysin: Associated with Stiff person's syndrome (5%) and paraneoplastic encephalomyelitis. It has been reported in patients with breast and small cell lung carcinoma. Correlation with other clinical findings is advised.

Positive Anti-Cv2/CRMP5: Associated with Paraneoplastic encephalomyelitis/sensory neuropathy. It has been reported in patients with

thymoma and small cell lung cancer. Correlation with other clinical findings is advised.

Positive anti-PNMA2: Anti-PNMA2 antibody (also known as anti-Ma2 or anti-Ta) is associated with cerebellar, limbic or brainstem encephalomyelitis. It has been reported in patients with testicular tumours. In a proportion of patients, there is co-existing anti-Ma1 antibody which has been associated with brainstem / cerebellar syndromes and various non-testicular tumours. Correlation with other clinical findings is advised.

Positive Anti-Recoverin Antibody: Anti-recoverin antibody is significant for tumour-associated retinopathy; a paraneoplastic syndrome mostly reported in patients with small-cell lung carcinoma but has also been reported in patients with thymoma, endometrial and prostate carcinoma. NOTE: These antibodies are not detected by IIF on cerebellum tissue as these antigens are not normally expressed in cerebellum, so Immunoblot results cannot be verified by IIF testing. Correlation with other clinical findings is advised.

Positive Anti-SOX1 Antibody: Anti-SOX1 antibody is associated with Lambert-Eaton Myasthenic syndrome (LEMS) with a specificity of up to 95% for small cell lung carcinoma in LEMS. It has also been reported in paraneoplastic cerebellar degeneration as well as paraneoplastic and non-paraneoplastic neuropathy. Correlation with other clinical findings is advised.

Positive Anti-Zic4 Antibody: Anti-Zic4 antibody is associated with paraneoplastic cerebellar degeneration and is often indicative of small-cell lung carcinoma. Up to 82% of patients can have positivity for other antibodies such as anti-Hu and anti-CV2/CRMP5 antibody. Correlation with other clinical findings is advised.

Positive Anti-Titin Antibody: Anti-Titin antibodies target Titin; a filamentous protein of striated muscle. These antibodies occur in myasthenia gravis alongside acetylcholine receptor antibodies. In many patients they are indicative of the additional presence of thymoma. Anti-Titin serum titre is thought to correlate with the severity of Myasthenia gravis. NOTE: These antibodies are not detected by IIF on cerebellum tissue as these antigens are not normally expressed in cerebellum, so Immunoblot results cannot be verified by IIF testing. Correlation with other clinical findings is advised.

Positive Anti-GAD65 Antibody: Anti-GAD65 antibody is associated with Stiff-person syndrome and paraneoplastic cerebellar ataxia. Small cell lung carcinoma, breast carcinoma and colon carcinoma are the most frequent tumours associated with anti-GAD antibodies. Correlation with other clinical findings is advised.

Positive Anti-Tr Antibody: Anti-Tr antibody is also known as Anti-PCA-Tr, and anti-DNER (Delta/Notch-like epidermal growth factor related receptor), and are

associated with paraneoplastic cerebellar degeneration. These are mostly associated with Hodgkin's lymphoma but have also been reported in non-Hodgkin's lymphoma. Correlation with other clinical findings is advised.

Paired Serum/CSF samples will be accepted for this screening test, results will be reported accordingly & must be interpreted within the clinical context. If you wish to discuss, please contact Senior Laboratory Staff or Prof. Keogan/Dr Khalib.

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.

2.26.25 *Autoimmune Encephalitis antibodies - Anti-NMDA and MOSAIC 6*

Autoantibodies against neuronal surface antigens are found in patients with autoimmune encephalopathies. The antibodies are directed against glutamate receptors (type NMDA and type AMPA), $GABA_B$ receptors, Voltage gated potassium channels or VGKC associated proteins (LGI1, CASPR2 and DPPX). The frequency of an underlying tumour ranges depending on the type of antibody. Early diagnosis can support a favourable prognosis. Prognosis for patients is improved with appropriate immunomodulatory therapy, and, in paraneoplastic syndrome, tumour detection and resection as early as possible. Antibodies can be either determined in Serum or CSF. In certain cases Plasma samples are acceptable. Paired CSF/Serum samples are the preferred sample type for this type of investigation since intrathecal synthesis of antibodies can occur even when the serum titre is low/absent. This is particularly the case with anti-glutamate receptor antibodies (type NMDA and AMPA) and also $GABA_B$ receptor antibodies. A positive serum result with an associated CSF negative results have been reported in the literature, particularly where the autoantibody is a paraneoplastic one. If you are concerned about some of the more recently described antibodies please discuss the case with Senior Laboratory Staff or Prof. Keogan/Dr Khalib

Indications:

- Suspected Limbic Encephalitis
- Seizures
- Neuropsychiatric symptoms
- Suspected Paraneoplastic syndrome
- Neuromyotonia

INTERPRETATION OF RESULTS

Normal result: Negative

Negative result: does not exclude these conditions, particularly where serum only has been tested. If clinical suspicion remains high please contact the Immunology laboratory on (01) 8092635 to discuss.

NMDA: N-methyl-D-aspartate

NMDA antibodies are found in patients with behavioural cognitive problems and seizures. These can commonly progress over time to a movement disorder, autonomic fluctuations and coma.

With the NMDA fixed assay, up to 14% of patients with anti-NMDA encephalitis have been reported to have anti-NMDA antibodies in CSF only. Therefore if

serum is negative suggest sending a CSF sample if clinical suspicion remains high. Additionally, if both serum and CSF are negative by NMDA fixed assay please contact the Immunology lab on (01) 8092635 to discuss further testing if anti-NMDA encephalitis remains a diagnostic possibility.

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazol-propionic acid

Anti-AMPA antibodies to the GluR1/GluR subunits of glutamate receptors type AMPA 1&2 have been reported in patients with limbic encephalitis. A significant number of patients with AMPA associated encephalitis have also been found to have tumours (Breast, Lung, Thymoma). Results should be interpreted in the context of clinical findings

$GABA_B$: γ-amino-butyric acid

Anti-$GABA_B$ receptor antibodies have been reported in patients with limbic encephalitis. It is also associated with a paraneoplastic syndrome in up to 47% of patients. Results should be interpreted in the context of clinical findings

DPPX: dipeptidyl aminoperoxidase like protein 6.

This protein is mainly produced in the brain tissue and interacts with the voltage-gated K+ channel Kv4. Anti-DPPX antibodies have been seen in patients with encephalitis with prominent delirium, GI symptoms, and movement disorders. Results should be interpreted in the context of clinical findings.

LGI1: Leucine-rich glioma inactivated protein 1

Anti-LGI1 antibodies are found in patients with limbic encephalitis with a low plasma sodium. These antibodies have also been seen in patients with seizure disorders (particularly facrobrachial dystonic seizures). Results should be interpreted in the context of clinical findings.

CASPR2: Contactin-associated protein 2

Anti-CASPR2 antibodies have been found in patient with neuromyotonia, limbic encephalitis and/or epilepsy and more recently in patients with cerebellar ataxia. There is also an association with a paraneoplastic syndrome in up to 30% of cases. Results should be interpreted in the context of clinical findings.

Positive anti-CASPR2 in Serum at 1:10 dilution ONLY: 'Anti-CASPR2 antibodies POSITIVE at 1:10 dilution, indicating this is a **BORDERLINE POSITIVE** result. Positive results at this titre should be interpreted with caution, within the clinical context.'

2.26.26 *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.

2.26.27 *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)

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

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.

2.26.28 *Acute Allergic Reaction Investigation - Beaumont GP Service only*

INDICATIONS – Clinical symptoms suggestive of actute allergy. (Service provided to Beaumont GP's currently only).

Despite advances in testing, allergy remains a clinical diagnosis, based on an allergy focussed history, supported by evidence of allergic sensitisation. Recent national and multiple international guidelines emphasise the need for allergy testing to only be performed as indicated by the allergy focussed history.

Specific IgE blood tests (when available to the allergen in question) can be helpful to indicate allergic sensitisation. However as specific IgEs are only markers of sensitisation, not necessarily a clinical allergy; results require interpretation within the context of the clinical history. Sensitised patients may be clinically allergic OR may be sensitised and tolerant, with on-going tolerance depending on on-going exposure. Unfortunately we continue to see patients that have had their diet restricted solely based on specific IgE test results; some of whom have lost tolerance as a result.

Therefore, from 1st of March 2020, there will be a change in the pathway for requesting specific IgE testing, to facilitate the implementation of the recommendations of these guidelines, align with MedLIS requesting and contribute to improving patient safety.

The form required for requesting specific IgE tests is available for download from the Beaumont Hospital website from both the Immunology Department and the Laboratory Medicine homepage. There is a section for allergens that will remain available for direct ordering (common inhalant allergens– house dust mites, cat, dog, grass and tree pollens). For other allergens, please complete the Acute Allergic Reaction Information (AARI) section by providing the clinical details associated with the episode(s).

http://www.beaumont.ie/media/LF-IMM-GEN00241.pdf

If further clinical details are required, you may be contacted by a member of the Immunology team. If we are not successful in making contact, you will receive a report requesting that you make contact for discussion of clinical details. The sample will be stored for 2 weeks pending this discussion.

Reference:

https://www.hse.ie/eng/about/who/cspd/ncps/pathology/resources/total-ige-and-specific-ige.pdf

The Acute Allergic Reaction Investigation will be resulted to you in one of the following ways:

1. Based on clinical details provided, relevant specific IgEs have been requested. Report to follow.

2. Specific IgE test is not available for the suspected allergen based on the provided clinical details. If there are any queries regarding this, please contact us at immunologylab@beaumont.ie (quoting the patient's episode number and AARI in the subject line). For patient confidentiality, please ensure that only a secure healthmail email account is used. Sample will be stored for 2 weeks from date of receipt.

3. Specific IgE not indicated based on clinical details provided. If there are any queries or updates of the clinical information please contact us at immunologylab@beaumont.ie (quoting the patient's episode number and AARI in the subject line). For patient confidentiality, please ensure that only a secure healthmail email account is used. Samples will be stored for 2 weeks from date of receipt.

4. Incomplete clinical information provided in the accompanying request form. We have not been successful in making contact using the provided contact

details. Please email us at immunologylab@beaumont.ie (quoting the patient's episode number and AARI in the subject line) to confirm contact details and suitable times between Tuesdays and Thursdays for clinical discussion. For patient confidentiality, please ensure that only a secure healthmail email account is used. Serum sample will be stored for 2 weeks pending this discussion. If no email is received within the next 2 weeks, it will be assumed that specific IgE testing is no longer required and serum will be discarded.

5. Further discussion of the clinical details is required to guide specific IgE testing if appropriate. We have not been successful in making contact using the provided contact details. Please email us at immunologylab@beaumont.ie (quoting the patient's episode number and AARI in the subject line) to confirm contact details and suitable times between Tuesdays and Thursdays for clinical discussion. For patient confidentiality, please ensure that only a secure healthmail email account is used. Serum sample will be stored for 2 weeks pending this discussion. If no email is received within the next 2 weeks, it will be assumed that specific IgE testing is no longer required and serum will be discarded.

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.

2.26.29 *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 Prof. 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

In October 2020 we changed the method for C3 & C4 from Nephelometry to Immunoturbidimetry with updated reference ranges as outlined in the table below.

Assay	Old Reference Range	New Reference Range
C3	0.75-1.65 g/L	0.9-1.8 g/L
C4	0.14-0.54 g/L	0.1-0.4 g/L

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.

2.26.30 *Complement Function CH100*

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 and the Alternative pathways. 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

CLS-PATH Normal: Classical pathway functioning normally
CLS-PATH 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.

ALT-PATH Normal: Alternate pathway functioning normally
ALT-PATH Reduced or Absent: Decreased complement activity may be caused by deficiencies of any of the individual components of the Alternate pathway, hereditary or acquired.

2.26.31 *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 October 2020 we changed the method for C1 inhibitor from Nephelometry to Turbidimetry with updated reference ranges as outlined in the table below.

		Normal Range
C1 Inhibitor	Old assay	0.21-0.39 g/L
	Current assay	0.21-0.38 g/L

INTERPRETATION OF RESULTS

C1 INH Low <0.15g/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 (0.21-0.38 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 (> 0.38 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.

2.26.32 *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

In October 2020 we changed the method for ASOT from_Nephelometry to Immunoturbidimetry. There was no change in reference range.

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.

2.26.33 *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.

2.26.34 *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 tesing 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.

2.26.35 ***Specific IgGs***

INDICATIONS

- Suspected APBA
- Suspected extrinsic allergic alveolitis eg
- Farmer's Lung or Bird Fancier's Lung

2.26.35.1 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.

2.26.35.2 Specific IgG to Micropolysporia 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.

2.26.35.3 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.

2.26.36 ***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, HMGCR, cN1A 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 T1F1γ 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-HMGCR Antibody: This antibody has been reported in up to 60% of patients with necrotising myopathy. Approximately 30 – 60% of these patients have been reported to have previous statin exposure. This antibody has also been reported with a high frequency of malignancy in this condition. Close correlation with clinical history, physical findings, muscle enzyme CK levels and histology is advised

Positive anti- cN1A Antibody: This antibody has been reported in 30 – 60% of patients with sporadic inclusion body myositis. However close correlation with clinical history, clinical findings, muscle CK enzyme and histology is strongly advised, as this antibody has also been reported in other patient cohorts such as Sjogrens Syndrome and SLE, other inflammatory myopathies and other non-autoimmune neuromuscular conditions. This antibody has also been reported in up to 5% of healthy population.

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.

2.26.37 ***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.

2.26.38 *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

In October 2020 we changed the method for IgG Subclasses from Nephelometry to Turbidimetry with updated reference ranges as outlined in the table below.

Assay	Old Reference Range	New Reference Range
IgG1	3.2-10.2 g/L	3.824 - 9.286 g/L
IgG2	1.2-6.6 g/L	2.418 - 7.003 g/L
IgG3	0.2-1.9 g/L	0.218 - 1.761 g/L
Total IgG	6-16 g/L	7 - 16 g/L

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.

2.26.39 *Anti- SARS-CoV-2 Antibodies*

Method: Roche Elecsys Anti-SARS-CoV-2 which uses a recombinant protein representing the nucleocapsid (N) antigen for the determination of antibodies against SARS‑CoV‑2. In April 2021 we added a quantitative assay for Anti-Spike S RBD antibody as part of of our serology service for Anti-SARS-CoV-2 antibdies. These antibody tests are now both available as a panel, measured by Electrochemiluminescence Immunoassay (ECLIA) on the Roche cobas e immunoassay platform.

The Anti-Nucleocapsid antibody tests are reported as either Not Detected, Equivocal or Detected.

The reference range for the Anti-Spike antibody test is as follows

<0.8 U/ml – Anti-SARS CoV-2 S antibody not detected
≥0.8 U/ml – Anti-SARS CoV-2 antibody detected

Interpretive comments will be added on all reports

2.26.40 *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.

2.26.41 *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.

Condition	**Typical Finding**	**Site to Biopsy**	**Age of lesion**	**Accompanying Serology**
Pemphigus	Linear IgG positivity in a chicken-wire pattern in the epidermis	Perilesional skin	Close to new lesion	Antibodies to epidermal intercellular substance

Condition	**Typical Finding**	**Site to Biopsy**	**Age of lesion**	**Accompanying Serology**
Pemphigoid	Linear IgG (+/- C3) along the dermoepidermal junction.	Perilesional skin	Close to new lesion	Antibodies to epithelial basement membrane
Dermatitis Herpetiformis	IgA (+/- C3 & fibrin) in granular or fibrillary pattern in the papillary dermis	Peri-lesional, non-erythematous skin	Close to new lesion	Anti-endomysial antibody.
Vasculitis	Granular deposition of C3 (+/- C4) with at least one isotype of immunoglobulin in dermal vessels	Lesion	Fresh, preferably <24 hours	C3, C4. Cryoglobulins ANF + follow ANCA RF
DLE	Granular deposition of one or more immunoreactants along the dermoepidermal junction (lupus band)	Lesion	>3 months	ANF, Anti-DNA Anti-ENA

2.27 MICROBIOLOGY

2.27.1 *General Sample Collection Guidelines*

As per MEMO-MIC-0236 circulated in 2020, due to a national shortage of medical scientists, courier delivered samples from General Practitioners and Nursing Homes for microbiological investigations other than urine C&S, urinary HCG and faecal *Helicobacter pylori* antigen are currently being referred to Eurofins Biomnis Ireland for processing until further notice.

- Collect specimens aseptically in appropriate CE-marked leak-proof containers and transport in sealed plastic bags.
- A sufficient volume of material must be submitted (See Section 3.6)
- Swabs in transport media are acceptable for throat, eye, ears, 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 to prevent samples becoming compromised and rejected. If this is not possible, store specimens in fridge until they can be transported.

2.27.2 *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)
- Faecal culture assay now includes *Cryptosporidium parvum/hominis and Giardia lamblia* as standard.
- 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* suspected and the first three samples are negative, ideally four additional samples should be submitted at weekly intervals.

FAECES – CLOSTRIDIOIDES DIFFICILE

- Testing for *Clostridioides 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.
- For *H. pylori* test only, ensure to clarify on form that sample is only for *H. pylori* and not for faecal C&S.
- *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.

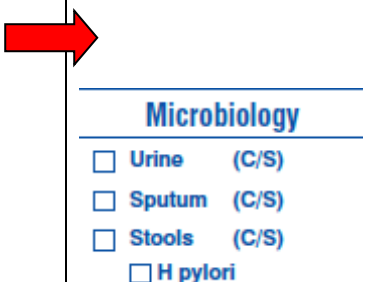
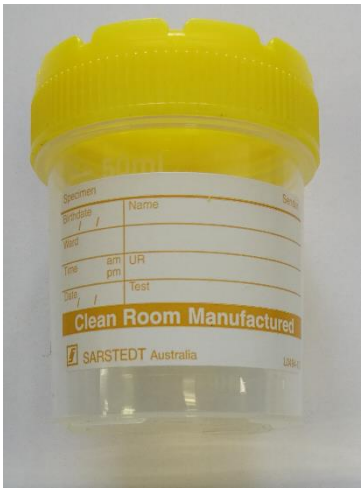
Urines for culture and sensitivity and pregnancy testing are now collected via the The Sarstedt NFT (Needle Free Transfer) system. This consists of a 100ml NFT primary container (Sarstedt Product Reference 75.562.900) and a 10mL Monovette tube (Sarstedt Product reference 10.252)

This system allows for the spill free collection and transfer of urine samples to the required 10mL Monovette tube (SeeTable 1 below).

- The 10mL Monovette **tube** is to be sent to the Microbiology lab for microbiology urine investigations (URCULT).
- ONLY 10mL Monovette tubes will be accepted and other container types will be rejected
- Both products are available from the Stores Department, Beaumont Hospital

.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 24 hours.

Table 1. Approved urine specimen collection containers.

Department	Test	Container
Microbiology	Urine culture and sensitivity Urine hCG	
Microbiology	Non-Urine Samples: Eg: Sputum Stool samples: *H.pylori* or culture and sensitivity 	

Select C/S **or** *H pylori* only		
Biochemistry	Urines for ACR PCRs etc	

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 >48hours 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 as per instructions on www.nvrl.ie.

All are available from the NVRL on request and the test is sent directly to the NVRL by the general practioner

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

2.27.3 *Serological Investigations*

HIV- VIRAL LOADS AND HEPATITIS C PCR.

- For HIV viral load, blood should be collected in an EDTA blood collection tube as per instructions on www.nvrl.ie.
- For hepatitis C PCR, a serum sample is requiredas per instructions on www.nvrl.ie.
- 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,** as per instructions on www.nvrl.ie.
- 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 as per instructions on www.nvrl.ie.
- 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 thrice daily by courier: 10.30am, 12.30pm and 2.30pm
- 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.

2.28 HISTOPATHOLOGY/CYTOPATHOLOGY/NEUROPATHOLOGY

2.28.1 *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.

2.28.2 *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.

2.28.3 *Coroners' s 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)

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.

2.29 MOLECULAR PATHOLOGY

2.29.1 *Sample selection*

All samples for solid tumour mutation analysis should be submitted as FFPE blocks. Samples for testing must arrive with a completed request form and a report on the patient sample.

All samples for Neuromolecular mutation analysis should be submitted as FFPE blocks with an accompanying H&E-stained slide. The H&E stained slide must be representative of the material in the block so a recent H&E is advised. The slide will be held in the Molecular laboratory in case the result needs to be queried in the future, for this reason a slide cut specifically for molecular testing is advised. Samples for testing must arrive with a completed request form and a report on the patient sample.

For germline BRCA testing, a peripheral blood sample should be submitted with the BRCA request form. Signed Patient consent must be obtained on this request form, the assay cannot be performed without this and will be rejected.

All samples should be sent to the following:

Molecular Pathology Laboratory
c/o Pathology Specimen Reception
Beaumont Hospital
Beaumont Road
P.O. Box 9063
Dublin 9

2.29.2 *Reporting of results*

External results are reported by email. Reports can be sent to at least two recipients by email. It is common practice to add the treating clinician or practice nurse to the email list to ensure that the result arrives to the clinic as rapidly as possible. As well as any individual's email a generic laboratory email (that can be checked by different individuals to cover periods of leave) should also be provided. This facilitates integration of the result into the sending hospital's laboratory information system (LIS). All email addresses must be specified in the recipients section of the test request form.

2.29.3 ***Contacting The Department***

Teresa Loftus	Chief Medical Scientist	018092856	molecular@beaumont.ie biomarkers@beaumont.ie teresaloftus@beaumont.ie

The Molecular Pathology laboratory provides a molecular pathology diagnostic and consultative service for hospitals throughout Ireland.

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

The Molecular Pathology Department is staffed from 08:00 – 17:00, Monday – Friday. The laboratory does not operate on Saturdays, Sundays or Bank Holidays.

2.30 NHISSOT

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 of both donors and recipients, HLA antibody screening for post transplant monitoring and HLA typing for disease association.

NHISSOT or H&I (Histocompatibility & Immunogenetics) is an accredited laboratory awarded by The European Federation for Immunogenetics (EFI). EFI is a European organisation that focuses on immunogenetics, tissue typing and transplantation. The EFI Accreditation Programme provides an internationally recognised accreditation scheme for laboratories providing Histocompatibility & Immunogenetics testing services in support of solid organ transplantation.

The H&I Department is committed to providing and maintaining a service of the highest quality by strictly adhering to policies and procedures that are in place to ensure the EFI standards are being maintained and updated.

We actively participate in well established external and internal quality control programmes to ensure best practice is being followed. We are continuously implementing ways to improve the service by assessing and validating new assays and techniques to provide the best level of service for our patients.

The NHISSOT provides H&I support for:

- The National Kidney Transplant Services at Beaumont Hospital
- The National Liver /Pancreas Transplant Services at St Vincent' s University Hospital
- The National Heart/ Lung Transplant Services at the Mater Misericordiae University Hospital
- Organ Donation Transplant Ireland

This document is intended as a guide to the services and tests available in the H&I Department. It provides details of the tests available, their specimen requirements, as well as appropriate background information.

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
D09 V2N0

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*

FUNCTION	CONTACT	TELEPHONE/EMAIL
Beaumont Hospital Reception	Switchboard	01-8093000/8377755
Directorate Management	Clinical Director	01-8092644
	Laboratory Manager	01-7977925
	Business Manager	01-8092508
Quality Management	Quality Manager	01-8092978
Appointments	Phlebotomy Appointements	01-7974675
BLOOD TRANSFUSION & HAEMOVIGILANCE		
Medical Enquiries	Prof. Philip Murphy	01-8093382
	Prof. John Quinn	01-8092664
	Prof Patrick Thornton	01-8092664
	Dr. Siobhan Glavey	01-8092664
	Haematology Registrar	Bleep 276
	SP Registrar	Bleep 887
	For Out-Of- Hours Service	Contact Switch Board
Scientific Enquiries	Chief Medical Scientist	01-8094733
	Senior Medical Scientists	01-8094734

FUNCTION	CONTACT	TELEPHONE/EMAIL
	Senior Scientist	01-8094734
	Routine Laboratory	01-8092705
	On Call	Bleep 252
Haemovigilance Enquiries	Haemovigilance Officers	01-8093334/2034 / Bleep 649
HAEMATOLOGY DEPARTMENT		
General Enquiries	HaematologyOffice-secretary/general enquiries Central Reception	01-8092655 haemtologyadmin@beaumont.ie 01-8093914/2674/2669/4075
Results	Phone number for the GP/External Lab Results Line	01-8092690
Medical Enquiries	Prof. Philip Murphy	01-8093382
	Prof. John Quinn	01-8092664
	Prof. Patrick Thornton	01-8092644
	Prof. Siobhan Glavey	01-8092664
	Dr Karl Ewins	018528832
	Dr Jeremy Sargent	01-8092150/2622
	Dr Elizabeth Smyth	01-8092150/2622
Clinical Advice and Laboratory Test Interpretation	Coleman. K. Byrne Unit	01-8092150/2622
	Haematology Registrars	'Contactable through switch'
	Haematology Senior House Officers	'Contactable through switch'
	Chief Medical Scientist	01-8092662
Clinic	Coleman. K. Byrne Unit	01-8092150/2622
	Warfarin Clinic	01-8092083/3982
Scientific Enquiries	Chief Medical Scientist	01-8092662
	Seniors office	01-8093952
	Haematology Laboratory	01-8092703
	Coagulation Laboratory	01-8092656
	Flow Cytometry Laboratory	01-8092763
	Morphology Special Haematology	01-8093226
IMMUNOLOGY DEPARTMENT		
General Enquiries	Departmental Secretary	01-8093026

FUNCTION	CONTACT	TELEPHONE/EMAIL
	Secretary to Prof. Keogan/ Dr Khalib	01-8092652
	Specialist Registrar	Bleep 797
Appointment Information	Secretary to Prof. Keogan/ Dr Khalib	01-8092652
Clinical Advice and Laboratory Test Interpretation	Specialist Registrar	immunologydepartment@beaumont.ie Bleep 797
Lab Enquiries	Lab Reception	01-809 3914/2674/2669/4075
Results	Pathology Reception	01-8092690
Scientific Enquiries	Chief Medical Scientist	01-8093174
	Immunology Laboratory	01-8092635/2421 immunologylab@beaumont.ie
CHEMICAL PATHOLOGY DEPARTMENT		
General Enquiries	Pathology Reception	01-8092507
Chemical Pathology	Chemical Pathology Office	01-8092765
Test Results	Pathology Reception	01-8092507
Medical Enquiries	Dr.Shari Srinivasan	01-8092676
	Dr. Ingrid Borovickova	Via Switchboard
	Specialist Registrar	01-8092666 Bleep 332
Scientific Enquiries	Chief Medical Scientist	01-8092670
	Chief Medical Scientist	01-7977811
	General Clinical Biochemistry	01-8092704/2668/2671/01-8528675
	Proteins	01-8092305
	Mass Spectrometry Laboratory	01-8092351 or 01-7977333
Out of Hours	Via Switchboard	Bleep 251 or DECT 8727
MICROBIOLOGY DEPARTMENT		
All Enquiries	Microbiology office	01-8092646
Results	Microbiology office	01-8092646
Medical Enquiries	Prof. B. Dinesh	01-8092646
	Prof. F. Fitzpatrick	01-8092646
	Prof. K. Burns	01-8092646
	Prof. K. O'Connell	01-8092646
	Dr. Ciara O' Connor	01-8092646
	Dr. Sinead O' Donnell	01-809 2646
	Dr. Helene McDermott	01-809 2646

FUNCTION	CONTACT	TELEPHONE/EMAIL
	Registrars	01 -8093320/3321/2667
	Out of Hours	Through Switchboard
Scientific Enquiries	Chief Medical Scientist	01-8092645
	Main Laboratory	01-8092647
HISTOPATHOLOGY & CYTOPATHOLOGY DEPARTMENT		
General Enquiries	Department Office	01-8092636/2353
	Department Email	histo@beaumont.ie
Medical Enquiries	Dr. Clíona Ryan	01-80922284
	Dr. Marie Staunton	01-8092997
	Dr. Anthony Dorman	01-8094240/ Bleep 322
	Prof. Brendan Doyle	01-8092636
	Dr. Anne Marie O'Shea	01-8093910
	Dr. Maeve Redmond	01-8092998
	Dr. Helen Barrett	01-8092641
	Dr. Christian Gulmann	01-8092078
	Dr. Keith Pilson	01-8093986
	Dr. Clive Kilgallen	01-8092284
	Dr. Odharnaith O'Brien	01-8094218
	Dr. Laura Mc Kenna	01-8093286
	Registrars Office	01-8092638/3435/ Bleep 448
Scientific Enquiries	Chief Medical Scientist	01-8092555
	Main Laboratory	01-8092353
	Specimen Reception	01-8092659
	Cytology Laboratory	01-8092640
	Molecular Histopathology	RCSI Building 01-8093726
Reports	Histopathology Office	01-8092636/2632/3919/3150/2154
RENAL PATHOLOGY		
Medical Enquiries	Dr. Anthony Dorman	01-8094240/ Bleep 322
	Prof. Brendan Doyle	01-8092636
Scientific Enquiries	Renal Pathology Laboratory	01-8528633 (dect phone)
General Enquiries	Renal Pathology Secretary	01-8092008
NEUROPATHOLOGY		
Medical Enquiries	Dr Jane Cryan	01-8093973
	Dr. Francesca Brett	01-8093143/ Bleep 324
	Dr. Alan Beausang	01-8092615
	Dr. Abel Devadass	01- 8097775
	Specialist Registrar	01-8092706

FUNCTION	CONTACT	TELEPHONE/EMAIL
Scientific Enquiries	Senior Medical Scientist	01-8092633
	Senior Medical Scientist (CJD)	01-8092633
	Research Scientist	01-8092706/ 3798
	Brain Bank	01-8092706
	Molecular Neuropathology	01-8098452/8453
Reports	Neuropathology Office	01-8092631/2072
NHISSOT		
General Enquires	Main Laboratory	01-8092650
	Chief Medical Scientist	01-8092661
Scientific Enquiries	Main Laboratory	01-8092650
	Molecular	01-8093955
	Scientists Office	01-8093238/2960
	Reporting Room	01-8092651/4246
	Antibody Screen	01-8094248
Email Addresses	General enquires	crossmatch@beaumont.ie
	Patient enquires	transplantlab@beaumont.ie
	Post transplant enquires	posttransplant@beaumont.ie
On-Call	Medical Scientist on duty	087-2615112
Clinical Enquiries	Consultant Immunologist	01-8092652
	Out of Hours	Through Switchboard
Renal Transplant Co-Ordinators Beaumont Hospital	Office	01-8092759
	E-Mail	transplantcoordina@beaumont.ie
	Urgent Call via Switch	01-809300/8377755
MOLECULAR PATHOLOGY		
General Enquiries	Chief Medical Scientist	01-8092856
HEALTHLINK SYSTEM		
General Enquiries/Test Result Issues	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 routine hours are 8am -5pm Monday to Friday and 9am-1pm on Saturday contactable on Ext. 2705. An Emergency On –Call service from 5pm -8am Monday to Friday and 1pm - 8am Saturday to Monday contactable on Bleep. 252
- Haematology Laboratory routine hours 8am to 8pm, Monday to Friday. There is a routine Saturday, 09.00 – 13.00. An Emergency On –Call service from 8pm - 8am Monday to Friday and 1pm - 8am Saturday to Monday. Contactable on Bleep. 852. A reduced service is offered between Christmas, New Year and Easter
- Only a limited Histopathology/Cytopathology/Neuropathology service is provided between 5pm to 8am and scientists on call can be contacted through switch.
- Microbiology laboratory hours are from 08:00am to 8.00pm, Monday to Friday and 9:00am to 1.00pm on Saturdays. After 8pm on weekdays, and from 1pm Saturday until 8:00am Monday morning, Microbiology provides emergency on-call service only.
- 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
- Molecular Pathology Laboratory hours are from 8am to 5pm Monday to Friday. The laboratory does not operate at weekends/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 Venepuncture 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.

Harmful

Corrosive

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.17 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.

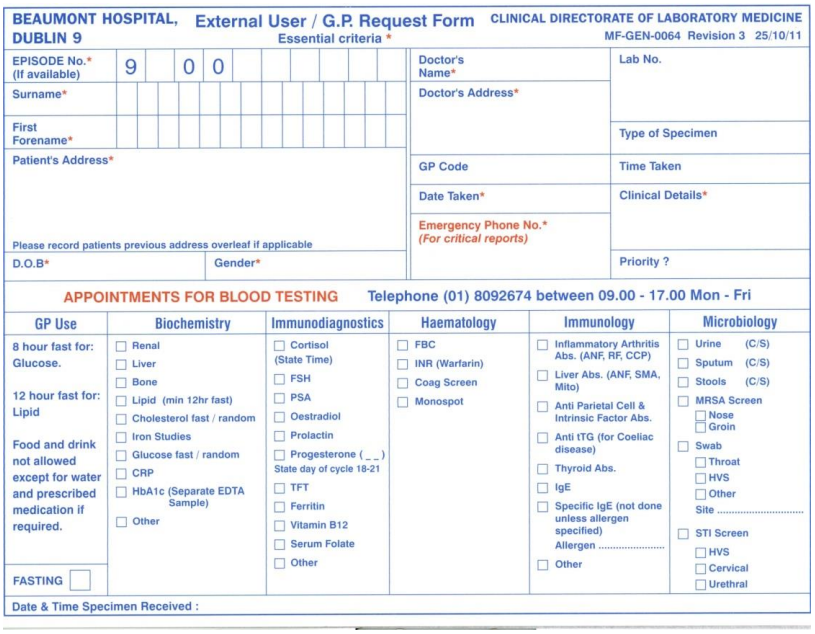
3.1.6 ***Specimen Labelling***

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 Histopatholoy/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 or if the request has come *via* another hospital, their Request Form is also acceptable provided all relevant demographics have been included. 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.



BEAUMONT HOSPITAL, DUBLIN 9 — **External User / G.P. Request Form** (Essential criteria *) — CLINICAL DIRECTORATE OF LABORATORY MEDICINE — MF-GEN-0064 Revision 3 25/10/11

EPISODE No.* (If available) 9 0 0 | Doctor's Name* | Lab No.

Surname* | Doctor's Address* | Type of Specimen

First Forename*

Patient's Address* | GP Code | Time Taken

Please record patients previous address overleaf if applicable | Date Taken* | Clinical Details*

D.O.B* | Gender* | Emergency Phone No.* (For critical reports) | Priority ?

APPOINTMENTS FOR BLOOD TESTING Telephone (01) 8092674 between 09.00 - 17.00 Mon - Fri

GP Use	Biochemistry	Immunodiagnostics	Haematology	Immunology	Microbiology
8 hour fast for: Glucose. 12 hour fast for: Lipid Food and drink not allowed except for water and prescribed medication if required. FASTING ☐	☐ Renal ☐ Liver ☐ Bone ☐ Lipid (min 12hr fast) ☐ Cholesterol fast / random ☐ Iron Studies ☐ Glucose fast / random ☐ CRP ☐ HbA1c (Separate EDTA Sample) ☐ Other	☐ Cortisol (State Time) ☐ FSH ☐ PSA ☐ Oestradiol ☐ Prolactin ☐ Progesterone (_ _) State day of cycle 18-21 ☐ TFT ☐ Ferritin ☐ Vitamin B12 ☐ Serum Folate ☐ Other	☐ FBC ☐ INR (Warfarin) ☐ Coag Screen ☐ Monospot	☐ Inflammatory Arthritis Abs. (ANF, RF, CCP) ☐ Liver Abs. (ANF, SMA, Mito) ☐ Anti Parietal Cell & Intrinsic Factor Abs. ☐ Anti tTG (for Coeliac disease) ☐ Thyroid Abs. ☐ IgE ☐ Specific IgE (not done unless allergen specified) Allergen ..... ☐ Other	☐ Urine (C/S) ☐ Sputum (C/S) ☐ Stools (C/S) ☐ MRSA Screen ☐ Nose ☐ Groin ☐ Swab ☐ Throat ☐ HVS ☐ Other Site ..... ☐ STI Screen ☐ HVS ☐ Cervical ☐ Urethral

Date & Time Specimen Received :

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.
NHISSOT request and consent 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.

Where additional samples are received and are not required to process the tests requested, these samples will be discarded without notification on the day of receipt.

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

- Lupus requests if the patient is on anticoagulation.
- Factor V Leiden sample, if the screening test APCR is not also requested. Exceptions allow for family history, In addition to Direct Factor Xa inhibitors and Direct Thrombin inhibitors . These cause a false elevation of the APCR , hence it will be rejected and the FV Leiden done directly.
    - Obvious inadequacy of specimen for the test(s) required i.e. only one coagulation specimen for a Thrombophilia screen.
- Protein C and S will be rejected if the patients is on warfarin and for at least 2 weeks post warfarin therapy.
- Antithrombin if the patients is on a Direct Thrombin inhibitor drug.
    - Haematology Molecular testing cannot be performed unless patient consent has been obtained and HAEMG-LF-084 Request form has been completed in full
    - HbA1c is analysed in the Biochemistry Laboratory. Patients requiring a FBC and HbA1c will require 2 EDTA 2.6mL 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 <4 hours old. In samples greater than 4 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/: 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: CD4Lymphocyte subsets, Lymphoproliferative and Acute Panels 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

Label code	Tube colour	Anticoagulant	Volume (ml)
ORAN	Orange	Lithium Heparin	4.9
ORAN	Orange	Lithium Heparin For Troponin Only	2.7
F.OR	Orange	Lithium Heparin in FOIL	4.9
F.WH	White	White Serum ,in FOIL	4.9
F.PK	Pink	EDTA wrapped in FOIL	
F.UR	Random Urine	Random urine wrapped in FOIL	
F.FA	Faecal Sample	Faecal Sample, foil wrapped.	

Label code	Tube colour	Anticoagulant	Volume (ml)
BRWN	Brown	Plain, With Gel Separator	4.9
4.9W	White	Plain, 4.9 mL volume	4,9
WHIT	White	Plain	7.5
ICEW	White On Ice	Plain White	7.5
OICE	Orange On Ice	Lithium Heparin	4.9
PINK	Pink	Potassium EDTA	2.6
ICEP	Pink on ICE	Potassium EDTA on ICE	2.6
YELL	Yellow	Sodium Fluoride	2.7
REDL	Pink- Large	Potassium edta	7.5
MSU	N/A	plain	
METL	**Orange	Special Metal Free Tube & Needle	7.5
24HU	N/A	Plain	
24AU	N/A	Pre-Acidfied	
CSF	N/A	CSF Plain	
DARK	CSF – Brown	Plain-Protect From Light	At least 1ml
BGAS	Arterial syringe	Lithium heparin	
PURP	Purple	Tri-sodium citrate 4NC	3.5 mL
PINK	Pink	EDTA-KE (Tri-potassium Ethylenediaminetetra-acetic acid)	2.6 mL 1.8 mL- Paediatric
GREN	Green	Tri-sodium citrate 9NC	2.9 mL 1.8 mL- Paediatric
EXAC	Red	0.82mg Magnesium/mL	2.7mL
SALI	n/a	Salivette	
BLUE	Blue tube with Blue cap	Potassium EDTA with Blood Bank label	4.9

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 The Sarstedt NFT (Needle Free Transfer) system. This consists of a 10ml NFT primary container (Sarstedt Product Reference 75.562.900) and a 10mL Monovette tube (Sarstedt Product reference 10.252)

- 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 3.1.16 on page 121 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, transaminases, LDH and Folic Acid. 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 in Section 3.1.2 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 the preferred method of result transmission. Beaumont Hospital Pathology Department does not issue paper reports unless specifically requested by a GP practice.. Beaumont Hospital uploads patient reports to the Healthlink service every 10minutes.

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 Requests for Results

A dedicated results telephone line is in place for blood sciences and a copy of a report can be emailed by administration staff to a secure email account (e.g @hse @healthmail etc.). The laboratory has previously issued details on obtaining a secure email account and this information is available at http://www.beaumont.ie/media/July20181.pdf.
The dedicated results line details are as follows:

Tel: 01 809 2690
Monday to Friday 9.30-12:30 and 14:30-16:00

Please note this does not affect the reporting of critical results or request for clinical interpretation. Requests for clinical interpretation will be handled as outlined in section 3.1.12 above

Other useful numbers:
Microbiology reports : 01 809 2646 (lines open 9am-4pm)
Histopathology reports: 01 809 2632 (lines open 9am-4pm
NVRL results: 01 716 4414 (lines open 8am-6pm)

3.1.12.2 Critical Values

Results falling outside defined alert limits will be telephoned to the appropriate GP/Nursing Home/External 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’.

3.1.12.3 Flagged Results.

A known limitation of the BHIS system is the failure of flags on results when a result is less than the analytical limit of the assay. The numerical file has to be changed to an alpha file with the consequent loss of the reference range and any result flags e.g. if a Vitamin B12 result if low - < 60, then the L flag will be lost from all reports and down-stream IT systems.

It is imperative that all values are checked at in conjunction with the reference ranges quoted in this User Guide.

3.1.13 ***Telephoning GP/Results Out of Hours***

Where a GP is not contactable by mobile telephone, the dedicated GP Results Out of Hours telephone may be used to alert the GP by text that urgent results are awaiting them.
If a mobile number for the GP is not available, a non-conformance will be raised. It will be passed to the relevant Consultant Pathologist for signing and the scanned

documentation will be emailed to the relevant GP's healthmail account.
Paper copies of patient reports are available from the Pathology Office, 8092507.
Beaumont Hospital has no access to the Healthlinks service.

3.1.14 *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.

3.1.15 *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.

3.1.16 *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 116 for correct specimen tubes to be used.

3.1.17 *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 NHISSOT 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 brought to the laboratory by a porter or sent via chute
  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.

3.1.18 *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

3.1.19 *Time Limits for Requesting Additional Examinations*

Please note that verbal requests for any examinations must be followed by a fully completed request form, by email request in order for results to be issued. Request forms must be received within the timeframe outlined for each department below.

Requests for Chemical Pathology add-on tests must be e-mailed to: chemicalpathology@beaumont.ie.

Requests for Haematology add-on tests must be e-mailed to: Haematology@beaumont.ie

3.1.20 *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

3.1.21 *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.

3.1.22 *Accreditation/Quality Standards*

Beaumont Hospital Clinical Directorate of Laboratory Medicine's current scope of Accreditation to ISO15189 is available from the INAB website, https://www.inab.ie/FileUpload/Medical-Testing/Beaumont-Hospital-225MT.pdf
The H&I Department is accredited by EFI (European Federation for Immunogenetics).

3.1.23 *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 office.

3.2 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.

3.2.1 *Repertoire of Haematology Tests*

Test	**Specimen Container**	**Minimum/ Container Volume**	**Adult Reference Range** (See Reports for Paediatric Ranges)			**TAT**	**Comment**
Full Blood count	EDTA (pink capped)	2.6ml standard	*Parameter*	*Male*	*Female*	1 Working Day	7.5ml and 10ml EDTA samples are not acceptable *Female >50 years
			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 0.355-0.52*		
			RCC	4-6.5 x10^{12}/L	3.8-5.8 x10^{12}/L 3.8-5.6*		
			RDW	11-15 %			
			MCV	79 -96 fL			
			MCH	27 -32 pg			
			MCHC	32.0-36.5 g/dL			
			PLTS	140 -400 x10^{9}/L			
			WBC	4.0 -11.0 x10^{9}/L			
			Neut	2.0 -7.5 x10^{9}/L			
			Lymph	1.0 -4.0 x10^{9}/L			
			Mono	0.2- 1.0 x10^{9}/L			
			Eosin	0.04- 0.4 x10^{9}/L			
			Baso	0.01- 0.1 x10^{9}/L			

Test	**Specimen Container**	**Minimum/ Container Volume**	**Adult Reference Range** (See Reports for Paediatric Ranges)	**TAT**	**Comment**
Platelet Check (Thromboexact)	0.82mgMg^{2+}/mL (Red)	2.7mL	140 -400 x10^9/L	1 Working Day	Arrange in advance with laboratory to obtain sample tube. Please write on the request form that a platelet count from a Thromboexact tube is required
ESR	Trisodium citrate 4NC /3.5 (purple)	3.5 ml **must** be filled to the line	*Male*: 1- 12 mm/hr; *Female*: 1-20 mm/hr	1 Working Day	Addressograph label must only be placed over the manufacturer's label on the bottle.
Reticulocyte Count	EDTA (pink capped)	2.6ml standard	Retic: 0.4-1.9% Male 0.4-1.8% Female Retic (Abs) 14-100 x10^9/L	1 Working Day	7.5ml and 10ml EDTA samples not acceptable
Infectious mononucleosis Screen	EDTA (pink capped)	2.6ml standard	Negative	1 Working Day	
Blood film examination	EDTA(pink capped)	2.6ml standard	N/A	5 Working Days	Sample must be <8 hrs old. Please specify that a blood film is required on the request form.
Malaria: Rapid Diagnostic Tests (RDT) and Blood Film	N/A	N/A	Negative	N/A	**Samples are required in the Laboratory < 2 hours post sample collection. Therefore, patients must attend the Hospital Phlebotomy/A&E Department for sample collection.**

3.2.2 ***Repertoire of Flow Cytometry Tests***

Test	**Specimen Container**	**Minimum/ Container Volume**	**Reference Range**	**TAT**	**Comment**	**Mnemonic**
CD4	EDTA (pink capped)	2.6ml Standard	502-1749 Cells/ul	2 Working days	Samples must be <24 hours old. Only processed Monday to Friday. Must be Received in Laboratory before 3pm on a Friday	**CD4**
Lymphocyte Subsets	EDTA (pink capped)	2.6ml Standard	CD3#797-2996Cells/ul CD3/4#502-1749Cells/ul CD3/8#263-1137Cells/ul CD19#99-618Cells/ul CD56#72-577Cells/ul	2 Working days	Samples must be <24 hours old. Only processed Monday to Friday. Must be Received in Laboratory before 3pm on a Friday	**LY_SUB**
Lymphoid Screening Tube	EDTA (pink capped)	2.6ml Standard	N/A	Written report: 10 working days Verbal report: 24 hours	All Samples must be <24 hours old.	**LST**
	Sodium Heparin (orange capped - BMA) (white capped, RPMI cytogenetics bottle – Lymph Node Aspirate)	2.7ml Standard			Immunophenotyping request form to be completed.	
Lymphoproliferative Panel	EDTA (pink capped)	2.6ml Standard	N/A	Written report: 10 working days Verbal report: 24 hours	All Samples must be <24 hours old.	**LY_PRO**
	Sodium Heparin (orange capped - BMA) with 1ml RPMI	2.7ml Standard			Immunophenotyping request form to be completed.	

Test	**Specimen Container**	**Minimum/ Container Volume**	**Reference Range**	**TAT**	**Comment**	**Mnemonic**
Acute Leukaemia Screen Acute Leukaemia Panel Blast Count	EDTA (pink capped)	2.6ml Standard	N/A	Written report: 10 working days Verbal report: 24 hours	Immunophenotyping request form to be completed.	**AL**
	Sodium Heparin (orange capped- BMA) with 1ml RPMI.	2.7ml Standard			EDTA Samples must be <24 hours old.	
					Sodium Heparin (orange capped - BMA) with 1ml RPMI must be < 48 hours old	
Paroxysmal Nocturnal Haemoglobinuria	Fresh EDTA (pink capped)	2.6ml Standard	N/A	Written report: 10 working days Verbal report: 24 hours	Immunophenotyping request form to be completed. Sample may be stored in fridge for <48 hours if not for immediate testing	**PNH**
T-Cell Panel	EDTA (pink capped)	2.6ml Standard	N/A	Written report: 10 working days Verbal report: 24 hours	All Samples must be <24 hours old.	**T_PANEL**
	Sodium Heparin (orange capped) with 1ml RPMI	2.7ml Standard			Immunophenotyping request form to be completed.	

3.2.3 ***Repertoire of Coagulation Tests***

Test	**Specimen Container**	**Number of Samples**	**Minimum Volume**	**Reference Range**	**TAT**	**Comment**
Coagulation Screen Prothrombin Time Activated Partial Thromboplastin Time	Trisodium citrate 9 NC/2.9 mL (green capped)	1	**Must** be filled to the line	PT: 10-13.2 seconds INR should only be used for monitoring Warfarin therapy. Refer to local treatment algorithm. APTT: 24 – 36 seconds	1 working Day	Sample must be <4 hours old
INR	Trisodium citrate 9 NC/2.9 mL (green capped)	1	**Must** be filled to the line	The INR should only be used for monitoring Warfarin therapy. Refer to local treatment algorithm.	1 working Day	INR only requests are stable for 24 hrs
Warfarin office INR	Trisodium citrate 9 NC/2.9 mL (green capped)	1	**Must** be filled to the line	The INR should only be used for monitoring Warfarin therapy. Refer to local treatment algorithm.	1 working Day	Warfarin Office contact no. 01-8092083 WINRs are stable for 24 hrs
D-Dimer	Trisodium citrate 9 NC/2.9 mL (green capped)	1	**Must** be filled to the line	<0.5 µg/ml	1 working Day	Sample must be <8 hours old

Test	Specimen Container	Number of Samples	Minimum Volume	Reference Range	TAT	Comment
Fibrinogen	Trisodium citrate 9 NC/2.9 mL (green capped)	1	**Must** be filled to the line	1.9 – 3.5 g/L	1 working Day	Sample must be <4 hours old For patients on Argatroban a Clauss Fibrinogen test is not appropriate & will be reported as follows: "Fibrinogen result is unavailable as the patient is on Argatroban which may cause a false low fibrinogen result in the Clauss fibrinogen assay. Please discuss with the Haematology team".
Mixing study	Trisodium citrate 9 NC/2.9 mL (green capped)	2	**Must** be filled to the line	Corrected to within the PT and APTT normal ranges	1 week	
Factor Assays	Trisodium citrate 9 NC/2.9 mL (green capped)	2	**Must** be filled to the line	FII 0.72 - 1.31 IU/mL FV 0.63 - 1.33 IU/mL FVII 0.51 - 1.54 IU/mL FVIII 0.60 - 1.36 IU/mL FIX 0.80 - 1.47 IU/mL FX 0.64 - 1.50 IU/mL FXI 0.72 - 1.52 IU/mL FXII 0.52 - 1.64 IU/mL	Case dependent, maximum 14 days	Tests done in batches. For urgent requests, contact the laboratory in the morning, may be able to facilitate testing that day.

Thrombophilia screen	Trisodium citrate 9 NC/2.9 mL (green capped)	4	**Must** be filled to the line	See individual requests APCR, PC, FPS, AT3 and LA.	4 weeks.	Batch tested. The Thrombophilia screen (TPSC) includes the following tests: PT, APTT, FIBN, D-DIMER, LA, AT3,PC, FPS, APCR 5LEIDEN*. Hence, these tests do **not** need to be ordered on an individual basis. See below for TAT for 5Leiden. If sending a TPSC from an External Hospital: **Separated Samples:** Plasma samples must be separated by a double centrifugation procedure according to the following instruction in order to prepare platelet poor plasma (plt< x10^9/L). • Check all samples for clots and adequate volume prior to centrifugation. • Centrifuge samples for 10 mins at 3,000 g at RT°C. • Pool plasma from all samples and centrifuge for a second time. • Aliquot approximately 1 ml of plasma into a minimum of seven 1.5mL Micro Tube PCR-PT • Frozen samples must be sent in appropriate frozen transport containers. All samples must remain in these containers.

Test	Specimen Container	Number of Samples	Minimum Volume	Reference Range	TAT	Comment
Protein C	Trisodium citrate 9 NC/2.9 mL (green capped)	1	**Must** be filled to the line	0.74 - 1.32 IU/mL	4 weeks.	Batch tested. Patient must be off warfarin for a minimum of 2wks to perform this assay.
Free Protein S	Trisodium citrate 9 NC/2.9 mL (green capped)	1	**Must** be filled to the line	Males: 0.76-1.46 IU/mL Females:0.65-1.33 IU/mL	4 weeks.	Batch tested. Patient must be off warfarin for a minimum of 2wks to perform this assay
Antithrombin	Trisodium citrate 9 NC/2.9 mL (green capped)	1	**Must** be filled to the line	0.82 - 1.18 IU/mL	4 weeks.	Batch tested
Activated protein C resistance (APCR)	Trisodium citrate 9 NC/2.9 mL (green capped)	1	**Must** be filled to the line	Negative	4 weeks	Batch tested
Von Willebrand factor	Trisodium citrate 9 NC/2.9 mL (green capped)	2	**Must** be filled to the line	0.49 - 1.73 IU/mL	Case dependent, maximum 14 days	"The presence of Rheumatoid Factor may produce an overestimation of the result"
Lupus anticoagulant	Trisodium citrate 9 NC/2.9 mL (green capped)	1	**Must** be filled to the line	DRVVS < 1.17 DRVVTR: <1.23 SCT TR < 1.14	4 weeks	Batch tested. Patients must not be on any anticoagulation as they interfere with the interpretation of the assay.

3.2.4 ***Repertoire of Haematology Molecular Tests***

Test	**Specimen Container**	**Number of Samples**	**Minimum/ Container Volume**	**Reference Range**	**TAT**	**Comment**
Factor V Leiden mutation (5Leiden) And APCR	EDTA sample (pink)	1	2.6ml Standard	Negative	6 weeks	Only tested if the Activated Protein C (APCR) is positive or a family history is indicated on the request form. For APCR sample requirements see previous table. Thrombophilia request form HAEMC-LF-023 **MUST fully** be completed. This form can be obtained from the Beaumont Hospital website, under Haematology Dept. If genetic consent is not obtained the molecular test will be rejected. The laboratory will no longer take receipt or store the form containing patient genetic consent. It is the responsibility of the ordering clinician to obtain and file a copy of genetic consent in the patient's record.
	Trisodium citrate 9 NC/2.9 mL (green capped)	1	**Must** be filled to the line	Negative		
Prothrombin G20210A mutation	EDTA sample (pink)	1	2.6ml Standard	Negative	6 weeks	Thrombophilia request form HAEMC-LF-023 **MUST fully** be completed. This form can be obtained from the Beaumont Hospital website, under Haematology Dept. If genetic consent is not obtained the molecular test will be rejected. The laboratory will no longer take receipt or store the form containing patient genetic consent. It is the responsibility of the ordering clinician to obtain and file a copy of genetic consent in the patient's record.

Test	Specimen Container	Number of Samples	Minimum/ Container Volume	Reference Range	TAT	Comment
HFE Haemo-chromatosis	EDTA (PINK cap) Whole blood	1	2.6ml	Not detected	4weeks	Must be accompanied by completed Haemachromatosis Genetic Screening Request Form (HAEMC-LF-077) This form can be obtained from the Beaumont Hospital website, under Haematology Dept. If genetic consent is not obtained the molecular test will be rejected. The laboratory will no longer take receipt or store the form containing patient genetic consent. It is the responsibility of the ordering clinician to obtain and file a copy of genetic consent in the patient's record.

3.2.5 ***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. GP verbal requests accepted by phone will then need to be emailed to haematologyadmin@beaumont.ie for the additional test.

Refer to table below for test cut-off times when requested to add a test to a sample already received in the Laboratory. Processing an additional request depends on the sample having the correct anti-coagulant, not too old for analysis, correct storageand not discarded.

3.2.5.1 Test Cut-Off Times

Test	Test Cut-off Times
FBC	<24 hours
Blood Film preparation	<8 hours
Platelet Exact for platelet clumping	<24 hours
Reticulocyte	< 24hours
ESR	< 6 hours
Malaria	< 2 hours (test should be performed within 2 hours of phlebotomy hence patient should present themselves to the Phlebotomy Department or AE with a request for a "malaria screen")
IM	<24 hours
Sickle Screen	<14 days if stored @ 2-8ºC
PNH	< 48 hours if stored at @ 2-8°C
Lymphocyte Subset Analysis & CD4	<24 hours
Lymphoproliferative Panel, T-Panel, , Lymphoid Screening Tube	All EDTA samples must be <24 hours old.
Acute Screen, Acute Leukaemia Panel, Blast Count	Sodium Heparin (orange capped - BMA) with 1ml RPMI must be < 48 hours old
Coagulation Samples	< 4hours
D-dimer	<8 hours
INR/WINR	<24 hours
Factor V Leiden, PT mutation	<28 days once stored at 2-8°C

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.

3.2.6 ***Critical Values***

- GP results are available on Healthlink.
- Results falling outside defined alert limits are telephoned to the appropriate personnel.
- It is imperative that the mobile phone number of the requesting doctor is on the request form so that critical results can be phoned when surgeries are closed.
- **If a mobile phone number is not available or contact number unanswered, the critical alert value will be telephoned the following day.**

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

Test	OPD(except CKB) GP/ Nursing Homes/ External Hospitals
Hb	<7.0g/dL & >19.0 g/dL (1st time)
PLT	<50 x 10^9/L & >1000 x 10^9/L (1st time)
Neutrophils	<0.5 x 10^9/L & >50 x 10^9/L (1st time)
FBC	Results indicating possible leukaemia i.e. numerous flags (especially blast), increase WCC, DIFF vote-out or very abnormal, plt<100 and low Hb. Phone ward/clinical team responsible for the patient and to bring these results to their attention.
INR/WINR	>5.0
Fibrinogen	<1.5 g/l
Flow Cytometry	New Acute Leukaemia/PNH patients CHB Blast counts >5%, contact Prof. Thornton/Dr Quinn directly
Morphology	New Acute Leukaemia TTP/MAHA
Malaria Screen & Film	Positive

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

3.3 CHEMICAL PATHOLOGY

3.3.1 *Services Offered*

The Chemical Pathology Department provides a comprehensive suite of routine and specialised tests including;

- 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 Plasma Free Metanephrines, urinary fractionated catecholamines, metanephrines and 5HIAA.

3.3.2 *Contact Details for Medical / Clinical Advice*

For test results please contact the dedicated results telephone line on (01) 8092690.

For medical advice contact;
Consultant Chemical Pathologist; Dr. Shari Srinivasan on (01) 8092676
Consultant Chemical Pathologist; Dr. Ingrid Borovickova via switchboard

During working hours medical advice can also be obtained by contacting;
Chemical Pathology Specialist Registrar; (01) 8092666 or 8093000 Bleep#332

During working hours scientific advice can be obtained by contacting;
Chief Medical Scientist; Alison Griffin (01) 8092670
Chief Medical Scientist; Miriam Shinners (01) 7977811
For information on test requirements please see below.

3.3.3 *Requests for Additional Tests*

To request additional tests please contact the relevant laboratory.
Samples are retained in the Department for 72 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 and if agreed an e-mail outlining the request must then be sent to Chemical Pathology chemicalpathology@beaumont.ie for the attention of the relevant chemical pathology staff member following the discussion.

Table D: Routine Profiles and their Components

Description	Mnemonic	Tests
Renal Profile	Renal	Urea, Na, K, Cl,Creatinine
Liver Profile	Liver	Bilirubin, ALT, ALK, γGT, AST, ALB, TP, Globulin
Lipid Profile - Fasting Lipid Profile - Non-fasting	FHDL HDL	Cholesterol, Triglyceride, HDL, Calculated LDL, non HDL Cholesterol
Bone Profile	Bone	Ca, ALB, Phosphate, Ca Adjusted, ALK
Thyroid Function Test	TFT	FreeT4 and TSH

3.3.4 *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.

3.3.5 *Tumour Marker Analysis*

Tumour markers should not be used to diagnose disease. Their use is 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 Consultants request included.

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

Please refer to National Pathology Handbook: Laboratory Testing for Tumour Markers.

3.3.6 *Lipid Profile for Cardiovascular Risk Assessment*

Please refer to National Pathology Handbook: Laboratory Testing for Lipids.

3.3.7 *Externally Referred Tests*

Samples from General Practitioners or other external service users are not referred to external laboratories. Beaumont Hospital is 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 this will be facilitated **if** the request from the clinician is sent with the test request form or noted very clearly on the request form.

3.3.7.1 Reports from External Laboratories

External test reports are issued on paper only. They will not be available on Healthlinks. However, they will be available electronically within Beaumont Hospital to the requesting clinician. The result will also be scanned into the patient file and available from the Consultant secretary.

3.3.8 *Fertility Clinics*

Chemical Pathology does not provide testing services for patients attending fertility clinics.

3.3.9 *Critical phoning limits*

- Results falling outside defined alert limits will be telephoned to the requesting GP or referring laboratory.
- Note: If it is not possible to contact the relevant GP out of hours the result will be communicated to the nominated out-of-hours service.
- It is the responsibility of the healthcare professional who requests a laboratory test to ensure that the result is reviewed and appropriate action taken.
- Results apply to the **current episode number.**
- **Urgency A** – rapid communication of results within 2 hours.
- **Urgency B**- results require communication within 24 hours, and preferably on the same working day. This would also apply to outpatients. For outpatients if there is no facility to phone on a Saturday then discuss with the on-call senior to determine the urgency.
- **Urgency C**- communication of these results on the next working day is deemed satisfactory.

RESULTS FOR URGENT COMMUNICATION						
	Units	**Action Limits**		**Urgency**	**Ref.**	**Comments**
Analyte		**Lower**	**Upper**			
Sodium	mmol/L	120 130 if< 16 yrs 125 GP/OPD	155	A A A	1 2 3	**Note different phoning limits for in-patients and GP/OPD**
Potassium	mmol/L	2.5 2.7 GP/OPD	6.5 6 GP/OPD	A A A	2 1 3	Check for haemolysis, age of sample & EDTA contamination. **Note different limits for in-patients and GP/OPD**
Urea	mmol/L	-	30 10 if< 16 yrs 35 CKD patients	A A A	1 2 3	
Creatinine	µmol/L	-	354 200 if < 16 yrs 800 CKD patients	A A A	1,2 2 3	
Glucose	mmol/L	2.5	25 30 GP/OPD/known diabetics 15 if < 16 yrs	A A A	1,2,3 2 2	
Calcium Adjusted (Total Calcium if no calculation available)	mmol/L	1.8*	3.5 3 GP/out-patients 3.2 CKD patients	A B A	1,2 1 3	*report with Albumin Request and perform U&E. All calcium results above upper action limit to be phoned regardless of previous critical result.
Phosphate	mmol/L	0.3 0.45 GP/OPD	-	A B	2 1	
Magnesium	mmol/L	0.4		A	1,2	
Creatine Kinase	U/L	-	5000 400 if <16 yrs	A A	1,2 3	
Amylase	U/L	-	500	A	1,2	
CRP	mg/L	-	300	A	1,2	

RESULTS FOR URGENT COMMUNICATION						
	Units	**Action Limits**		**Urgency**	**Ref.**	**Comments**
Analyte		**Lower**	**Upper**			
AST	IU/L	-	500 female 600 male	A A	1,2 1,2	
ALT	IU/L	-	500 female 600 male	A A	1,2 1,2	
Cortisol	nmol/l	50	-	A	1	Unless part of dexamethasone suppression test **Do not assume a dexamethasone test has been undertaken.**
Cortisol (SST)	nmol/L	250		A	2	As part of short synacthen test
Bicarbonate	mmol/L	10	-	A	2	Excluding ICU patients
Ethanol	mg% (mg/dL)		400 All levels in <16 yrs	A A	2 3	
CSF results	All Xanthochromia results to be phoned			A	3	
Paracetamol	mg/L	All results		A	2	
Digoxin	ug/L	-	2.5	A inpatients B GP/OPD	2 1,2	Check timing > 6 hrs from last dose. Give U&E results also. More urgent if K^+< 3 mmol/L. Phone **immediately** to GP/OPD requestor if overdose suspected or K^+ low
Carbamazepine	mg/L		15	A inpatients B GP/OPD	3 3	
Phenobarbitone	mg/L	-	70	A inpatients B GP/OPD	3	
Phenytoin	mg/L	-	25	A inpatients B GP/OPD	2,3 1,2,3	
Valproate (Valproic acid)	mg/L	-	120	A inpatients B GP/OPD	3	
Theophylline	mg/L		25	A inpatients B GP/OPD	2 2	
Lithium	mmol/L	-	1.5	A inpatients B GP/OPD	2 1,2	
Salicylate	mg/L	-	300	A	2	
Triglycerides	mmol/L	-	20	B	1	If specimen lipaemic, measure and report

RESULTS FOR URGENT COMMUNICATION						
	Units	**Action Limits**		**Urgency**	**Ref.**	**Comments**
Analyte		**Lower**	**Upper**			
						direct ISE Sodium and Potassium.
Haem 4+ samples	Phone all URGENT haem 4+ (this will include all ED)			A	3	
PSA	ng/mL	-	40	C	3	If no previous critical result
Ferritin	ng/mL	-	5000	C	3	
TSH	mU/L		30	C	3	
fT4	pmol/L	-	50	C	1,3	
Prolactin	mU/L	-	2000	C	3	
Testosterone female	nmol/L	-	5	C	3	
Gamma-globulins	g/L	IgG<3		C	1	With low IgA and IgM
Serum FLC Ratio		-	>100	C	3	First time detection
Paraprotein	g/L	Any IgE/IgD	IgG>15 IgA>10 IgM>10	C	1	First time detection
		Monoclonal free light chain- any size, whether or not with intact paraprotein		C	3	First time detection

3.3.9.1 Reference Intervals

All reference intervals and/or clinical decision values apply only to non-pregnant adults unless specifically stated otherwise.

3.3.9.2 Interference in Laboratory Tests

Many laboratory tests are subject to interference by endogenous or exogenous factors which may alter the true concentration of a substance within the body, or cause an analytical interference giving a potentially erroneous or misleading result.

All samples are routinely checked for Haemolysis, Lipaemia and Icterus which can interfere with laboratory tests to varying extents. Significant levels of any of these may affect the quality of some test results which will be highlighted and/or removed from the individual report.

Test results should be interpreted in conjunction with clinical findings and if interference is suspected please contact the laboratory where further information on each test method is available.

Drug interferences are also commonly encountered, a summary list is available at http://www.beaumont.ie/media/Interference_in_Laboratory_Tests1.pdf.

3.3.9.3 Repertoire of Test Services - Routine Chemistry

Test	Sample Required	Specimen Container	Minimum Volume	Reference Range/ Unit of Measurement	TAT	Comment
5HIAA	24 hour Urine collection	Pre-acidified (50% acid)	N/A	2.5 - 50 µmol/24hr	12 working days	If the patient is <15 years old, please contact the laboratory (01-7977333) for information regarding sample collection. Age-related reference ranges will accompany results for paediatric samples.
Alpha1 Antitrypsin	Plasma	Orange Cap	4.9ml	0.9 – 2.0 g/L	72 hours	
Adrenocortico-trophic Hormone (ACTH)	EDTA PLASMA on Ice	Blue Cap	4.9mL	7.2-63.3 pg /mL	10 days STAT-Contact laboratory	Patient must attend Beaumont Hospital phlebotomy for sample collection. Sample is labile, must be sent to lab immediately on ice for separation.
Adrenaline	24 hour Urine collection	Pre-acidified (50% acid)	N/A	See Table Below	24 working days	If the patient is < 15 years old, please contact the laboratory (01-7977333) for information regarding sample collection. Age-related reference ranges will accompany results for paediatric samples
Alanine aminotransferase (ALT)	Plasma	Orange Cap	4.9 mL	Female: < 33 IU/L Male: < 41 IU/L	24 hours	
Albumin	Plasma	Orange Cap	4.9 mL	35 – 52 g/L	24 hours	
Albumin Creatinine Ratio	Spot urine sample	Plain MSU	N/A	mg/mmol Creatinine Males: <2.5 Females: <3.5	96 hours	

Test	**Sample Required**	**Specimen Container**	**Minimum Volume**	**Reference Range/ Unit of Measurement**	**TAT**	**Comment**
Alkaline Phosphatase (Total Alk Phos)	Plasma	Orange Cap	4.9 mL	Female 35 – 104 IU/L Male 40 – 129IU/L	24 hours	
Amylase	Plasma	Orange Cap	4.9mL	28 - 100 IU/L	24 hours	
Amylase Urine	Spot urine sample	Plain MSU container	N/A	M: 16 – 491IU/L F: 21 – 447 IU/L	24 hours	
Angiotensin Converting Enzyme (ACE)	Serum	Plain (White Cap)	4.9mL	8 – 65 U/L	7 working days	
Aspartate aminotransferase (AST)	Plasma	Orange Cap	4.9mL	Female < 32 IU/L Male < 40 IU/L	24 hours	
B12 (Vitamin)	Plasma	Orange Cap	4.9mL	197 – 771 ng/L	3days	
B2M	Plasma	Orange Cap	4.9mL	0.8 – 2.2 mg/L	72 hours	
bHCG	Serum	Brown Cap	4.9mL	Non-pregnant,pre-menopausal women: <1 Postmenopausal women: <7	72 hours	For preganacy testing purposes only.
Bicarbonate (TCO2)	Plasma	Orange Cap	4.9mL	22 – 29 mmol/L	24 hours	Fresh sample required – patient should attend Beaumont Hospital phlebotomy for sample collection.
Bilirubin - total	Plasma	Orange Cap	4.9mL	< 21 µmol/L	24 hours	
Bilirubin Direct	Plasma	Orange Cap 22 wrapped in foil to exclude light	4.9mL	<5.0µmol/L	72 hours	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.

Test	**Sample Required**	**Specimen Container**	**Minimum Volume**	**Reference Range/ Unit of Measurement**	**TAT**	**Comment**
BNP (Nt-Pro BNP)	Plasma or serum	Orange Cap or White Cap	4.9ml	18-44yrs < 97pg/ml 45-54yrs < 121pg/ml 55-64yrs < 198pg/ml 65-74yrs < 285pg/ml ≥ 75yrs < 526pg/ml	24hrs	
CRP C Reactive Protein	Plasma	Orange Cap	4.9ml	0 – 5mg/L	24hrs	
CA 12-5	Serum	Brown Cap	4.9ml	<35 kU/L	72 hours	Refer to Ovarian Cancer GP Referral For Symptomatic Women May 2016
Caeruloplasmin	Plasma	Orange Cap	4.9ml	Male 0.15 – 0.30g/L Female 0.16 – 0.45g/L	72 hours	
Calcium	Plasma	Orange Cap	4.9mL	2.15 – 2.50mmol/L	24 hours	
Calcium Adjusted	Plasma	Orange Cap	4.9ml	2.21 – 2.52mmol/L	24 hours	Locally derived equation.
Calcium 24 Hour Urine	24 hour Urine collection	No Preservative	N/A	2.5 - 7.5 mmol/24hrs	72 hours	Container available from Phlebotomy Department
Carbamazepine	Serum	Plain (White Cap)	4.9mL	4.0 – 12.0 mg/L	24 hours	Samples should be taken immediately prior to next dose.
Catecholamines (Noradrenaline, Adrenaline & Dopamine)	24 hour Urine collection	Pre-acidified (50% acid)	N/A	See Table Below	24 working days	If the patient is <15 years old, please contact the laboratory (01-7977333) for information regarding sample collection. Age-related reference ranges will accompany results for paediatric samples.
Chloride	Plasma	Orange Cap	4.9mL	95 – 108 mmol/L	24 hours	
Cholesterol	Plasma	Orange Cap	4.9mL	< 5.0 mmol/L	24 hours	
Cortisol AM (8-10AM)	Serum	Brown Cap	4.9mL	166-507 nmol/ L	24hrs	
Creatine Kinase (CK)	Plasma	Orange Cap	4.9mL	Males 39-308 IU/L Females 26 – 192 IU/L	24 hours	

Test	**Sample Required**	**Specimen Container**	**Minimum Volume**	**Reference Range/ Unit of Measurement**	**TAT**	**Comment**
Creatinine	Plasma	Orange Cap	4.9mL	Male: 59-104µmol/L Female: 45-84µmol/L	24 hours	
Creatinine Urine - 24 Hour	24 hour Urine collection	Plain	N/A	Female: 6000-13000 µmol/ 24hrs Male: 9000-19000 µmol/ 24hrs	72 hours	
Creatinine Clearance (GFR)	24 hour Urine collection	Plain	N/A	80 – 125 mL/min (adults)	72 hours	Blood creatinine level also required for GFR calculation.
Cyclosporin A	Whole blood	EDTA (Pink Cap)	2.6mL	N/A (ng/ml)	10days	Trough level sample.
Dehydroepi-androsterone Sulphate (DHEAS)	Serum	Plain (White Cap)	4.9mL	(µmol/L) *Years* / *Female* / *Male* 15-20y: 1.8-10.0 / 1.9-13.4 20-25y: 4.0-11.0 / 5.7-13.4 25-35y: 2.7-9.2 / 4.3-12.2 35-45y: 1.7-9.2 / 2.4-11.6 45-55y: 1.0-7.0 / 1.2-9.0 55-65y: 0.5-5.6 / 1.4-8.0 65-75y: 0.3-6.7 / 0.9-6.8 ≥ 75y: 0.3-4.2 / 0.4-3.3	3days	Age and gender specific ranges are applied to individual reports.
Dexamethasone overnight	Serum	Brown Cap	4.9 mL		24hrs	
Digoxin	Serum	Plain (White Cap)	4.9mL	0.6 -1.2 µg/L	24 hours	With hypokalaemia toxicity may occur within the therapeutic range

Test	**Sample Required**	**Specimen Container**	**Minimum Volume**	**Reference Range/ Unit of Measurement**	**TAT**	**Comment**
Dopamine	24 hour Urine collection	Pre-acidified (50% acid)	N/A	See Table Below	24 working days	If the patient is <15 years, please contact the laboratory [(01)7977333)] for information regarding sample collection. Age-related reference ranges will accompany results for paediatric samples.
Ethanol	Plasma	Fluoride oxalate (Yellow Cap)	2.7 mL	Unit: mg %	24 hours	
Ferritin	Plasma	Orange Cap	4.9mL	Female(17-60yr):13-150ng/mL Male (20-60yr):30-400ng/mL	3days	No reference range for >60yr.
Folate/ Folic Acid	Plasma	Orange Cap	4.9mL	3.9 – 26.8µg/L	3days	Affected by light and recent food intake. Please note, results reported as >20 µg/L.
Follicle Stimulating Hormone (FSH)	Serum	Brown Cap	4.9mL	Male : 1.5 – 12.4 U/L Female: Follicular : 3.5-12.5 U/L Mid Cycle : 4.7 – 21.5 U/L Luteal : 1.7-7.7 U/L Post Menopausal : 25.8-134.8 U/L	3days	
Free T3	Plasma	Orange Cap	4.9ml	3.1 - 6.8 pmol/L	3days	
Free Thyroxine (fT4)	Plasma	Orange Cap	4.9mL	11.9-21.6 pmol/L	3days	
Gamma-glutamyl transferase (GGT)	Plasma	Orange Cap	4.9mL	Males < 59 I.U/L Females < 39 I.U/L	24 hours	

Test	**Sample Required**	**Specimen Container**	**Minimum Volume**	**Reference Range/ Unit of Measurement**	**TAT**	**Comment**
Glucose - Fasting	Plasma	Fluoride Oxalate (YELLOW cap)	2.7mL	Normoglycaemia = 3.5 - 5.5 mmol/L Impaired Fasting Glucose = 5.6 –6.9 mmol/L Diabetes = ≥7.0 mmol/L	24 hours	Fast for at least 8 hours. See Interpretive Comment (ADA 2019)
Glucose- Random	Plasma	Fluoride Oxalate (YELLOW cap)	2.7mL	Values >7.8 and <11.1 recommend Fasting Glucose Values ≥ 11.1 (with symptoms of hyperglycaemia) are consistent with Diabetes.	24 hours	See Interpretive Comment (ADA 2019)
Growth Hormone (hGH)	Serum	Brown Cap	4.9mL	N/A (ng/ml) See Interpretive Comment	20 days	hGH test most useful in dynamic tests where states of hypoglycaemia or hyperglycaemia are induced. Samples need to be separated and frozen immediately.
HbA_{1C}	Whole blood	EDTA (PINK cap)	2.6mL	IFCC: 20–42 mmol/mol Reference range for people without diabetes. The target range for patients with diabetes will be set by the clinician.	96 hours	
HMMA (VMA)	24 hour Urine collection	Pre-acidified (50% acid)		< 45 µmol/24 hr (Adult)	12 working days	If the patient is <15 years, please contact the laboratory [(01)7977333)] for information regarding sample collection. Age-related reference ranges will accompany results for paediatric samples.

Test	**Sample Required**	**Specimen Container**	**Minimum Volume**	**Reference Range/ Unit of Measurement**	**TAT**	**Comment**
HVA	24 hour Urine collection	Pre-acidified (50% acid)		< 40 µmol/24 hr (Adult)	12 working days	If the patient is <15 years, please contact the laboratory [(01)7977333)] for information regarding sample collection. Age-related reference ranges will accompany results for paediatric samples.
Immmunoglobulin G, A, M	Serum	Brown Top	4.9ml	IgG: 7.0 - 16.0g/L IgA: 0.7 - 4.0 g/L IgM: 0.4 - 2.3 g/L	35 days	

<table>
<tr>
<td>IGF1</td>
<td>Serum</td>
<td>White Cap</td>
<td>4.9mL</td>
<td>(ng/ml)

<table>
<tr><th>Years</th><th>Female</th><th>Male</th></tr>
<tr><td>0-1y</td><td>17.9-125.6</td><td>27.0-157.0</td></tr>
<tr><td>1-2y</td><td>19.5-132.3</td><td>29.7-166.8</td></tr>
<tr><td>2-3y</td><td>22.2-145.4</td><td>33.9-183.9</td></tr>
<tr><td>3-4y</td><td>25.9-164.2</td><td>39.0-204.5</td></tr>
<tr><td>4-5y</td><td>30.7-187.8</td><td>44.3-225.0</td></tr>
<tr><td>5-6y</td><td>26.2-214.4</td><td>50.0-245.5</td></tr>
<tr><td>6-7y</td><td>42.0-240.4</td><td>56.2-267.1</td></tr>
<tr><td>7-8y</td><td>48.6-269.6</td><td>63.4-291.9</td></tr>
<tr><td>8-9y</td><td>56.9-305.3</td><td>72.4-323.1</td></tr>
<tr><td>9-10y</td><td>67.2-349.4</td><td>83.6-361.6</td></tr>
<tr><td>10-11y</td><td>79.5-400.3</td><td>96.9-406.6</td></tr>
<tr><td>11-12y</td><td>92.6-452.6</td><td>111.6-454.4</td></tr>
<tr><td>12-13y</td><td>105.3-499.1</td><td>126.1-498.7</td></tr>
<tr><td>13-14y</td><td>115.9-533.4</td><td>138.6-532.5</td></tr>
<tr><td>14-15y</td><td>123.4-552.0</td><td>147.5-551.2</td></tr>
<tr><td>15-16y</td><td>127.4-554.2</td><td>152.2-553.5</td></tr>
<tr><td>16-17y</td><td>127.9-541.5</td><td>152.9-541.8</td></tr>
<tr><td>17-18y</td><td>125.3-517.3</td><td>150.6-520.6</td></tr>
<tr><td>18-19y</td><td>120.5-485.8</td><td>146.2-493.6</td></tr>
<tr><td>19-20y</td><td>114.4-450.8</td><td>140.2-462.7</td></tr>
<tr><td>20-21y</td><td>107.8-416.0</td><td>133.1-430.0</td></tr>
<tr><td>21-26y</td><td>92.9-342.0</td><td>115.2-354.8</td></tr>
<tr><td>26-31y</td><td>78.4-270.0</td><td>97.9-281.6</td></tr>
<tr><td>31-36y</td><td>73.1-243.0</td><td>88.3-246.0</td></tr>
<tr><td>36-41y</td><td>69.0-227.0</td><td>83.4-232.7</td></tr>
<tr><td>41-46y</td><td>61.5-204.4</td><td>74.9-216.4</td></tr>
<tr><td>46-51y</td><td>56.8-194.5</td><td>66.9-205.1</td></tr>
<tr><td>51-56y</td><td>53.0-189.6</td><td>60.6-200.3</td></tr>
<tr><td>56-61y</td><td>45.6-172.4</td><td>54.3-194.2</td></tr>
<tr><td>61-66y</td><td>42.2-169.0</td><td>48.8-187.7</td></tr>
<tr><td>66-71y</td><td>38.3-162.5</td><td>46.5-191.9</td></tr>
<tr><td>71-76y</td><td>36.6-164.7</td><td>40.9-179.2</td></tr>
<tr><td>76-81y</td><td>34.7-164.8</td><td>37.1-172.0</td></tr>
<tr><td>81-86y</td><td>34.4-172.4</td><td>33.8-165.4</td></tr>
<tr><td>86-91y</td><td>33.6-177.8</td><td>32.2-166.1</td></tr>
</table>
</td>
<td>20days</td>
<td>Sample must be spun and separated immediately.</td>
</tr>
</table>

Test	**Sample Required**	**Specimen Container**	**Minimum Volume**	**Reference Range/ Unit of Measurement**	**TAT**	**Comment**
Iron	Plasma	Orange Cap	4.9mL	5.8 – 34.5µmol/L	24 hours	
Lactate Dehydrogenase (LDH)	Plasma	Orange Cap	4.9mL	Males 135 – 225 U/L Females 135 – 214 U/L	24 hours	Significantly increased by haemolysis and/or if sample is left on cells overnight.
Lithium	Serum	Plain (White Cap)	4.9mL	0.6 – 1.2 mmol/L	24 hours	Therapeutic range: Up to 1.3 in acute mania. Severe Toxicity likely if level > 2.0
Lipid Profile (Fasting)	Plasma	Orange Cap	4.9ml	Cholesterol ≤ 5.0mmol/L LDL(calculated) ≤ 3.0mmol/L HDLCholesterol ≥ 1.0mmol/L Triglycrides ≤ 1.7mmol/L Non HDL Cholesterol ≤ 3.8mmol/L	24 hours	Refer to National Pathology Handbook: Laboratory Testing for Lipids
Lipid Profile (Non Fasting)	Plasma	Orange Cap	4.9ml	Cholesterol ≤ 5.0mmol/L LDL (calculated) ≤3.0mmol/L HDLCholesterol ≥ 1.0mmol/L Triglycrides ≤ 2.0mmol/L Non HDL Cholesterol ≤ 3.8mmol/L	24 hours	Refer to National Pathology Handbook: Laboratory Testing for Lipids

Test	**Sample Required**	**Specimen Container**	**Minimum Volume**	**Reference Range/ Unit of Measurement**	**TAT**	**Comment**
Luteinising Hormone (LH)	Serum	Brown Cap	4.9mL	Male: 1.7 – 8.6 U/L Female: Follicular: 2.4 - 12.6 U/L Ovulation: 14.0 - 95.6 U/L Luteal: 1.0 - 11.4 U/L Post-Menopausal: 7.7-58.5 U/L	3days	
Magnesium	Plasma	Orange Cap	4.9mL	0.66 – 1.07 mmol/L	24 hours	Increased by haemolysis or if sample is left on cells overnight.
Magnesium 24 Hour Urine	24 hour Urine collection	No Preservative		3.0 – 5.0 mmol/24hr	72 hours	Container available from Phlebotomy Department
Metanephrine (Urine Total)	24 hour Urine collection	Pre-acidified (50% acid)		See Table Below	24 working days	Container available from Phlebotomy Department
Neuroblastoma Screen (HMMA, HVA, Dopamine, Adrenaline and Noradrenaline).	Spot Urine Sample carefully acidified to pH 4 or less.	Plain container but sample should be protected from light	1-2 mLs	All results are reported in mmol/Mol of Creatinine	12 days for Routine samples 1-2 working days for Urgent samples- must be discussed with laboratory	

Test	**Sample Required**	**Specimen Container**	**Minimum Volume**	**Reference Range/ Unit of Measurement**	**TAT**	**Comment**
Noradrenaline	24 hour Urine collection	Pre-acidified (50% acid)	N/A	See Table Below	24 working days	If the patient is <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.
Normetanephrine (Urine Total)	24 hour Urine collection	Pre-acidified (50% acid)	N/A	See Table Below	24 working days	Container available from Phlebotomy Department
Oestradiol	Serum	Brown Cap	4.9mL	Follicular: 114-332 pmol/L Ovulation: 222-1959 pmol/L Luteal: 222-854 pmol/LPost Menopausal:18.4-505 pmol/L Male: 41.4 – 159 pmol/L	3days	
Osmolality	Plasma	(Orange Cap)	4.9mL	275 – 295 mOsm/Kg	24 hours	
Osmolality Urine	Spot urine sample	Plain MSU container	N/A	400 – 1000 mOsm/Kg	24 hours	Results are interpreted in conjunction with the plasma Osmolality
Paracetamol	Serum	Plain (White Cap)	4.9 mL	Units: mg/L	24 hours	In suspected overdose, take sample more than 4 hours post ingestion.
Parathyroid Hormone (PTH)	Whole blood	EDTA (Blue cap)	4.9ml	15 – 65 pg/ml	3days	
Phenobarbital	Serum	Plain (White Cap)	4.9mL	10.0 – 40.0 mg/L	24 hours	Therapeutic range ill-defined due to ‘tolerance’
Phenytoin	Serum	Plain (WhiteCap)	4.9mL	5.0 – 20.0 mg/L	24 hours	Severe toxicity likely if > 40.0mg/L

Test	**Sample Required**	**Specimen Container**	**Minimum Volume**	**Reference Range/ Unit of Measurement**	**TAT**	**Comment**
Phosphate	Plasma	Orange Cap	4.9mL	0.81 – 1.45 mmol/L	24 hours	Can be dramatically increased if sample is left on cells overnight.
Phosphate 24 Hour Urine	24 hour Urine collection	No Preservative	N/A	13.00 – 42.00 mmol/24hr	72 hours	Container available from Phlebotomy Department
Plasma Free Metanephrines	Plasma	EDTA 7.5ml	7.5ml	See table Section 3.4.11	12 working days	Sample must be taken on ice, spun within 1hr of collection aliquoted, frozen for transport.
Potassium	Plasma	Orange Cap	4.9mL	3.5 – 5.3 mmol/L	24 hours	Greatly increased if sample is left on cells overnight or refrigerated.
Potassium 24 Hour Urine	24 hour Urine collection	Plain	N/A	30.0 – 100.0 mmol/24hr (for person on average diet)	72 hours	
Potassium Urine	Spot urine sample	Plain MSU container	N/A	N/A See Interpretive Comment	24 hours	Reference ranges are not available for spot urine tests, results must be considered in conjunction with the age, sex and hydration status of the patient
Prolactin	Serum	Brown Cap	4.9mL	(mIU/L) **Total Prolactin** Female: 102-496 mIU/L Male: 86-324 mIU/L **Bioactive Prolactin:** Female: 75-381mIU/L Male: 63-245 mIU/L	7 days	Bioactive prolactin is the biologically active form of prolactin. For further information please consult the National Laboratory Handbook- Laboratory Testing for Hyperprolactinaemia 2019.

Test	**Sample Required**	**Specimen Container**	**Minimum Volume**	**Reference Range/ Unit of Measurement**	**TAT**	**Comment**
Progesterone	Brown	Brown Cap	4.9mL	Male: 0.159 - 0.474 nmol/L Female: Follicular: 0.159 – 0.616 nmol/L Ovulation: 0.175– 13.2nmol/L Luteal: 13.1 - 46.3 nmol/L Post Menopasal: 0.159 - 0.401 nmol/L	3days	Note date of cycle on test request form.
Protein (Total)	Plasma	Orange Cap	4.9mL	60 – 80 g/L	24 hours	
Protein (TUP) 24 Hour Urine	24 hour Urine collection	Plain	N/A	0.05 – 0.140 g/24 hour	72 hours	
Protein : Creatinine Ratio	Spot urine sample	Plain MSU container	N/A	3 – 14 mg/mmol	96hours	
Protein Electrophoresis	Serum	Brown Cap	4.9mL	N/A See Interpretive Comments	35 days	
PSA (total) Roche Method (e801)	Plasma	Orange Cap	4.9mL	**Age related PSA (non-suspicious DRE)** <50 = <2ug/L 50 – 59 = <3ug/L 60 – 69 = <4ug/L 70+ = <5ug/	3days	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. Refer to National Pathology Handbook: National Prostate Cancer GP Referral Guideline
Salicylate	Serum	Plain (White Cap)	4.9 mL	Units: mg/L	24 hours	Concern level 280 mg/L if age <5 years; Severe toxicity likely if level >700 mg/L

Test	**Sample Required**	**Specimen Container**	**Minimum Volume**	**Reference Range/ Unit of Measurement**	**TAT**	**Comment**
SHBG	Serum	Plain (White Cap)	4.9ml	Male (20-49yr): 18.3 – 54.1nmol/L Male (≥ 50yr): 20.6 – 76.7nmol/L Female (20-49yr): 32.4 – 128.0nmol/L Female (≥ 50yr): 27.1 – 128.0nmol/L	3days	No SHBG reference intervals for <20yr old.
Sodium	Plasma	Orange Cap	4.9mL	133 – 146 mmol/L	24 hours	
Sodium 24 Hour Urine	24 hour Urine collection	Plain	N/A	40.0 – 220.0 mmol/24hr	72 hours	
Sodium Urine	Spot urine sample	Plain MSU container	N/A	N/A See Interpretive Comment	24 hours	Reference ranges are not available for spot urine tests, results must be considered in conjunction with the age, sex and hydration status of the patient
Tacrolimus (FK506)	Whole blood	EDTA (Pink Cap)	2.6mL	(ngml) N/A See Interpretive Comment	72 hours	Trough level sample.
Testosterone	Serum	Plain (White cap)	4.9mL	Male: 19 - 50y: 8.6 - 29.0 nmol/L ≥50y: 6.7 - 25.7 nmol/L Female: 19 - 50y: 0.3 - 1.7 nmol/L ≥50 y: 0.1 –1.4 nmol/L	3 days	
Theophylline	Serum	Plain (White Cap)	4.9mL	10.0 – 20.0 mg/L	24 hours	Lower levels ≥ 5.0mg/L may be effective. Concern level 14.0mg/L if age < 3months. Severe toxicity likely if > 60.0mg/L.

Test	**Sample Required**	**Specimen Container**	**Minimum Volume**	**Reference Range/ Unit of Measurement**	**TAT**	**Comment**
Total Thyroxine (TT4)	Lithium Heparin	Lithium Heparin (Orange Top)	4.9ml	66 – 181nmol/L	72 hours	
Transferrin	Plasma	Orange Cap	4.9mL	A Fasting Transferrin Saturation > 55% in Males OR > 50% in Females indicates Iron accumulation.	24 hours	If Transferrin Saturation > 50% Please repeat on a morning fasting sample. Refer to: BCSH Guidelines.
Thyroid Stimulating Hormone (TSH)	Plasma	Orange Cap	4.9mL	0.27- 4.20 mU/L	72 hours	
Triglycerides	Plasma	Orange Cap	4.9mL	0.5 -2.0nmol/L (Non fasting) 0.5 - 1.7mmol/L (Fasting)	72 hours	Refer to National Pathology Handbook: Laboratory Testing for Lipids
Troponin T (TNT) Must have a dedicated sample	Lithium Heparin	Lithium Heparin (Orange Top)	2.7mL	< 14 ng/L	1.5 hours	
Urate/Uric Acid	Plasma	Orange Cap	4.9mL	Males 202 - 416µmol/L Females 143 - 340µmol/L	24 hours	
Urate 24 Hour Urine	24 hour Urine collection	Plain	N/A	1.20 – 5.90 mmol/24hr	72 hours	
Urea	Plasma	Orange Cap	4.9mL	2.8 – 8.1 mmol/L	24 hours	
Urea 24 Hour Urine	24 hour Urine collection	Plain	N/A	428.0 – 714.0 mmol/24hr	72 hours	
Valproic Acid	Serum	Plain (White Cap)	4.9mL	50 – 100 mg/L	24 hours	Therapeutic range ill-defined as toxic effects shows no clear relationship to plasma levels.
Vitamin D	Serum	Plain (White Cap)	4.9mL	Deficient: < or = 50 nmol/L	10 days	Vitamin D analysed by LCMS Please refer to: National Pathology Handbook: Laboratory Testing for Vitamin D

Test	Sample Required	Specimen Container	Minimum Volume	Reference Range/ Unit of Measurement	TAT	Comment
Xanthochromia	CSF	Brown cap wrapped in foil to exclude light	1mL	N/A See Interpretive Comment	24 hours (Mon-Fri)	See Protocol for Sending CSF Samples to Beaumont for Xanthochromia Analysis Beaumont Hospital - Chemical Pathology **Service available during routine working hours only.**

3.3.9.4 Calculated / Derived Tests

Calculated Parameter	Formula	Reference Range	Units	Important Notes
Calcium Adjusted	[Ca] + (46.18 – [Alb]) * 0.01516 (Locally derived equation)	2.21 – 2.52	mmol/L	The calculation is unsuitable if the albumin result is < 30g/L or > 52g/L
Globulin	Total Protein - Albumin	N/A	g/L	Interpret in conjunction with Total Proteim amd Albumin values.
LDL (Low Density Lipoprotein) Cholesterol	Cholesterol – HDL – (triglyceride / 2.2) (Friedwald Equation)	See Above	mmol/L	The calculation is unsuitable if the triglyceride level is > 4.5mmol/l Refer to National Pathology Handbook: Laboratory Testing for Lipids
Non-HDL Cholesterol	Total Cholesterol – HDL Cholesterol.	See Above	mmol/L.	Refer to National Pathology Handbook: Laboratory Testing for Lipids
Transferrin saturation (TfS)	(Iron / Transferrin) * 398	See Interpretive Comment	%	If transferrin saturation > 50% please repeat on a morning fasting sample. Refer to: BSCH guidelines. A fasting transferrin saturation > 55% in males or >50% in females indicates iron accumulation.

Calculated Parameter	Formula	Reference Range	Units	Important Notes
Unconjugated Bilirubin	Total Bilirubin – Conjugated (Direct) Bilirubin	N/A		Patient must attend Beaumont Hospital phlebotomy so that a fresh sample can be taken and protected from light. Must be interpreted in conjunction with Total and Conjugated Bilirubin.

3.3.10 *Urinary Catecholamines and Metabolites Reference Ranges:*

Adult reference ranges:

Analyte	Reference Interval
Noradrenaline	< 0.900 umol/24hrs
Adrenaline	< 0.230 umol/24hrs
Dopamine	< 3.300 umol/24hrs
Metanephrine	< 1.80 umol/24 hrs
Normetanephrine	< 2.80 umol/24 hrs

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

Age Group (yrs)	Noradrenaline	Adrenaline	Dopamine
< 1	< 0.43	< 0.08	< 1.95
1 – 3	< 0.20	< 0.08	< 1.45
3 – 5	< 0.19	< 0.08	< 0.95
5 – 8	<0.18	< 0.08	< 0.85
8 – 11	<0.17	< 0.08	< 0.75
> 11	<0.13	< 0.08	< 0.65

3.3.11 *Plasma Metanephrine Reference Ranges*

Analyte	Reference Interval (Seated)

Plasma Free Methanephrine	0 – 510 pmol/L
Plasma Free Normetanephrine	0 – 1180 pmol/L
Plasma Free 3-Methyoxytyramine	0 – 180 pmol/L

The below table provides Supine Reference Intervals, i.e. after 30 minutes rest.

Analyte	Reference Interval (Supine)
Plasma Free Methanephrine	0 – 450 pmol/L
Plasma Free Normetanephrine	0 – 730 pmol/L
Plasma Free 3-Methyoxytyramine	0 – 180 pmol/L

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.

3.4 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.

3.4.1 *Clinical Service*

There is a general immunology outpatient's clinic held in Clinic A on Monday mornings and an allergy clinic held in Clinic F on Thursday afternoons. Additionally, ANP led allergy clinics are held in the department on Monday, Tuesday, Wednesday and Thursday mornings. The Department also has an established home therapy programme for patients on immunoglobulin replacement therapy and an ANP-led review clinics for these patients are held in the department on Thursday afternoons.

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 Prof. 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.

3.4.2 *Laboratory Service*

The Laboratory provides a large range of immunological investigations focussing on investigations for autoimmune and allergic disorders. 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.

3.4.3 *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, Prof. 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 Prof. Keogan/Dr Khalib by a senior member of the clinical team who is familiar with the patient's history.

3.4.4 ***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.

Test	**Specimen**	**Minimum Volume**	**Method**	**Reference Range**	**TAT**	**Urgent Service**	**Comment**	**Frequency of Retesting**
Acute Allergic Reaction Investigation AARI (Service for Beaumont GP's only)	Serum Gel Brown tube	4.9 mls Note 200µl serum plus an extra 50µl serum per specific allergen needed	FEIA (IMMUNOCAP)	N/A	N/A		See Section 2.4.28 for reporting pathway	As requested and discussed as per interpretive comments
Anti-Adrenal Antibodies	Serum Gel Brown tube	4.9 mls	Indirect immunofluorescence	Negative	4 weeks			6 months
Anti-Beta2Glycoprotein 1	Serum Gel Brown tube	4.9 mls	EliA (IMMUNOCAP)	<10 U/ml	8 days			12 weeks
Anti-Cardiolipin Antibodies (IgG and IgM)	Serum Gel Brown tube	4.9 mls	EliA (IMMUNOCAP)	IgG: 0-10 GPLU/mL IgM: 0-10 MPLU/mL	8 days			12 weeks
Anti-CCP	Serum Gel Brown tube	4.9 mls	EliA (IMMUNOCAP)	< 7 U/ml	8 days			3 Months
Anti-Double-Stranded-DNA Antibodies	Serum Gel Brown tube	4.9 mls	EliA (IMMUNOCAP) & IIF by DNA crithidia	EliA:<10 IU/mL IIF: Negative	EliA:<3-5 days IIF: 8 days	On Request		>3 weeks (unless plasma-apheresis/ discussion)

Test	Specimen	Minimum Volume	Method	Reference Range	TAT	Urgent Service	Comment	Frequency of Retesting
Anti-ENA (Extractable Nuclear Antigen) Antibodies – includes anti-Ro, La, RNP, Sm, Jo-1 & Scl-70)	Serum Gel Brown tube	4.9 mls	EliA with confirmation by EliA &Immunoblot	Negative for all 6 components	2-3 weeks		ENA Typing only preformed on Equivocal and Positive ENA Screens	>1 year unless patient is pregnant
Anti-Endomysial (IgA) Antibodies	Serum Gel Brown tube	4.9 mls	Indirect Immunofluorescence	Negative	8 days			>3 months
Anti-Endomysial (IgG) Antibodies	Serum Gel Brown tube	4.9 mls	Indirect Immunofluorescence	Negative	8 days		Only performed when IgA deficiency	
Anti-Gastric-Parietal Cell antibodies (Anti-GPC)	Serum Gel Brown tube	4.9 mls	Indirect Immunofluorescence	Negative	3-5 days			>3 months
Anti-Glomerular Basement Membrane antibodies (Anti-GBM)	Serum Gel Brown tube	4.9 mls	EliA (IMMUNOCAP)	Negative: <7U/ml Equivocal: 7- 10U/ml Positive: >10 Uml	1-3 days	On Request		As requested & discussed
Anti-Histone Antibodies	Serum Gel Brown tube	4.9 mls	Immunoblot	Negative	4-6 weeks			Once Off
Anti-Intrinsic Factor Antibodies	Serum Gel Brown tube	4.9 mls	EliA (IMMUNOCAP)	Negative: <7 U/ml Equivocal: 7- 10U/ml Positive: >10 U/ml	8 days			>6 months
Anti-Liver-Kidney Microsomal (LKM) Antibodies	Serum Gel Brown tube	4.9 mls	Indirect Immunofluorescence + Immunoblot if IIF positive	Negative	3-5 days			>1 month

Test	**Specimen**	**Minimum Volume**	**Method**	**Reference Range**	**TAT**	**Urgent Service**	**Comment**	**Frequency of Retesting**
Anti-Mitochondrial Antibody (including M2 subtyping)	Serum Gel Brown tube	4.9 mls	Indirect Immunofluorescence + ELISA if positive	Negative M2 ELISA <10 IU/ml	3-5 days (1 month if IIF positive)			>3 months M2 performed only once
Anti-Myeloperoxidase antibodies (Anti-MPO)	Serum Gel Brown tube	4.9 mls	EliA (IMMUNOCAP)	<3.5IU/mL	3-5 days, or as required	On request	Follow-up of patients with know MPO-ANCA positive disease	3 Weeks, unless discussed
Anti-Myeloperoxidase antibodies (Anti-MPO) & Anti-Proteinase 3 antibodies (Anti-PR3)	Serum Gel Tube	4.9mls	EliA (IMMUNOCAP)	<3.5IU/mL MPO <2IU/mL PR3	3-5 days, or as required	On request		3 Weeks, unless discussed
Anti-Neuronal Antibodies incorporating Anti-Hu, Anti-Yo, Anti-Ri, Anti-PNMA2, Anti-Amphiphysin, Anti-Cv2/CRMP5, Anti-Recoverin, Anti-SOX1, Anti-Zic4, Anti-Titin, Anti-GAD65, Anti-Tr	Serum Gel Brown tube	4.9 mls	Indirect Immunofluorescence & Immunoblot	Negative	15 days	On request	Paired Serum/CSF samples will be accepted. Results of both must be interpreted in the clinical context.	>6 months
	CSF Specimen, minimum volume 1ml	1ml						

Test	Specimen	Minimum Volume	Method	Reference Range	TAT	Urgent Service	Comment	Frequency of Retesting
Anti-Neutrophil Cytoplasm Antibodies (ANCA) (IIF)	Serum Gel Brown tube	4.9 mls	Indirect Immunofluorescence	Negative	8 days			3 Weeks, unless discussed
Anti-Nuclear Factor	Serum Gel Brown tube	4.9 mls	Indirect Immunofluorescence	Negative. Weak positive (1:80) are commonly seen particularly in healthy older women.	3-5 days			No more than 3 monthly
Anti-Nucleosome Antibodies	Serum Gel Brown tube	4.9 mls	Immunoblot	Normal value: Negative	4-6 weeks		Strong clinical suspicion of lupus with negative routine serology. Must discuss with Consultant Immunologist.	Once Off
Anti-Pneumococcal antibodies	Serum Gel Brown tube	4.9 mls	ELISA	Normal response to vaccination is a four fold increase in the level of titres. Units mg/L.	4-8 weeks		Should only be used to assess vaccine responses.	Pre-vaccine, 1 month post, 3-6 months & then annually for Pneumococcal. Pre & Post vaccine for others.

Test	Specimen	Minimum Volume	Method	Reference Range	TAT	Urgent Service	Comment	Frequency of Retesting
Anti-Proteinase 3 antibodies (Anti-PR3)	Serum Gel Brown tube	4.9 mls	EliA (IMMUNOCAP)	<2IU/mL	3-5 days, or as required	On request	Follow-up of patients with known PR3-ANCA positive disease.	3 Weeks, unless discussed
Anti-Ribosomal-P-Protein antibodies	Serum Gel Brown tube	4.9 mls	Immunoblot	Normal value: Negative	4-6 weeks		Strong clinical suspicion of lupus with negative routine serology. Must discuss with Consultant Immunologist.	Once Off
Anti- SARS-CoV-2 Antibodies	Serum Gel Tube	4.9mls	Immunoassay	Nucleocapsid: Not Detected Anti-Spike: <0.8 U/ml Not Detected	16 days		Nucleocapsid and Spike Antibody	
Anti-Scleroderma Antibodies	Serum Gel Brown tube	4.9 mls	Immunoblot	Negative	4-6 weeks			
Anti-Skin Antibodies	Serum Gel Brown tube	4.9 mls	Indirect immunofluorescence	Negative	8 days			6 months but Positive ICS as requested
Anti-Smooth Muscle Antibodies	Serum Gel Brown tube	4.9 mls	Indirect Immunofluorescence	Negative	3-5 days			>3 months
Anti-Streptolysin-O Titre (ASOT)	Serum Gel Brown tube	4.9 mls	Immunoturbidimetry	<200IU/ml	3-5 days			3 weeks

Test	Specimen	Minimum Volume	Method	Reference Range	TAT	Urgent Service	Comment	Frequency of Retesting
Anti-Thyroid Peroxidase Antibodies (anti-TPO)	Serum Gel Brown tube	4.9 mls	Immunoassay	Negative: <=34 IU/mL Positive: > 34 IU/mL	8 days			>6 months; if equivocal >3 months
Anti-Tissue Transglutaminase Antibodies (anti-tTG)	Serum Gel Brown tube	4.9 mls	EliA (IMMUNOCAP)	Negative: < 4 U/ml Equivocal: 4-10 U/ml Positive: 10 U/ml	8 days			>3 months
Autoimmune Encephalitis MOSAIC incorporating anti-NMDA, anti-AMPA 1/2, anti-$GABA_B$, Anti-DPPX, anti-LGI1, anti-CASPR2 antibodies	Serum Gel Brown tube	4.9 mls	Indirect Immunoflourescence	Negative	8 days	On request	Serum/CSF paired samples are preferable particularly in the initial diagnostic phase. In certain cases Plasma may be acceptable.	Discussed
Complement Function	Serum Gel Brown tube	4.9 mls	ELISA	Normal	3 months		It is essential that serum is separated and frozen within 3 hours maximum after venepuncture	3 weeks
C1 Esterase Inhibitor (C1IN)	Serum Gel Brown tube	4.9 mls	Turbidimetry	0.21-0.38 g/L	4-6 weeks			Once off if normal. As required if low.
C3	Serum Gel Brown tube	4.9 mls	Immunoturbidimetry	0.9-1.8 g/L	1-5 days	On request		As Requested

Test	Specimen	Minimum Volume	Method	Reference Range	TAT	Urgent Service	Comment	Frequency of Retesting
C4	Serum Gel Brown tube	4.9 mls	Immunoturbidimetry	0.1-0.4 g/L	1-5 days	On request		As Requested
CTD Screen	Serum Gel Brown tube	4.9 mls	EliA (IMMUNOCAP)	Negative	2-5 days			No more than 3 monthly
Direct Immunofluorescence (DIF) on Skin Biopsies	Fresh skin punch biopsy wrapped in saline gauze		Direct Immunofluorescence		4 weeks		Unless special arrangements have been agreed specimen MUST reach the immunology laboratory by 4pm	
IgG Subclasses	Serum Gel Brown tube	4.9 mls	Turbidimetry	IgG 7 - 16 g/L IgG1 3.824 - 9.286 g/L IgG2 2.418 - 7.003 g/L IgG3 0.218 - 1.761 g/L Note: These are adult specific reference ranges	8 weeks			Annually
Mast Cell Tryptase	Serum Gel Brown tube	4.9 mls	FEIA (IMMUNOCAP)	2-14 µg/L (Anti-mortem specimens only)	1 month			As requested/discussed
Myositis Screen	Serum Gel Brown tube	4.9 mls	Immunoblot, correlated with ANF appearance	Negative	4-6 weeks			Once off
Query Test	Serum Gel Brown tube	4.9 mls	Consultant, SPR or Chief Medical Scientist will select appropriate tests	As appropriate	As per assay		Full clinical details & bleep number required	

Test	Specimen	Minimum Volume	Method	Reference Range	TAT	Urgent Service	Comment	Frequency of Retesting
Rheumatoid Factor	Serum Gel Brown tube	4.9 mls	Immunoturbidimetry	<14 IU/mL	3-6 days			>3 Months
Specific IgE	Serum Gel Brown tube	4.10 mls Note 200µl serum plus an extra 50µl serum per specific allergen needed	FEIA (IMMUNOCAP)	<0.35 Class 0 Negative 0.35-0.7 Class 1 Weakly Positive 0.7-3.5 Class 2Positive 3.5-17.5 Class 3 Positive 17.5-52.5 Class 4 Strongly positive 52.5-100 Class 5 Strongly positive >100 Class 6 Strongly positive	15days 21 days for sIgE to Drugs		For GP users,please use AARI form LF-IMM-GEN0024, refer to section 2.4.28	1 year for same allergens
Specific IgGs sIgG Aspergillus sIgG M. Faeni sIgG Budgie sIgG Pigeon	Serum Gel Brown tube	4.9 mls	FEIA (IMMUNOCAP)	<40 mgA/l <22 mgA/l <30 mgA/l <38 mgA/l	30 days			>6 months
Total IgE	Serum Gel Brown tube	4.9 mls	FEIA (IMMUNOCAP)	Range is age related. Adult reference range 0-100 kU/L	15 days			1 year

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 Prof. 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.

3.4.4.1 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.

Profile	*Tests Included*	*Indication*	*Comment*
Acute Renal Failure Screen ARF SCR	CTD ANCA GBM C3/C4 ASOT	Acute or acute –on-chronic renal failure.	Please discuss all pulmonary-renal syndrome or ?rapidly progressive GN, as urgent service available.
Inflammatory Arthritis Antibodies INFL ABS	RF CCP CTD	Isolated inflammatory arthritis, in the absence of systemic features.	ANCA should be added if urinalysis is abnormal. NB: 2 separate samples are required for INFL ABS.
Liver Autoantibodies LIV ABS	ANF Anti-Smooth Muscle Anti-Mitochondrial Anti-LKM	Suspected chronic liver disease.	If MITO pos, M2 subtyping will be performed, on the first occasion only.
Vasculitis Screen VAS SCR	CTD ANCA RF C3/C4	Suspected vasculitis or connective tissue disease.	This battery is intended for diagnosis only. More selective tests should be used for monitoring once diagnosis established.
Asthma sIgE RASTA	sIgE - House dust mite sIgE – Aspergillus sIgE – Cat	Allergic asthma.	
Rhinitis sIgE RASTR	sIgE – House dust mite sIgE – Cat sIgE – Trees sIgE - Grass	Perennial Rhinitis, thought to be allergic.	
Shellfish sIgE RSTSHELL	sIgE – Lobster sIgE - Crab sIgE - Shrimp sIgE - Mussel	Suspected allergy to shellfish.	Negative result does not rule out shellfish allergy. If this is suspected clinically referral to a Clinical Immunologist is advised.
Pollen sIgE RSTPOLL	sIgE – Mixed grass sIgE –Mixed trees		

Profile	*Tests Included*	*Indication*	*Comment*
Coeliac Screen COEL SCR	Anti-tTG Anti-EmA (if tTG is Equivocal or Positive)	Suspected Coeliac Disease. Malabsorption. Anaemia. Gastrointestinal symptoms.	

3.4.4.2 Immunological Tests performed in other Laboratories in Beaumont Hospital

Test	**Specimen**	**Contact**
Immunoglobulins	Serum Gel	Proteins (809) 2305
C Reactive Protein	Heparin	Clin Chem (809) 2668
Protein electrophoresis	Serum Gel	Proteins (809) 2305
Urine electrophoresis (Bence Jones Protein)	24 hour urine collection	Proteins (809) 2305
β2 Microglobulin	Serum Gel	Proteins (809) 2305
Cryoglobulins NOTE: Instruct patient to fast for 8 hrs prior to phlebotomy	Contact laboratory for details	Proteins (809) 2305
Lymphocyte subsets	EDTA	Haematology (809)2763

3.5 MICROBIOLOGY

3.5.1 *Repertoire of Test Services*

Test	Target Pathogens	Specimen	Minimum Volume	Reference Range	TAT	Comment
URINE						
Microscopy	N/A	The Sarstedt NFT (Needle Free Transfer) system. 100ml NFT primary container Reference 75.562.900) and a 10mL Monovette tube reference 10.252)	2 mls	N/A	Within 24 hours of receipt	
Culture	Urinary pathogens	The Sarstedt NFT (Needle Free Transfer) system. 10ml NFT primary container Reference 75.562.900) and a 10mL Monovette tube reference 10.252)	2 mls	N/A	6 days	
Pregnancy test	N/A	The Sarstedt NFT (Needle Free Transfer) system. 10ml NFT primary container Reference 75.562.900) and a 10mL Monovette tube reference 10.252)	2 mls	N/A	Same day	

Test	**Target Pathogens**	**Specimen**	**Minimum Volume**	**Reference Range**	**TAT**	**Comment**
TB culture	*Mycobacterium* spp.	Approved yellow screw-capped (Sarstedt) container	10 mls	N/A	8 weeks	3 consecutive EMUs needed – For diagnosis of disseminated or urinary tract mycobacterial infection only
FAECES						
Enteric pathogens	Enteric pathogens including *Cryptosporidium parvum/hominis and Giardia lamblia*	Approved yellow screw-capped (Sarstedt) container	1-2g	N/A	5 days	Perfomed **only** on specimens which take the shape of the container
C. difficile	*C. difficile*	Approved yellow screw-capped (Sarstedt) container	1-2mls	N/A	2 days	Perfomed **only** on specimens which take the shape of the container and >2 years of age.
Rota/adeno virus	Rota/adeno virus	Approved yellow screw-capped (Sarstedt) container	1-2g	N/A	4 days	Performed routinely on children <2years
Ova/parasites	Ova & Parasites	Approved yellow screw-capped (Sarstedt) container	1-2g	N/A	5 days	Clinical/travel details essential or discussion with CMT.
Helicobacter pylori antigen	*Helicobacter pylori*	Approved yellow screw-capped (Sarstedt) container	1-2g	N/A	4 days	

Test	Target Pathogens	Specimen	Minimum Volume	Reference Range	TAT	Comment
SPUTUM						
Routine culture	Respiratory pathogens	Approved yellow screw-capped (Sarstedt) container	As available	N/A	6 days	Salivary samples are unsuitable
TB	*Mycobacterium* spp.	Approved yellow screw-capped (Sarstedt) container	As available	N/A	70 days	3 consecutive morning samples
SKIN SCRAPPINGS/ NAIL CLIPPINGS						
Microscopy	Fungal Elements	Screw capped container (as above) or Dermapak	As much as Possible	N/A	7 days	
Culture	Dermatophytes, moulds & Yeasts	Screw capped container (as above) or Dermapak	As much as possible	N/A	35 days	Swabs are not an appropriate specimen for fungal culture. Hair must contain root
SWABS						
MRSA Screen	MRSA	Charcoal Transswab	N/A	N/A	4 days	MRSA- Only from Nasal, Groin & Wound sites
Non uro-genital (e.g. wound, eye, ear, nasal, throat)	Pathogens appropriate to site	Charcoal Transswab	N/A	N/A	6 days	Relevant clinical details essential, e.g. surgery, post-partum
Penile/vulval	Non -STI pathogens	Charcoal Transswab	N/A	N/A	4 days	
HVS –microscopy	Bacterial vaginosis	Charcoal Transswab	N/A	N/A	Within 24 hours of receipt	
HVS –Culture	Bacterial vaginosis	Charcoal Transswab	N/A	N/A	48-72hrs	

Test	Target Pathogens	Specimen	Minimum Volume	Reference Range	TAT	Comment
1. Ova & parasites only performed when specifically requested **and** with relevant clinical details eg. Foreign travel. 2. Microscopy is prioritised over culture if insufficient sample is received.						
SUSPECTED STI SPECIMEN REQUIREMENTS						
MALE Urine	*Chlamydia trachomatis*	GenProbe Collection Device[1]	25 mls	N/A	5 days	First void urine (FVU) required
Swab - urethral	*N. gonorrhoeae*[2]	Charcoal Transwab or GenProbe Collection Device[1]	N/A	N/A	4 days	Clinical details eg urethral disharge/ ?STI essential[1]
- rectal	*N. gonorrhoeae*	Charcoal Transwab or GenProbe Collection Device[1]	N/A	N/A	4 days 48-72 hours	Clinical details essential[1]
- pharyngeal	*N. gonorrhoeae*	Charcoal Transwab or GenProbe Collection Device[1]	N/A	N/A	4 days	Clinical details essential[1]
FEMALE Swab endocervical	*N. gonorrhoeaee*	Charcoal Transwab or GenProbe Collection Device[1]	N/A	N/A	4 days	Clinical details essential[1]
	Chlamydia trachomatis	r GenProbe Collection Device[1]	N/A	N/A	5 days	Clinical details essential[1]
- cervical	*N. gonorrhoeae*	Charcoal Transwab or GenProbe Collection Device[1]	N/A	N/A	4 days	Clinical details essential[1]
- urethral	*N. gonorrhoeae*	Charcoal Transwab or GenProbe Collection Device[1]	N/A	N/A	4 days	Clinical details essential[1]
- HVS	Bacterial vaginosis Candida and non-STI pathogens	Charcoal Transwab	N/A	N/A	4 days	Clinical details essential[1]

Test	Target Pathogens	Specimen	Minimum Volume	Reference Range	TAT	Comment
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.						

3.5.2 ***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.

3.5.3 ***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 and occasionally requiring referral of an isolate to a Reference Laboratory for typing or confirmation

3.5.4 *Samples sent to External Laboratories e.g., NVRL for analysis*

Please refer to the NVRL user manual for information on range of tests available in the NVRL, the type of specimen required and turn around time information on these tests. Information available on www.nvrl.ie

3.5.4.1 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
- Specimens that are referred out from Beaumont Hospital are not covered by the scope of Beaumont Hospital ISO15189 accreditation.

3.5.5 *Abbreviations Used on Microbiology Reports*

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

3.5.6 *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

3.6 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.

3.6.1 *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.

3.6.2 ***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.

3.6.3 ***Reports***

Printed reports are sent to the Clinical Consultant, source (wards / OPD) or requesting GP. Reports are available through PIPE/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.

3.6.4 ***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.

Tissue Type	**Fixative Required**	**Comment**
Specimen for Frozen Section.	Send fresh to the laboratory - immediately.	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.
Renal biopsies	Send in saline (Dublin Hospitals) Send in Formalin/Zeus (Regional Centres) (full details in section 3.6.9)	Please inform Renal Office Ext. 2765 of specimen. The Main Histology Lab can be contacted @ 2353. The EM lab on 8633.
Lymph nodes (for lymphoma diagnostics)	Send fresh to the laboratory - immediately.	Please supply all relevant clinical details.
Solid Tumours (Colon, Breast, Lung etc.)	Send fresh to the laboratory - immediately.	Please supply all relevant clinical details.
Liver biopsies*	Where possible, send two specimens – one in 10% Neutral Buffered Formalin and one wrapped in saline moistened gauze.	Please supply relevant clinical details. Referred to St. Vincents University Hospital for copper analysis where required*

Tissue Type	Fixative Required	Comment
Oncotyping*	Paraffin Block	Referred to Genomic HealthCare (US) for Oncotyping
Mitochondrial Studies*	Send fresh to the laboratory - Immediately	Referred to Mitochondrial Research Group in Newcastle University for analysis/St James Hospital (CMD)
CSF for RT-QuIC Analysis	CSF frozen at -70°C within 30 minutes of aspiration and transported to the Neuropathology Dept, Beaumont Hospital on dry ice.	Volume CSF: 1 - 2ml. Sample must be clear and colourless (not blood stained) with a white cell count of <10x10^6/L and have a total protein concentration of <1 g/L1. Red blood cells (>1250 x 10^6/L) inhibit the RT-QuIC response resulting in false negatives. High CSF total protein concentrations of >1.0 g/L and raised white blood cell counts can result in false positives.
Primary Ciliary Dyskinesia*	Nasal Scraping	Referred to Southhampton General Hospital for analysis
Flow Cytometry*	CSF Or Lymph Node	Referred to Haematology in St. James's Hospital Dublin for Analysis
Amyloidosis*	Paraffin Block	Referred to National Amyloidosis Centre, London, Univeristy College London .
PDL1* metastatic breast carcinoma , Head & Neck Cancer & metastatic oesophageal SCC. (NCCP recommendation)	Paraffin Block	Referred to Poundbury Cancer Institute, Dorchester, London HSL-Advanced Diagnostics HEALTH SERVICES LABORATORIES (A Sonic Healthcare UK laboratory) for metastatic oesophageal SCC.
Molecular Studies* – MY88		Referred to Royal Victoria Hospital, Belfast
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

Tissue Type	Fixative Required	Comment
		twice the volume of the tissue to be fixed. Small specimens should be placed in biohazard bags.
Histology Blocks *		outsourcing of blocks for cutting & staining to HTS

***** Specimens referred out from Beaumont Hospital - the results of these tests are not covered by the scope of Beaumont Hospital Histopathology Department ISO15189 accreditation.

3.6.5 ***REQUIREMENT FOR EXTERNAL CENTRES***

The responsibility for sending slides/blocks/material lies with the external centre (Sender). External centres may send slides/blocks/material to Pathology for review/conferences etc. Ensure that packaging and transportation comply with the European Agreement for the Carriage of Dangerous Goods by Road, ADR Regulations.
Address the package to:

Histopathology/Neuropathology (as appropriate to the analysis required)
Beaumont Hospital,
Dublin 9
Include the Consignee address and telephone number.

3.6.6 ***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

3.6.7 *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
CSF for RT-QuIC –10-15 working days
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.

3.6.8 *Cytopathology Specimen Requirements*

Specimen	**Specimen requirements**
Bronchial brushings	- Place material in a sterile container labelled with patient and specimen details, including the time of specimen collection.
	-
Fluids (Pleural, Ascitic etc.)	- 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.
Urine	- Total voided specimen is required for cytology. - The first morning specimen is not suitable. - Place in a container labelled with patient and specimen details.
Fine Needle Aspiration Cytology/EUS/EBUS	Sample from EUS/EBUS is sent to the cytology lab in cytolyt (available in the lab – Ext 2640)
Cerebrospinal Fluid for Cytology.	- Specimen must be collected in a sterile container labelled with patient and specimen details and delivered to the Neuropathology laboratory.
Flow Cytometry*	- ***Cytometry placed directly into RPMI are viable for up to 18Hrs****. (Contact Cytology on Ext. 2640)*

* Specimens referred out from Beaumont Hospital - the results of these tests are not covered by the scope of Beaumont Hospital Histopathology Department ISO15189 accreditation

ITEMS THAT CAN BE OBTAINED FROM THE CYTOLOGY 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
- Cytolyt containers

- **NOTE: EACH SAMPLE SHOULD BE ACCOMPANIED BY A HISTOPATHOLOGY/CYTOPATHOLOGY REQUEST FORM (FOUND ON ALL WARDS) – PLEASE PUT AS MUCH INFORMATION AS POSSIBLE.**

TURN AROUND TIMES FOR CYTOLOGY SAMPLES

Non-Gynae Cytology Samples – 3-4 Days

3.6.9 *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.

3.6.10 *Renal Pathology Requirements for External Centres*

- The Renal Pathology Department should be notified before sending biopsy via email to: electronmicroscopy2@beaumont.ie
- 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 01-8528633 (Dect phone)
- 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:

Consultant Renal Pathologist
Renal Pathology/Electron Micrscopy/Histopathology,
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.

The remaining cores can be placed in Formalin. It is not necessary for external laboratories to make and keep a stock of glutaraldehyde. A piece of the core can be taken for EM from the Formalin fixed tissue on arrival at Beaumont Hospital Histopathology Department.

3.6.11 *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 the renal pathologist on duty 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.

3.6.12 *Electron Microscopy*

The Electron Microscopy (EM) Laboratory was initially set up to serve diagnostic Renal Pathology which comprises the bulk of the caseload but a small number of Neuropathology cases are also handled.

The Laboratory is equipped with a JEOL 1400 Plus Transmission Electron Microscope and an AMT XR50 4 megapixel Digital Camera system. Samples 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 Primary Ciliary Dyskinesia (PCD)*. This is a highly specialised investigation and requires expertise which cannot be gained in this hospital due the low volume of requests. Please contact the EM laboratory for instructions, request form and fixative. When the procedure has been carried out, the sample should be sent to the EM Laboratory from where it will be referred to the UK National Centre for PCD Analysis in Southampton.

*Specimens referred out from Beaumont Hospital - the results of these tests are not covered by the scope of Beaumont Hospital Histopathology Department ISO15189 accreditation.

3.6.13 ***Specimen Requirements for Neuropathology***

Tissue Type	**Means of Delivery to Neuropathology**	**Comment**
Specimen for urgent frozen section	Send fresh. Hand deliver immediately.	The Neuropathology consultation form must include a bleep number or intercom number to deliver the report
Muscle Biopsy*	Send on gauze that is barely dampened in saline. Do not fix in formalin. Hand deliver immediately. See Section 3.6.10 for requirements from external centres.	Must be received during normal working hours unless previously arranged.
Nerve Biopsy	Send on gauze that is barely dampened in saline. Do not fix in formalin. Hand deliver immediately. See Section 3.6.10 for requirements from external centres.	Must be received during normal working hours unless previously arranged.
Hippocampus & Amygdala	Send fresh. Hand deliver immediately to the laboratory.	
Temporal Lobe (Epilepsy)	Send fresh. Hand deliver immediately to the laboratory.	
Temporal Artery	Send in 10% Neutral Buffered Formalin.	Send Immediately/ASAP
Laminectomy/Disc	Send in 10% Neutral Buffered Formalin.	
Tumour fluid for cytology	Hand delivery immediately.	Must be received during normal working hours.
CSF for cytology	Hand delivery immediately.	Must be received during normal working hours.
CSF for RT-QuIC Analysis	CSF frozen at -70°C within 30 minutes of aspiration and transported to the Neuropathology Dept, Beaumont Hospital on dry ice.	Must be received during normal working hours unless previously arranged. CJD Questionnaire must accompany specimen.

Tissue Type	**Means of Delivery to Neuropathology**	**Comment**
Autopsy & Biopsy tissue (e.g/ Brain / Tonsil) for Prion Protein Analysis	Hand delivery immediately.	Must be received during normal working hours. Contact Rachel Howley 017977766
All other tissue	Sent in 10% neutral buffered formalin indicating volume.	Must be received during normal working hours.

***** Specimens referred out from Beaumont Hospital - the results of these tests are not covered by the scope of Beaumont Hospital Histopathology Department ISO15189 accreditation.

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 RT-QuIC Analysis: There is an approximate turn around of 10-15 days from receipt of the sample to results.

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 RT-QUIC ANALYSIS

Requirements

The sample should be sent to the Neuropathology lab immediately after aspiration for freezing as sub optimal sample storage may give unpredictable results. Alternatively the CSF sample must be frozen at -70°C within 30 minutes of aspiration and transported to the Neuropathology Dept, Beaumont Hospital on dry ice. All samples must be logged in with the Neuropathology Lab prior to sending. All samples must accompany a completed questionnaire (LF-NCJD-CSF Questions), copies of which are available from the Neuropathology Laboratory (Ext. 2633) or are available for download from www.cjd.ie.

The sample volume should be between 1-2mls and be clear and colourless (not blood stained) with a white cell count of <10x10^6/L and have a total protein concentration of <1 g/L1. Red blood cells (>1250 x 10^6/L) inhibit the RT-QuIC response resulting in false negatives. High CSF total protein concentrations of >1.0 g/L and raised white blood cell counts can result in false positives.

Safety Precautions

CSF is considered to be a low risk sample for all types of Prion Disease . Take appropriate precautions when sampling..*[see www.cjd.ie]*

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.

3.6.13.1 Test Request Forms

Test request forms are available to download via the Beaumont Hospital Histopathology department website at http://www.beaumont.ie/index.jsp?p=105&n=349 or by contacting the laboratory.

3.6.13.2 Delivery of Specimens for Analysis

Courier Services Specimens can be delivered via courier directly to the Department of Histopathology.

3.6.13.3 Test Results

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.

3.6.13.4 Specimen Referral

When we are unable to provide a request or required follow-on analysis, 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.

3.6.13.5 Details Required for All Specimens

Regardless of the specimen type, the minimum essential information and minimum criteria that must be supplied **legibly** include:

On the specimen block/slide:
Histopathology block number

On the request form
- Name of patient
- Date of Birth
- Requesting Clinician/Pathologist
- Referring Hospital
- Relevant clinical details
- Specimen type

Note: Please send the pathology report relating to the sample to be tested and give ***as much clinical information on the form / letter*** as possible, as this will be required by the Pathologist when considering interpretations and advice.

Specimens will not be accepted without a minimum of three forms of identification on the request form and will be returned to the source of origin to be completed / labelled correctly.
All hazard labels where appropriate must be used for the health and safety of the staff that will be handling the specimen.Turnaround Times for Results (TATs)

The turnaround time of specimens 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 times for different specimen types from time of receipt in the laboratory:

Histopathology IHC or ISH	5-10 days
HER2 IHC testing	10-15 days
Histopathology (referred to external institute)	20 days

Notes

- TATs refer to working days from receipt of specimen until report has been authorised. Time refers to 95% of referrals.
- Any request forms requesting a phoned report will be phoned to the Clinician or his/her Secretary.
- There is no time limit for requesting additional examinations but requests should be made by contacting the laboratory or the Pathologist dealing with the case.
- Urgent specimens will be “fast tracked”. From receipt of specimen to interim report status can be performed in two days.

3.6.13.6 Reports

Reports are not available through the laboratory.

- Reports are sent to the Clinical Consultant and/or source.
- Reports are normally sent via the Fortimail email encryption System. Please contact the laboratory if you wish to receive reports by an alternative means.
- Reports are available by phoning the Pathology Office at (01) 8092632.
- Only authorised reports are available through the office/PIPE
- If an interim report, clinical advice or result interpretation is required please contact the Consultant Histopathologist

3.6.14 *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-Coroner cases (ie "Hospital" or "House" consent cases) before an autopsy is performed. (LAB 358B6)

- In Coroner's 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.

3.7 MOLECULAR PATHOLOGY

Molecular Pathology Department

CONTACTING THE DEPARTMENT

Teresa Loftus	Chief Medical Scientist	018092856	molecular@beaumont.ie biomarkers@beaumont.ie teresaloftus@beaumont.ie

The Molecular Pathology laboratory provides a molecular pathology diagnostic and consultative service for hospitals throughout Ireland.

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

3.7.1 ***HISTOMOLECULAR MUTATIONAL ANALYSIS***

The laboratory provides a solid tumour mutation testing service using next generation sequencing (NGS).

The AmpliSeq for Illumina Focus NGS Panel is a targeted resequencing assay for biomarker analysis of 52 genes with known relevance to solid tumours (Table 1). The Focus Panel can simultaneously analyse both DNA and RNA extracted from the same specimen. The Focus Panel is part of a workflow that includes AmpliSeq for Illumina PCR-based library preparation, Illumina sequencing by synthesis (SBS) next-generation sequencing technology and automated analysis.

Starting with 10 ng of DNA and RNA, the panel enables the analysis of genes associated with multiple cancer types, including lung, colon, breast, and melanoma. The low-input requirement allows use with various sample types, including formalin-fixed, paraffin-embedded (FFPE) tissues. As part of the AmpliSeq for Illumina targeted resequencing solution, the Focus Panel enables quick and accurate assessment of genomic variation. A similar approach is used for BRCA1&2 germline and somatic analysis. Currently germline only analysis is performed for HER2 negative locally advanced or metastatic breast cancer patients.

In conjunction with germline analysis, FFPE material is required for somatic analysis for prostate, ovarian, fallopian tube or primary peritoneal cancer. Sequencing of germline and somatic testing for these tumour types is performed in accordance with National testing guidelines.

3.7.1.1 Relevant gene content

The AmpliSeq for Illumina Focus Panel targets hundreds of mutations across 52 key genes associated with solid tumours (Table 2). Gene content for this panel was selected based on published literature, current guidelines (National Comprehensive Cancer Network [NCCN], Association for Molecular Pathology [AMP], College of American Pathologists [CAP], European Society for Medical Oncology [ESMO], etc.), and relevant clinical trials.

Like most similar panels, this is a hotspot panel and does not cover all exons for all genes. Please contact the laboratory if you have a specific variant to analyse.

3.7.1.2 Colorectal Cancer (CRC) Mutation Panel:

KRAS & NRAS

KRAS & *NRAS* mutation status are critical when evaluating patients with a view to placing them on *EGFR*-targeted monoclonal antibody therapy. The presence of an activating *KRAS* or *NRAS* mutation is generally associated with a lack of response to anti-*EGFR* therapy.

BRAF

Mutations in position p.V600 in *BRAF* have been associated with poor prognosis, especially in patients with metastatic disease. Currently there is insufficient evidence to recommend *BRAF* V600 mutational status as a predictive molecular biomarker for response to anti-*EGFR* therapy but this is a rapidly evolving field.

MICROSATELLITE INSTABILITY (MSI)/MISMATCH REPAIR DEFICIENCY (DMMR)

MSI/dMMR CRC have been shown to have increased sensitivity to immune-oncological (IO) agents such as PD-L1 inhibitors. In addition, while the majority of these tumours are sporadic, MSI/dMMR tumours are more likely to be associated with Lynch syndrome than MSS/MMR intact tumours. MSI and/or immunohistochemistry (IHC) testing is performed on tumour tissue samples to predict likely response to IO agents. In addition, the results of this testing allow for risk stratification in relation to Lynch syndrome (in certain circumstances this will be the primary indication for this testing).

Initially samples are tested with a panel of 4 mismatch repair protein IHC markers (MLH1, PMS2, MSH2 & MSH6). The laboratory will routinely reflex cases for MSI testing in the event of equivocal IHC or if indicated by clinical parameters. MSI testing is performed using a multiplex PCR approach for thirteen different microsatellite loci followed by DNA fragment analysis using the SeqStudio™ Genetic Analyser. PCR is carried out using the Applied Biosystems TrueMark™ MSI Assay which can identify microsatellite instability in FFPE samples from multiple tumour tissue types.

3.7.1.3 Lung Cancer Mutation Panel:

EGFR

EGFR mutation status is critical when evaluating patients with a view to placing them on anti-*EGFR* Tyrosine Kinase Inhibitors (TKIs). The presence of a sensitising mutation is associated with a favourable response to treatment with *EGFR* TKIs. The presence of resistance mutations need to be interpreted in the context of any previous treatment regimes.

ERBB2 (HER2) and KRAS G12C

Clinical trials are currently enrolling patients with EBRB2 exon 20 insertions and KRAS variants. The results of these trials will determine whether these treatments become part of standard care for patients with these mutations.

BRAF

BRAF mutation status is critical when evaluating patients with a view to placing them on *BRAF* targeted therapies. The presence of a mutation in codon 600 of *BRAF* is required for treatment with *BRAF* targeted therapies.

ALK

ALK translocation has been associated with response to anti-ALK targeted therapies such as crizotinib. ALK translocations can be assessed by a number of different methodologies. Any of immunohistochemistry, in-situ hybridisation or next-generation sequencing is acceptable methodologies for assessing the presence of translocations.

ROS1

ROS1 translocation has been associated with response to targeted therapies, including crizotinib. ROS1 translocations can be assessed by a number of different methodologies. Any of in-situ hybridisation or next-generation sequencing is acceptable methodologies for assessing the presence of translocations. While antibodies exist for ROS1 immunohistochemistry it is not currently an accepted method for assessing ROS1 translocations.

Note: *ALK* and ROS1 FISH testing is routinely performed on all lung cases

3.7.1.4 Melanoma Mutation Panel:

BRAF

BRAF mutation status is critical when evaluating patients with a view to placing them on *BRAF* targeted therapies. The presence of a mutation in codon 600 of *BRAF* is required for treatment with *BRAF* targeted therapies.

KIT

KIT gene analysis enables the selection of those melanoma patients with KIT variants that will benefit from TKIs.

3.7.1.5 Breast Cancer Mutation Panel:

PIK3CA

PIK3CA mutation status provides information to guide treatment with PIK3CA inhibitors. It may also have a role in predicting response to chemotherapy.

3.7.1.6 BRCA 1 & 2 analysis and Multi Ligation dependant Probe Amplification (MLPA)

This assay detects variants in BRCA1 or BRCA2, helping to identify patients that may benefit from treatment with PARP inhibitor. MLPA analysis also looks for largescale alterations in the BRCA 1 and 2 genes.

There is also an important role in BRCA testing for identifying germline variants that may be responsible for Hereditary Breast and Ovarian Cancer (HBOC). The results of these tests can then be used by clinicians to guide family testing as required. MLPA is performed on patients samples referred for germline testing.

Currently, we offer germline only analysis to HER2 negative locally advanced or metastatic breast cancer patients.

We offer both germline and somatic analysis for ovarian, fallopian tube or primary peritoneal cancer patients. MLPA testing is also performed on patients being tested for germline alterations. We offer somatic analysis and MLPA for patients with prostate cancer. Germline testing is performed reflexively if a significant variant is detected. Ideally, both a germline (EDTA blood) and a tumour sample (FFPE) will be provided on the same patient.

A separate request from for BRCA analysis is available on the Beaumont internet and intranet.

Service	Specimen type	Specimen Requirements
IHC & ISH	FFPE Blocks/slides	Ensure that sections are mounted on adhesive slides.
Mutational Analysis	FFPE Blocks	A representative block of tumour (resection, biopsy or cytology preparation) should be provided. The analysis can

		only be performed on specimens where there is adequate tumour material.
MSI	FFPE Blocks	Send representative blocks of tumour and normal tissue and if these are not available then contact us to discuss alternatives. The normal tissue sample need not be from the same specimen. If no normal tissue is available the analysis will be performed using a generic normal control. However, this can affect the interpretation of results.
Germline BRCA1 & 2 (including MLPA)	EDTA blood	Germline only analysis is currently performed for HER2 negative locally advanced or metastatic breast cancer patients. FFPE material will be required is somatic analysis is required for prostate, ovarian, fallopian tube or primary peritoneal cancer.

3.7.2 ***Neuromolecular Pathology Tests and Requirements:***

Molecular Test performed	**Requirement:**
1p19q Array CGH	10x5micron sections of requested block on unbaked glass slides.
MGMT methylation analysis	10x5micron sections of requested block on unbaked glass slides.
BRAF Fusion qPCR	5x5micron sections of requested block on unbaked glass slides.
IDH 1&2 sequencing analysis	10x5micron sections of requested block on unbaked glass slides.
DNA Methylation profiling	10x5micron sections of requested block on unbaked glass slides.

DNA/RNA NGS CNS Tumours (External Referral to SIHMDS-AG)	10x5micron sections of requested block in two separate labelled sterile 1.5mL tubes.

From an external referral centre the samples must arrive with adequate documentation and request form, outlining patient details as detailed in 10 below.

3.7.2.1 Array CGH

Microarray - Comparative Genomic Hybridization (array-CGH) is a molecular cytogenetic method for analysing Copy Number Variations (CNVs) relative to ploidy level in the DNA of a test sample (eg. tumour) compared to a reference control sample. Test (eg. tumour DNA) is labelled in one fluorescent dye (eg. Cy3) while reference (sample with a normal complement of chromosomes) is labelled in another fluorescent dye (eg. Cy5). Fluorescent intensities from both dyes are then scanned and compared to each other for every locus that is represented on the microarray. The final output is a genome-wide graph of copy number gains (gain or amplification) or deletions.

3.7.2.2 MGMT methylation analysis

MGMT in gliomas is a useful predictor of the responsiveness of tumours to alkylating agents. The protein O6-methylguanine-DNA methyltransferase (MGMT) functions to repair alkylated guanine in DNA by transferring the alkyl group at the O-6 position to a cysteine residue in the enzyme. This activity confers a certain chemoresistance to tumour cells and the silencing of *MGMT* through promoter methylation results in a better response to alkylating chemotherapy. In 2005 Hegi *et al*., reported that patients with methylated *MGMT* demonstrated a significant survival advantage with temozolomide treatment in a prospective phase III trial. The assessment of the methylation status of the *MGMT* promoter has therefore become an important genetic marker which is associated with response to alkylating chemotherapy and subsequent increased overall and progression free survival in GBM patients.

Assay Principle

The MGMT assay is based on the pyrosequencing of 8 CpG sites within the MGMT gene modified from Dunn J et al. 2009. The average methylation across the 8 CpG sites is calculated automatically by the PyroMark software. Methylated samples are defined as having an average methylation of $\geq$ 9% methylation in accordance with the clinically significant thresholds reported by Dunn *et al*.

3.7.2.3 BRAF Fusion

Molecular detection of the *BRAF-KIAA1549* fusion gene on chromosome 7q32 has been identified in up to 70% of PAs and is therefore of diagnostic value in these tumours (JONES, D. T. et al, Cancer Research, 2008). A qPCR based method is employed which based on the amplification of the 3 most common fusion partners in pilocytic astrocytoma. Primers specific for each of the exons above are used to amplify the fusion product. Fluorescent probes specific for the fusion junctions are used to detect the amplified product. A positive control (*GAPDH)* is included in each analysis to ensure the quality of tumour RNA. The assay is based on the publication by Tian *et al*. Journal of Molecular Diagnostics, 2011.

3.7.2.4 IDH 1&2 Sequencing

IDH1 mutations have been reported in 60-80% of WHO grade II and III gliomas, and secondary glioblastomas, whilst 2-5% of these tumours have *IDH2* mutations. Approximately 5% of primary GBM harbour *IDH* mutations. *IDH1* mutations have been associated with better clinical outcome; they are suitable predictive markers for adult glioma patients. In terms of diagnosis the presence of an *IDH* mutation can help to distinguish oligodendrogliomas from other tumours such as clear cell ependymomas and dysembryonic neuroepithelial tumours, as well as helping to differentiate between ganglioliomas and diffuse gliomas[2-5]. Mutations affecting *IDH1* and *IDH2* have been shown to be limited to the binding site of the proteins –cDNA positions 394 and 395 in *IDH1* and 514, 515 and 516 in *IDH2*, with mutations thought to be mutually exclusive.

3.7.2.5 DNA Methylation profiling

DNA methylation plays an important and dynamic role in regulating gene expression. It allows cells to become specialized and stably maintain those unique characteristics throughout the life of the organism, suppresses the deleterious expression of viral genes and other non-host DNA elements, and provides a mechanism for response to environmental stimuli. Aberrant DNA methylation (hyper or hypomethylation) and its impact on gene expression have been implicated in many disease processes, including cancer. By providing quantitative methylation measurement at the single-CpG–site level for normal and formalin-fixed paraffin-embedded (FFPE) samples, this assay offers powerful resolution for understanding epigenetic changes.

Following bisulfite conversion of DNA samples, DNA restoration is carried out using the Infinium HD FFPE Restoration Kit to optimise the processing of DNA previously extracted from FFPE tissue. The Illumina EPIC array Kit is then used to amplify, fragment and hybridise DNA to a beadchip which can be analysed on the Illumina iScan instrument to determine the methylation profile of the sample DNA.

3.7.2.6 DNA/RNA NGS for CNS Tumours

CNS tumours are referred to the external laboratory listed below for the purpose of DNA and RNA next generation sequencing. The purpose of this testing is to identify variants and fusions clinically relevant to CNS tumours as either diagnostic markers or indications for treatment.

SIHMDS Acquired Genomics Laboratory,
North Thames Genomic Laboratory Hub,
Level 4 Barclay House,
37 Queen Square,
London
WC1N 3BH

Samples are referred as cut sections in sterile tubes which undergo nucleic acid extraction at the referral site. This material is then sent to the Clinical Genomics laboratory at the Royal Marsden Hospital (also part of the North Thames genomic hub), where it undergoes DNA and RNA NGS using their custom gene/fusion panels. The data output is then returned to SIHMDS-AG for analysis, interpretation and reporting. For more information regarding the NGS panels and genes included, please contact the Molecular Pathology Laboratory, Beaumont Hospital.

3.7.3 *Test Request Forms*

Test request forms are available to download via the Beaumont Hospital Molecular department website at http://my.beaumont.ie/Pages/Departments/Pathlogy/Lab-user-guides-and-forms.aspx or by contacting the laboratory.

3.7.4 *Delivery of Specimens for Analysis*

Courier Services Specimens can be delivered via courier directly to the Molecular Pathology Department care of Pathology Specimen reception in the Laboratory Directorate addressed to the following.

Molecular Pathology Laboratory
c/o Pathology Specimen Reception
Beaumont Hospital
Beaumont Road
P.O. Box 9063
Dublin 9

3.7.5 ***Test Result Queries***

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.

3.7.6 ***Specimen Referral***

When we are unable to provide a request or required follow-on analysis, 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.

3.7.7 ***Details Required for All Specimens***

Regardless of the specimen type, the minimum essential information and minimum criteria that must be supplied **legibly** include:

On the specimen block/slide:
Histopathology block number

On the request form:
- Name of patient
- Date of Birth
- Requesting Clinician/Pathologist
- Referring Hospital
- Relevant clinical details
- Specimen type

Note: Please send the pathology report relating to the sample to be tested and give ***as much clinical information on the form / letter*** as possible, as this will be required by the Pathologist when considering interpretations and advice. Specimens will not be accepted without a minimum of three forms of identification on the request form and will be returned to the source of origin to be completed / labelled correctly.
All hazard labels where appropriate must be used for the health and safety of the staff that will be handling the specimen.

3.7.8 ***Turnaround Times for Results (TATs)***

The turnaround time of specimens 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 times for different specimen types from time of receipt in the laboratory:

Solid tumour mutation analysis	15 days
ALK & *ROS1* FISH testing	15 days
Microsatellite Instability analysis (MSI)	20 days
MLH1 Hypermethylation analysis	20 days
BRCA 1&2 testing (including MLPA)	48 days
Neuromolecular testing (aCGH, MGMT, BRAF fusion, IDHSeq,DMET. NGS)	42 days

Notes

- TATs refer to working days from receipt of specimen until report has been authorised. Time refers to 90% of referrals.
- All reports are emailed by the secretarial staff to requesting clinical and referral site. No results are issued over the phone.
- There is no time limit for requesting additional examinations but requests should be made by emailing the laboratory at molecular@beaumont.ie including an updated request form and stating the patient's name, DOB and original sample number of available.
- Urgent specimens will be "fast tracked" as appropriate.
- Microsatellite instability analysis TAT refers to time post MMR IHC result.

3.7.9 ***Reports***

Reports are not available through the laboratory.

- Reports are sent to the Clinical Consultant and/or source.
- Reports are normally sent via secure email System. Please contact the laboratory if you wish to receive reports by an alternative means.
- Reports are available by phoning the (01) 8092632 for histomolecular reports and (01) 8092631 for neuromolecular reports.
- Only authorised reports are available through the office.

- If an interim report, clinical advice or result interpretation is required please contact the Consultant Histopathologist/Neuropathologist.

3.8 NHISSOT

3.8.1 *How to Order Tests*

As per the EFI standard for sample acceptance, all samples received and accepted into the laboratory **must** have the patient's name, date of birth and sample date.

- The Histocompatibility Testing Request and Consent Form **must** have the name, date of birth, requesting clinician/consultant, centre and sample date.
- Samples that do not comply with this EFI standard will be rejected and repeat samples for H&I will be required.

Note: It is the responsibility of the requesting clinician to ensure that the patient has read and understood the permission statement on the consent form. This must be initialled by the patient. For more information on the permission statement please contact the laboratory.

Note: Patients for HLA antibody screening only (HLA antibodies) must meet the same standards of identification. An HLA antibody Screening request form must be completed and can be emailed to crossmatch@beaumont.ie or posted with the samples to the H&I Department. This form is checked against the specimens received and the referring dialysis centre is notified if the samples are unsuitable and are rejected. For Beaumont Hosital patients the information is automatically generated on the specimen label by the Beaumont Hospital Information System

Request and Consent forms for HLA typing and HLA antibody screening are available from the H&I department. Please phone or email crossmatch@beaumont.ie if a request form is required.

3.8.2 ***Repertoire of Tests***

Test		**Specimen Container**	**Minimum Volume**
HLA typing of patients for solid organ transplants		Citrated Blood	Paeds: 5ml Adult: 10ml
HLA antibody screening for solid organ transplants Pre transplant		Clotted Blood	Paeds: 3ml Adult: 5ml
Urgent requests: Antibody Mediated Rejection		Clotted Blood	5ml
HLA-B27		Citrated Blood	10ml
HLA-B57		Citrated Blood	10ml
Potential Transplant Recipients: bloods required forcrossmatching for deceased donors		Clotted Blood	5ml
Potential deceased donor work-up:		Citrated Blood Clotted Blood EDTA	60ml 5ml 7.5ml
Living donor work-up: Potential Donors	1st Workup	Citrated Blood EDTA	10ml 4.9ml
	2A Workup	Citrated Blood EDTA	10ml 4.9ml
	2B Workup	Citrated Blood EDTA	50ml 4.9ml
	3rd and Final Workup	Citrated Blood Clotted Blood	50ml 5ml
	Full house potential donor	Citrated Blood Clotted Blood	10ml 5ml
Living donor work-up: Potential Recipients	2B Workup	Citrated Blood Clotted Blood	40ml 5ml
	3rd and FinalWorkup Clotted blood should be within 7 days of the proposed transplant date	Citrated Blood Clotted Blood	40ml 5ml
Autocrossmatch		Clotted Blood	5ml
	CDC Autocrossmatch Only	Citrated Blood	10ml
	Flow Autocrosmatch	Citrated Blood	40ml
Post transplant monitoring for all solid organ transplants: See section on post-transplant Monitoring		Clotted Blood	5ml
Post transplant monitoring – Urgent antibody screening request for query graft rejection: See section on post-transplant Monitoring		Clotted Blood	5ml
HLA typing for disease association		Citrated Blood	10ml
HLA typing for partners		Citrated Blood	10ml
ABO blood grouping		EDTA	7.5ml

3.8.3 *HLA Typing of Patients for Solid Organ Transplantation*

Human Leucocyte Antigen (HLA) type is defined by the presence of different HLA antigens on the cell surface. These antigens enable the immune system to recognise foreign organisms and destroy them.

In solid organ transplantation the major HLA antigens involved are HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ and HLA-DP.

Mismatches between donor and recipient HLA type are a major stimulus of the development of donor specific HLA antibodies leading to rejection of the transplanted organ.

Potential recipients are HLA typed by serology and low to medium resolution molecular techniques. These techniques use commercial sera and probes and primer sets selected according to the EFI standards for HLA typing.

Note: Specimens received for HLA typing have DNA isolated and stored, serum stored and a sample sent for blood grouping. When instructed by the Transplant Co-Ordinator, HLA typing and antibody screening will be carried out. A NHISSOT patient report will be issued prior to the patient's appointment at the renal and pancreas transplant clinics.

Samples and tests required prior to a transplant clinic appointment

- 1x10ml citrated sample for HLA typing
- 1x5ml clotted sample for Antibody Screening
- 1x7.5ml EDTA sample for Blood Grouping

Histocompatibility Testing Request and Consent Form

MOLECULAR (DNA) TYPING

Patient's DNA is isolated from citrated blood and typed by molecular 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. 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. Assignment of a HLA type is based on the reaction patterns associated with published HLA gene sequences.

Note: Luminex® technology for SSO typing allows for multiplex, high throughput testing. This method is therefore particularly suited for the routine HLA typing of multiple DNA samples. SSO typing is not usually done outside of routine hours but it is a backup method if required for donors on call.

Note: DNA samples may be sent to the National Histocompatibility and Immunogenetics Reference Laboratory (NHIRL) for high resolution or sequencing for confirmation of rare HLA types.

Note: Patients are HLA typed on two separate samples taken on two different occasions.

3.8.4 *Antibody Screening*

Antibodies to HLA antigens can develop through pregnancy, transfusion, previous solid organ transplant or cardiac mechanical assist device placement. These antibodies can potentially react with a transplant organ causing graft rejection. All patients for solid organ transplantation are tested for HLA antibodies and these are recorded in the H&I database.

3.8.4.1 ScreeningTests

Luminex Single Antigen

Microbeads coated with purified HLA antigens are used to detect HLA antibodies. The Luminex flow analyser detects the fluorescent emission from the beads and the amount of fluorescence can help to estimate the amount of antibody present.

3.8.4.2 Antibody Analysis and Identifcation

The antibody screening results are analysed and any HLA antibody identified is recorded in the patient's antibody profile as an unacceptable antigen.Therefore, if a donor is identified and a recipient list is generated, any potential recipient with an antibody to the potential donors's HLA antigens are excuded. This significantly reduces the liklihood of a positive crossmatch.

3.8.4.3 Pgen

PGEN - GENERATED OR CALCULATED PRA

Using the H&I database of donor HLA types, we can calculate how many donors are unsuitable due to the presence of HLA antibody. This is referred to as generated PRA - Pgen. The Pgen value can be used as an indicator of how difficult it is to find a compatible graft.

Pgen example:

HLA-A2 is present in approx 25% of Irish donor population. If a recipient has an antibody to HLA-A2, the Pgen is calculated at 25%. This tells us that 25% of donors in the Irish population are unsuitable for this recipient. The more antibodies the patient has, the higher the Pgen.

3.8.4.4 Specimen Requirements for Antibody Screening

Renal/pancreatic patients who are active on the transplant waiting list:

- HLA antibody screening sample (clotted sample) every 90 days
- CAPD and pre-emptive patients can have the samples taken by their local GP and posted to the department -see section 3 for transport requirements
- Following a transfusion (blood products or platelets) a clotted sample is required 14 days post transfusion or as soon as possible thereafter. It is **vital** we receive these samples to monitor a patient for donor specific HLA antibodies.

Note: 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 for transplant.

Renal/pancreatic patients who are *not yet* on the transplant waiting list:

- All patients transfused (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

Cardiothoracic patients

- Patients identified as positive for HLA antibody – **Sample every month**
- Patients with no identified HLA antibody **– Sample every 3 months**

Due to time constraints in cardiothoracic transplants the following schedule applies to ensure that a sample within an acceptable time frame is available for crossmatch:

- Following a blood transfusion (blood products or platelets), we require a sample at week 2, 3 and 4

Cardiothoracic patients with cardiac mechanical assist device placement – Samples required every month unless otherwise notified.

3.8.5 ***Solid Organ Transplant Pools Work-Up***

3.8.5.1 Renal/Pancreatic Patients

If a patient is approved for the transplant waiting list at the transplant clinic by the Consultant Transplant Surgeon, the Transplant Co-ordinator will contact the laboratory by email to confirm patient's approval for activation.

The patient will appear on the monthly transplant waiting list as 'NHISSOT workup'.

A Patient is 'activated' on the transplant list when all the documentation and immunological work is completed.

A letter is then issued from the H&I Department to the patient, their Consultant Nephrologist and Transplant Co-ordinator to confirm activation on the transplant list.

3.8.5.2 Cardiothoracic Patients

On receipt of a request by email from the Cardiothoracic Transplant Co-ordinator, the patient is HLA typed and tested for HLA antibodies. The report issued will indicate if the patient will need a prospective crossmatch when listed for transplant.

Note: Additional samples are required on patients listed for lung transplant for auto crossmatch. These bloods will be requested by the Cardiothoracic Transplant Co-ordinators when the patient has been approved for the active lung transplant waiting list

3.8.5.3 LIVER PATIENTS

On receipt of a request by email from the Liver Transplant Co-ordinator, a patient is HLA-B typed and ABO blood grouped. A Confirmatory HLA-B type is performed following transplantation.

3.8.6 *Deceased Donor Work-up and Potential Recipient List Generation*

The ODTI Transplant Co-ordinator (Organ Donation and Transplant Ireland) contacts the H&I Department whena potential donor is identified. Donor bloods are sent to the laboratory.

On receipt

- Potential donor is HLA typed
- ABO blood group requested
- Match programme to identify suitable recipients is generated
- Potential recipient list is complied according to agreed criteria andcontains information on the following :

- Priority Patients/Paediatric Patients/Acceptable Mismatched Patients
- Significantly Sensitised Patients (Highly Sensitised) PGen ≥ 50
- Favourable Match / Reasonable Match Patients

- Longest Waiting Patients
- HLA incompatiable patients (HLAi)
- Simultaneaous Pancreas and Kidney (SPK) Patients

- The list of immunological suitable recipients is sent to the Consultant Transplant Surgeon and the Renal Transplant Co-ordinator.

3.8.7 *Matchability Scores*

A database of HLA types of previous deceased donors from the Irish population is used 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.

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 a further transplant the degree of sensitisation following a well matched graft is usually less than that following a poorly matched graft.

DEFINING MATCHABILITY

For patients of blood groups A and O:

Score	Reported
5% or under	Low
5.1-7.9%	Medium
8% and over	High

3.8.8 *Living Donor Work-Up*

3.8.8.1 What is living donation?

Living donation is where a living person donates an organ (or part of an organ) for transplantation to another person. Living Donation 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.

The following forms must accompany potential donor samples for work-up:

- HLA Request form for 1st Living Donor Workup
- Activation Request form for Living Donor Workup

Samples should be forwarded to the H&I Department, either directly from the transplant co-ordinator, or by post if from abroad. Only 2 potential donors per recipient will be processed by the laboratory at any one time. If either is deemed unsuitable, two further potential donors can be evaluated once a signed activation form has been received.

3.8.8.2 What makes a Living Donor Suitable?

- **Compatible blood group**
  The living donor and recipient blood groups should be compatible.

- **Compatible HLA type**
  HLA antigens are inherited therefore blood relatives are more likely to have similar HLA type. A brother and sister have a one in four chance of having an identical type.

  Those genetically unrelated can also be assessed for living donation. Any potential living donor is HLA typed to ensure that their HLA type is compatible with the potential recipient

  HLA antigens assessed for matching are HLA-A, -B, -C, -DR, -DQ, -DP.

- **Compatible Antibody Profile**

A potential living donor can be eliminated at the first stage of living donor work-up, if the potential recipient has an antibody to the donor's HLA antigens. This antibody can pose a risk to the graft.

3.8.8.3 Summary of stages for Living Donor work-up

Note: Families who wish to donate **must** initially contact the Transplant Co-ordinators. Any samples received into the laboratory **will not be processed** without prior contact with the Transplant Co-ordinators.

Note: Samples required are listed in the repertoire of tests.

First living donor work-up – virtual crossmatch

- Potential donor HLA type and blood group
- Risk assessment issued

Second living donor work-up

2a Workup – virtual crossmatch

- Confirmatory HLA type and blood group
- Risk assessment issued

2b Workup – 'wet' crossmatch

- Confirmatory HLA type and blood group
- Crossmatch using the potential donor cells and recipient sera
- Autocrossmatch of the potential recipient
- Risk assessment issued

Final living donor work-up

- This final stage of the work-up takes place no more than one week pre-transplant
- 'Wet' crossmatch
- Risk assessment issued

3.8.8.4 Risk Assesment

Using antibody screening data, sensitisation history and crossmatch results the immunological risk for a donor/recipient pair is assigned by the Consultant Immunologist

3.8.8.5 Reporting

Reports for the first and second work-up are issued to the Transplant Co-ordinator. The final work-up report is sent to the Consultant Surgeon and Transplant Co-ordinator.

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

3.8.9 *Crossmatching for Solid Organ Transplantation*

Transplanting an organ into a patient who has circulating antibodies to donor HLA antigens could result in hyperacute rejection and immediate organ loss.

The crossmatch prior to transplantation will detect any donor specific antibodies and thus prevent hyperacute rejection, greatly reduce acute rejection and the risk of graft loss.

A positive crossmatch is not necessarily a bar to transplant. A patient's sensitisation history and antibody screening profile is also taken into account for the risk assessment.

The crossmatch uses a selection of both current and historic sera:

- Detection of historic antibody can be an indication of prior sensitisation (exposure) of the patient to donor antigen and the presence of memory T and B cells. This can lead to a rapid immunological response if challenged with the same antigen.
- Detection of current antibody, if directed against HLA antigens present on the graft, can cause hyperacute rejection of the organ, or an acute rejection.
- A day of transplant (DoTX) sample is required for crossmatch where a patient has had a recent sensitising event, 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**

3.8.9.1 Crossmatch tests

The crossmatch techniques used in the laboratory are flow cytometry and complement dependent cytotoxicity (CDC). They can detect both HLA class I and class II donor specific antibodies.

3.8.9.2 Virtual crossmatching

In limited circumstances a patient may be suitable for transplant without a prospective crossmatch due to theatre time constraints.

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 may not be suitable for a virtual crossmatch.

All patients transplanted using virtual crossmatching require a flow crossmatch retrospectively in accordance with EFI standards

3.8.9.3 Autocrossmatch

This assay involves a crossmatch of the recipient's lymphocytes with autologous (own) serum. This can identify auto-reactive antibodies.

Knowledge of the presence and type of autoantibody can be helpful in interpreting positive crossmatches.

- Samples for autocrossmatches should reach the laboratory within 24 hours
- Please contact the H&I deparment to book in the samples for autocrossmatch

3.8.10 *Post Transplant Monitoring*

Antibody testing post transplant can detect the presence of donor specific antibodies (DSA) that may develop clinical and sub-clinical. Screening for DSA post transplant and early intervention could prevent graft rejection and improve graft outcomes.

3.8.10.1 Graft Rejection

Transplant rejection occurs when a transplanted organ is rejected by the recipient's immune system, which destroys the transplanted tissue.

Rejection of solid organ grafts is conventionally classified as hyperacute, acute and chronic.

- Hyperacute rejection causes rapid activation of complement, platelet aggregation, thrombosis and ischaemic necrosis. 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 to prevent a severe inflammatory response.

- Acute rejection usually occurs early following transplantation (typically within 4 weeks). It is a classical cell-mediated immune response involving presentation of foreign antigens to T cells by antigen presenting cells, proliferation and activation of T cell clones and destruction of the graft by cytoxic T cells.

- Chronic rejection occurs later (typically months or years after transplantation). It leads to a gradual deterioration of renal function with biopsy appearances of fibrous intimal thickening, interstitial fibrosis and tubular atrophy. The most consistent predisposing factor is that of previous episodes of acute rejection.

3.8.10.2 Renal/Pancreatic Patients

Specimen Requirements

- 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 when clinically indicated - at biopsy, when there are concerns regarding graft function or a change to the immunosuppressive regimen.

Note: Samples are tested according to their post transplant testing schedule and a post transplant report sent to the requesting Clinican.

Note: Please email posttransplant@beaumont.ie or phone the H&I department when screening is clinically indicated. Please include any clinical indicators such as creatinine levels and a contact number for urgent results. If antibody mediated rejection is suspected, this should be discussed with a Senior Medical Scientist who will contact the Consultant Immunologist with patient clinical details.

3.8.10.3 Cardiothoracic 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 when clinically indicated - at biopsy, when there are concerns regarding graft function or a change to the immunosuppressive regimen.

Note: Samples are tested according to their post transplant testing schedule and a post transplant report sent to the requesting Clinican.

3.8.10.4 Liver Patients

Graft versus Host Disease (GvHD) can pose significant risks to liver transplant patients. If GVHD is suspected, please contact the department with clinical details by phone or email posttransplantlab@beaumont.ie.

3.8.11 *Patients for Disease Association*

There are many thousands of different HLA types as a result of the differences in our HLA genes.
Some of these tissue types are associated with disease including ankylosing spondylitis, Behcet's disease, coeliac disease, narcolepsy, rheumatoid arthritis and selective IgA deficiency

Only Beaumont Hospital patients and GP patients in the catchment area are HLA typed for disease association. All disease association typing from other hospitals are carried out in the NHIRL, National Blood Centre, James's Street, Dublin 8.

3.8.12 *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, anantiretroviral drug. Patients who require treatment are HLA typed for HLA-B57. Patients found to be HLA-B57 positive by low resolution are typed by high resolution to define the B*57allele.

3.8.13 *HLA Typing for Partners of Recipients*

During pregnancy or birth the baby's cells can cross the placenta and expose the mother to paternal's HLA antigens.

Occasionally this can induce an immune response and the mother can subsequently develop HLA antibodies. These only become clinically relevant if the mother requires a transplant.

Paternal HLA typing is helpful to identify the antigens the mother may have been exposed to.This can aid antibody identification and help to build up an antibody profile on a patient.

3.8.14 *ABO blood group typing*

Beaumont Hospital Blood Transfusion Department carries out all donor and recipient blood grouping on request.

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

The H&I department provides an out-of-hours service for solid organ transplantation.
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

3.8.16 *Data Protection Act and freedom of information*

The H&I computer database is used to maintain patient data. A back-up paper copy is also retained. All data is stored in compliance with General Data Protection Regulation. .

Data can include the following:

- 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.
- Related donor information, where patients have been transplanted.
- Related family information, where a family study has been performed.Partner's HLA type where applicable.

3.8.17 ***Reports Issued/Expected Turn Around Times (TAT)***

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

TESTS	**TURN AROUND TIMES**
HLA typing for Solid Organ Transplant	3 weeks – *Urgent service available*
HLA Antibody Screening	2-4 weeks – *Urgent service available*
HLA Antibody Screening HLA typing requests for emergency transplantation	Same day service if requested
NHISSOT Patient report for the transplant clinic	4 weeks from request to issuing a report
Transplant pool work-up	2-6 weeks
Deceased donor work-up	6 hours
Potential donor recipient list	8 hours
Crossmatching for renal transplants	6 hours
Crossmatching for pancreatic/cardiothoracic transplants. Time taken from receipt of bloods in H&I laboratory and potential names for crossmatch given to the on-call scientist	6.5 hours for processing a single donor with a standard workup of a maximum 4 names. This time may change due to additional names or for technical issues. Users will be informed
Living donor work-up	1st work-up 4 weeks 2nd work-up 3 weeks Final work-up 48 hours
Autocrossmatch	2-3 days
Post Transplant Monitoring Non Urgent. *If contacted by the referring clinician for a more timely report the sample can be set on the next screen*. Further testing/ typing	2 weeks. 3 weeks
Post Transplant monitoring - Urgent antibody screening request for possible graft rejection *Requires discussion with Antibody Screening Senior. The level of urgency must be stated by the referring clinician*.	Same day service available if required, otherwise the sample is set on the next screen.
HLA typing for disease association	4 weeks
HLA typing for BMT/HSCT	2 weeks (unless awaiting further potential donors from overseas)

TESTS	TURN AROUND TIMES
HLA typing for B57	4 weeks
HLA typing for partners	3 weeks
ABO Blood Grouping	2-3 hours

3.8.18 *Abbreviations on H&I Reports and Printouts*

DIALYSIS CENTRES

AM Antrim Area Hospital
BE Beacon Clinic Sandyford, Dublin
BD Beacon Clinic Drogheda, Dublin
BT Beacon Clinic Tallaght, Dublin
BF Belfast City Hospital
BH Beaumont Hospital, Dublin
CA Cavan General Hospital
CB Mayo General Hospital, Castlebar
CO Cork University Hospital
CR Our Lady's Hospital for Sick Children, Crumlin, Dublin
EU Patients dialysing in hospitals overseas within the EU
FR Fresensius Limerick
GA Galway University Hospital
GW Wellstone Clinic, Galway
JA St. James's Hospital, Dublin
KK Wellstone Clinic, Kilkenny
LE Letterkenny General Hospital
LI Limerick University Hospital
MA Mater Misericordiae University Hospital, Dublin
MK Merlin Park Hospital, Galway
MW Midlands Wellstone Clinic
NC Northern Cross Clinic, Dublin
NE Daisy Hill Hospital, Newry
OM Omagh General Hospital
SL Sligo General Hospital
SV St. Vincent's University Hospital, Dublin
TA Tallaght Hospital (AMNCH), Dublin
TE Children's University Hospital, Temple Street, Dublin
TR University Hospital Kerry
TU Tullamore General Hospital
WA University Hospital Waterford
WW Wexford Wellstone

3.8.19 *RENAL/PANCREATIC TRANSPLANT POOL PRINTOUT*

ABBREVIATIONS

Age	Age in years
Blood Grp	Blood Group
BMI	Body Mass Index
Compno	H&I computer number
Cyto Due Date	Date a sample for antibody screening is required
Days Sample	Number of days sample is due. Minus number indicates the number of days the sample is outstanding.
Dial Cen	Dialysis centre
Dial	Dialysis type: P = CAPD/CCPD H =Haemodialysis
KG	Weight in kilos
Match %	Matchability score
PGen	Generated PRA PGen4: calculated on 4 HLA loci PGen10: calculated on 10 HLA loci
Prev Tx	Previous transplant(s): Number is printed
Ref Hosp	Referring Hospital
Urgent	Highest urgency- ABO compatible kidney
Wait	Length of time on transplant pool in months

3.8.19.1 *Crossmatch Codes*

Potential deceased donor offer list

DoTx	Day of transplant sample required
Std	Standard – sample(s) available in the laboratory and suitable for crossmatch
VXM	Suitable for virtual crossmatch