A Guide to the Histocompatibility and Immunogenetics Services Provided to Support Haemopoietic Stem Cell Transplantation

March 2017
This guide outlines the Histocompatibility and Immunogenetics (H&I) services provided by the Transplantation Laboratory, Manchester Royal Infirmary in support of haemopoietic stem cell transplant (HPCT) programmes. The guide is of use to clinical and support staff in bone marrow transplant units.

Revised in March 2017 by Alison Logan, Natalia Diaz Burlinson, Dr Helena Lee, Julie Kane & Dr Kay Poulton.

Next review due April 2018
# Contents

1. **Introduction** ........................................ 4  

2. **General Information** ................................ 7  
   2.1 Postal Address ........................................... 7  
   2.2 Business hours .......................................... 7  
   2.3 Laboratory Key Personnel ............................. 7  
   2.4 Essential Telephone Numbers ...................... 8  
   2.5 Essential Email Addresses ........................... 8  

3. **Use of the Laboratory** .............................. 9  
   3.1 Service Availability .................................... 9  
   3.2 Labelling of sample containers ..................... 9  
   3.3 Transportation of routine samples to the laboratory ... 10  
   3.4 Urgent Samples ......................................... 10  
   3.5 Acceptance time limit after sample drawing ...... 11  

4. **General Information and Notes on Tests Available** ........ 14  
   4.1 Descriptions of Standard Tests and Services .......... 14  

5. **Requesting Test & Samples Required** .............. 18  

6. **Reporting of Results** ................................ 23  

7. **Appendix** ............................................. 24  
   7.1 Requirements for Sending Specimens by Post .......... 24  
   7.2 List of Tests and Samples Required .................. 25  
   7.3 HSCT Donor Selection Procedure – Related Transplantation ... 26  
   7.4 HSCT Donor Selection Procedure – Unrelated Transplantation ........ 27
1. Introduction

The Transplantation Laboratory is a regional speciality pathology service and as such offers a wide range of high quality, efficient and cost-effective services using state of the art technologies to Central Manchester Foundation Trust, other regional Trusts, and healthcare providers.

The main services provided by the Transplantation Laboratory are described below:

a) Haemopoietic Stem Cell Transplantation

The Transplantation Laboratory provides Histocompatibility & Immunogenetics (H&I) support for the haemopoietic stem cell transplantation programmes at Central Manchester Foundation Trust (MRI and RMCH) and other regional trusts. The laboratory utilises state of the art molecular HLA typing technologies for patients and their potential donors who may need a stem cell or bone marrow transplant. The laboratory is one of the leading laboratories in the country in the application of chimaerism monitoring using short tandem repeats post stem cell transplantation. The laboratory provides additional KIR typing and interpretation of results for haploidentical stem cell transplants.

The laboratory offers a rapid and professional Graft Information and Advisory Service (GIAS) to undertake donor selection. This service is delivered by highly qualified and experienced HCPC staff and is led by an RCPath qualified H&I Consultant Clinical Scientist.

b) Solid Organ Transplantation

The Transplantation Laboratory provides H&I support for –

- Kidney, kidney and pancreas, pancreas and islet cell transplantation programmes at Manchester Royal Infirmary.
- Cardiothoracic organ transplantation at Wythenshawe Hospital
- Corneal transplantation at the Manchester Royal Eye Hospital, Victoria Hospital in Blackpool and North Manchester Hospitals.

There is a 24 hour on-call service for kidney / kidney and pancreas / pancreas only / islet transplantation and all thoracic organ transplants.

c) Immunogenetics testing

The Transplantation Laboratory provides testing to support disease diagnosis and management for the Central Manchester Foundation Trust, Primary Care Centres and hospitals. A range of tests is provided, including HLA-B27 and HLA-B*57:01 determination and HLA typing to support the diagnosis of Actinic Prurigo, Uveitis, Birdshot Retinopathy, Narcolepsy and Coeliac Disease. On request the laboratory can perform additional HLA typing to aid disease diagnosis and drug hypersensitivity investigations for tests in addition to those outlined.
d) Research and Innovation

The Transplantation Laboratory participates in research and innovation relevant to the clinical services provided to ensure that we continually improve our service provision in line with the current clinical evidence base. Projects are closely tailored to local clinical practice to ensure the most appropriate services are provided for the patients.

The Transplantation Laboratory is part of a network, which is cross-directorate and is known as the Manchester Institute of Nephrology and Transplantation (MINT). MINT is a multi-professional body of physicians, surgeons, nursing staff, scientists, other professions allied to medicine and managers. Its aim is to improve and develop the research and educational activities of the transplantation, nephrology and dialysis services to achieve the best possible care for transplant patients.

e) Audit

The Transplantation Laboratory is actively involved in audit related to laboratory activities as well as clinical audit in conjunction with the services we support. The process of clinical audit directly relates to the Trust’s Clinical Effectiveness Strategy that aims to improve the quality and outcome of patient care. The laboratory also has an internal audit cycle against ISO 15189:2012 and European Federation for Immunogenetics standards to ensure continual compliance and continual improvement in transplant outcome.

f) Quality assurance

The Transplantation Laboratory has full UKAS ISO 15189 accreditation (UKAS Reference No 7878) for medical laboratory services and EFI (European Federation of Immunogenetics) accreditation (Reference No: 03-GB-009.991).

The laboratory has a well-established quality management system in operation which allows the laboratory to be focused on continual improvement in line with needs and requirements of our users. The QMS provides a structured framework for the laboratory and is monitored and maintained by the Laboratory Operations and Quality Manager. The Quality Policy which is reviewed annually describes the aims of the services.

Participation in external quality assurance programmes such as UK NEQAS and UCLA schemes together with continual internal quality assessment monitoring of our tests ensures that the laboratory’s high quality standards are maintained.

UK NEQAS schemes conform to high standards of professionalism, impartiality, clinical relevance and strict financial accountability across all disciplines and specialities, so that all concerned with the quality of laboratory investigations may have confidence in the service provided.

A highly experienced consultant team offers support to clinicians and service users 24 hours a day, seven days a week. The team provides information related to using the service, interpretation of test results and clinical advice. Reviews and changes to
the service provision will be in consultation with our users and will be clearly defined in revised Service Level Agreements (SLAs), where applicable.

The Transplantation Laboratory actively supports and encourages staff training and continual professional development. It is recognised by both the Royal College of Pathologists, the National School for Healthcare Scientists and the British Society for Histocompatibility and Immunogenetics as a training laboratory in Histocompatibility and Immunogenetics. Where appropriate staff members are registered with the Health and Care Professions Council (HCPC).

Details of our accreditation, including current certificates and performance data, are available upon request from the Laboratory Operations and Quality Manager (Julie.Kane@cmft.nhs.uk).

In order to help us improve our service, you may be asked to complete a questionnaire. We greatly appreciate and value your input and would like to thank you for your assistance and suggestions.

**g) Complaint Procedure**

The Transplantation Laboratory is continually aware of, and takes into consideration the requirements of its users and staff, whilst striving to create the best standards of professional care. According to Trust policy, any complainants are referred to the Patient Advice and Liaison Service (PALS) who can support staff and patients to achieve speedy solutions. Also, complaints can be directed to the Laboratory Director, a Consultant Clinical Scientist or any Transplantation Laboratory representatives at Multi-disciplinary Team meetings. Please make any concerns you have about the quality of the service known to us as soon as possible; we take your complaints seriously.

Any suggestions from users regarding any aspect of our service provision, or indeed how the User Guide could be improved, are very welcome. Please forward any suggestions to the Laboratory Operations & Quality Manager (Julie.Kane@cmft.nhs.uk).

**h) Clinical Liaison and Advice**

A Consultant Clinical Scientist or deputy will always be available to attend multi-disciplinary team meetings as required in order to ensure optimum communication between the laboratory and clinical teams and provide advice relating to the Haemopoietic Stem Cell Service.

An experienced consultant team offers support to clinicians and service users 24 hours a day, seven days a week. The team provides interpretation of test results and clinical advice.

A 24-hour, 365-day on-call service is provided for deceased donor HLA typing and crossmatching and a Consultant Clinical Scientist is always available for the provision of advice.
2. General Information

2.1 Postal Address
Transplantation Laboratory
2nd Floor, Purple Zone
Manchester Royal Infirmary
Oxford Road
Manchester  M13 9WL

Tel 0161 276 6397
Fax 0161 276 6148

2.2 Business Hours
Opening Hours for routine work: 08.30am – 17.00pm
Out of hours, weekends and Bank holidays: On call staff & Consultant Clinical Scientist can be paged via CMFT switch Tel: 0161 276 1234

2.3 Laboratory Key Personnel
Laboratory Director
Dr Kay Poulton PhD, FRCPath
Consultant Clinical Scientist,
0161 276 6397
Email: kay.poulton@cmft.nhs.uk

Consultant Clinical Scientists
Mr Stephen Sheldon, FRCPath
Consultant Clinical Scientist
Tel: 0161 276 6397
Email: stephen.sheldon@cmft.nhs.uk

Ms Natalia Diaz Burlinson, FRCPath
Consultant Clinical Scientist
Tel: 0161 276 6397
Email: Natalia.DiazBurlinson@cmft.nhs.uk
HPCT Support Services Enquires
Dr Helena Lee
Principal Clinical Scientist
0161 276 6662
Email: helena.lee@cmft.nhs.uk

Alison Logan MPhil
Principal Clinical Scientist
0161 276 6661
Email: alison.logan@cmft.nhs.uk

Laboratory Operations and Quality Manager
Julie Kane MSc
Principal Clinical Scientist
0161 276 6424
Email: julie.kane@cmft.nhs.uk

General Enquires
Admin Manager
Judith Spencer
Tel: 0161 276 6397
Fax: 0161 276 6148
Email: judith.spencer@cmft.nhs.uk

2.4 Essential Telephone Numbers
Specimen Reception: 0161 276 6471
Admin office: 0161 276 6397
HPCT support Service and GIAS enquiries: 0161 276 6661/6662
Chimaerism monitoring enquiries: 0161 276 6662/6661
Immunogenetics team – General Enquires: 0161 276 6661/6662

2.5 Essential Email Addresses
HPCT Enquiries: cmm-tr.Transplantationlabhsct@nhs.net
Transplantationlaboratory.HSCT@cmft.nhs.uk

Solid Organ Enquiries: cmm-tr.Histocompatibility@nhs.net
3. Use of the Laboratory

3.1 Service Availability

The laboratory is open for receipt of routine specimens from 08:30 to 17:00 between Monday to Friday. Internal on site samples may be sent directly to the laboratory using the pneumatic pod system (Transplantation Pod No 805).

There is an on-call service provision available outside of normal working hours provided by an on call team consisting of a HCPC registered Clinical Scientist, a technologist and a Consultant Clinical Scientist. This service is generally restricted to the solid organ transplant programme and the laboratory on call team are contactable by pager via the hospital switchboard (0161 276 1234).

3.2 Labelling of sample containers

The Transplantation Laboratory will make every effort to ensure requests are processed in a safe and timely manner but it is essential that request forms and samples are labelled appropriately and legibly. The minimum acceptance criteria for request are normally 3 key identifiers that should include at least:

- Patient’s name (forename and surname)
- Date of birth
- Hospital number and or MRI District number
- NHS number
- Home Address of the patient.

These are all identifiers specific to the patient which help us to confirm identity and are essential.

It is also important to clearly identify the investigations required when completing the request card, please only select the test required and send only the appropriate sample tube.

If you have any concerns regarding this please ring 0161 276 6471 / 6397 for further advice.
Specimens will not be accepted for analysis if:

- There is insufficient unique identification of the patient i.e. they do not meet the minimum data set for identification
- Incorrect sample type or tube
- Incorrect transportation conditions mean that the sample is unsuitable
- Sample is received in a hazardous condition e.g. leaking or sharps attached.
- Sample is unlabelled or incorrectly labelled with less than the minimum data set required for patient identification
- Mismatch of details between the form and sample(s)
- The information provided is illegible

Samples that fail to meet the above criteria will be discarded as unsuitable for analysis, and the sender will be informed. The only exception to this is for patients whose identity is anonymous and they have their own unique identifier, for example patient samples from Genitourinary Medical Centres or potential stem cell donors. In other circumstances samples may be accepted without the 3 key identifiers at the discretion of the laboratory.

3.3 Transportation of routine samples to the laboratory

All users are advised to refer to P650 Packaging Instruction which applies to UN No. 3373 (Diagnostic Specimens) for information on the correct procedures for packaging and transporting samples. When sending samples to the laboratory it is important to follow the correct courier and postal procedures and ensure the specimens are appropriately packaged. (See appendix 7.1)

All specimens should be transported at room temperature (22°C - 25°C), unless otherwise instructed, avoiding where possible prolonged over exposure to heat. The samples should be transported directly to the laboratory as quickly as possible after collection to maintain the integrity of the sample and avoid compromising the results.

Internal on site specimens may be transported directly to the Transplantation laboratory via normal portering rounds during the normal working day or by pneumatic pod system to Pod No 805. Samples should be placed in a specimen bag with the request for transportation around the trust.

Please contact the laboratory on 0161 276 6471 / 6397 if there are specific questions regarding transportation of specimens.

3.4 Urgent samples

If a result is required urgently and the sample will arrive during working hours the laboratory MUST be notified by telephone so that we can prioritise your request.

All samples should be packaged and transported as above.

If you need to submit a sample out of normal working hours for testing on-call please contact the Clinical Scientist on-call via the hospital switchboard (0161 276 1234).
3.5 **Acceptance time limit after sample drawing**
For all tests requests complete the request card or an ICE request for users within CMFT.

**Chimaerism Analysis**

**Whole Blood Analysis:** Send 5ml EDTA blood No time limit

**Bone Marrow Aspirate:** Send **minimum** 100ul

Samples may be received in the laboratory at any time during normal working hours.

**Single or Multiple Lineage Analysis:**

A minimum sample of 3ml EDTA blood **per cell lineage** and the patient’s current WBC is required for this test. Samples can be received in the laboratory Monday 09:00 – Friday 13:00, and **must be received within 24 hours of the sample being taken**. The 24 hour time limit is important to maintain the integrity of the sample.

In some circumstances a buccal swab may be requested as reference material. Please contact the laboratory team as detailed in the contacts section of this user guide for instruction on how to take such samples.

**HLA and KIR genotyping**

5 ml EDTA blood for HLA or KIR typing No time limit

Buccal or dried blood spot samples may be used if necessary see page 12 & 13 for detailed instructions.

**HLA antibody screening**

5 ml Clotted blood (no anticoagulant) for HLA specific antibody testing Up to 48 hours

The 48 hour time limit is important to maintain the integrity of the sample.

EDTA blood samples should be kept at room temperature whilst waiting and during transport to the laboratory.

Request cards can be obtained from the Admin Manager, please call on 0161 276 6397 or email judith.spencer@cmft.nhs.uk. These request cards are also available in electronic format upon request.

See Appendix 7.1 for Specimen Transportation
See Appendix 7.2 for List of tests and samples required
See Appendix 7.3 HPCT selection of donor in related Transplantation
See Appendix 7.4 HPCT selection of donor in unrelated Transplantation
INSTRUCTIONS FOR COLLECTION OF BUCCAL CELL SAMPLES USING THE CYTOLOGY BRUSH TECHNIQUE

1) Wash hands thoroughly using soap and warm water to avoid sample contamination.

2) Ensure that the individual providing the sample has not consumed either food or drink for 30 minutes prior to sample collection.

3) Using the cytology brush provided scrape against the inside of the individual’s cheek 10 times. At this stage cells should be visible on the brush. If they are not, repeat the procedure.

4) Wrap the cytology brush head with the parafilm provided. It is not necessary for the cytology brush to remain moist after collection and the sample will remain stable for several days.

5) Return the sample to the Transplantation Laboratory at the above address in approved packaging (first class post) as with blood specimens.

Please note that if this procedure is carried out incorrectly we may be unable to isolate DNA from the sample provided.
COLLECTION OF BLOOD SPOT SAMPLES USING THE ACCU-CHEK® SAFE- T-PRO PLUS STERILE SINGLE-USE LANCING DEVICE

1. Ensure the collection paper is fully labelled with surname, forename, date of birth and date of specimen.
2. Wash hands thoroughly using soap and warm water and dry well to ensure a clean puncture site.
3. Twist the sterility cap and remove it.
4. The penetration adjustor is pre-set to the medium depth (~1.8mm) and is suitable for most adults. For children adjust to the low penetration depth (~1.3mm).
5. Hold the lancing device between the middle and index finger with the thumb on the release button.
6. Press the lancing device firmly against the puncture site, the side of the finger is recommended, and press the release button.
7. Squeeze the finger to encourage blood flow and collect at least 4 blood drops on each of the four circles (labelled 1-4) on the collection card (ensure the whole circle is filled with blood). A second puncture of the finger may be necessary.
8. Cover puncture site(s) with a plaster, dispose of the lance in an appropriate medical waste container and allow the blood spots to dry completely before packaging.
4. General Information and Notes on Tests and Services Available

4.1 Descriptions of standard tests

What is HLA typing (Tissue Typing)?
HLA typing is performed predominantly to match a donor and recipient for solid organ or haemopoietic stem cell transplantation (HPCT). Minimising the number of HLA mismatches between donor and recipient maximises the opportunity for optimal transplant survival.

HLA molecules are crucial to normal immune processes by enabling the cells involved in immune responses to recognise foreign organisms and react against them. Following transplantation the recipient’s immune cells can recognise donor HLA molecules as foreign and react against them causing rejection.

For HPCT it is essential to have minimal HLA mismatching to reduce the risk of graft versus host disease (GvHD) and mortality. GvHD is mainly caused by immunocompetent T cells transferred with the graft that recognise recipient cells as foreign and mount an immune mediated reaction.

HLA typing refers to the series of laboratory tests whereby the HLA molecules expressed on the surface of an individual’s body cells are identified. HLA molecules are on the surface of all nucleated cells (i.e in humans, all cells apart from red blood cells) but lymphocytes are routinely used for tests because they can easily be isolated from anti-coagulated peripheral blood.

The technique used for testing varies according to the clinical requirement. For example, a rapid, but low resolution technique (PCR SSP) may be used to HLA type a potential donor in a solid organ transplant setting. LABType® SSO offers intermediate level resolution and facilitates rapid batch testing of samples. A patient requiring HPCT will require extensive next generation sequenced based typing analysis to provide a high resolution HLA type. This is to ensure that the recipient and donor are matched as closely as possible. The Transplantation laboratory employs state of the art sequenced based HLA typing technology to identify the best matched donor for a patient.
An individual’s HLA type defines the combination of HLA molecules on the surface of their body cells. These are determined genetically. The HLA genes are unique in the human genome because of their considerable variability, which results in many different HLA types. The HLA genes are identified by letters (e.g. HLA-A) and the different gene products (specificities) by numbers (e.g. HLA-A2). Each individual inherits one set of HLA molecules from each parent thus they have two HLA-A, two HLA-B types and so on. For the purpose of matching for HPCT we consider matching HLA-A, B, C, DRB1 and DQB1 alleles to be of importance. Whenever possible, donors and recipients are matched for HLA DPB1.

In unrelated HPCT other factors such as donor CMV status, blood group, age and gender may be taken into consideration when there are two donors of equivalent HLA match for a patient to rank the donors in priority order.

The samples used for HLA typing tests are 5ml EDTA blood (if WBC>4.0x10^6/l). If the WBC<4.0x10^6/l, please send 20ml EDTA blood). Buccal samples or blood spot cards may be used if necessary.

**What is Chimaerism Monitoring?**
The Transplantation Laboratory is one of the leading centres in the UK in the field of Chimaerism monitoring.

In the post-transplant period, engraftment can be monitored by analysis of “short tandem repeat (STR)” markers in the patient’s peripheral blood or bone marrow aspirate. This post-transplant engraftment monitoring is referred to as Chimaerism monitoring. STR markers are variable DNA sequences, which differ in length by multiples of repeated units. The variability of these markers means that in most cases, the donor and recipient will not share the same sized STR marker. By monitoring these size differences in the post-transplant sample we can measure the success of the engraftment by calculation the percentage of donor derived cells in the recipient's sample.

In some scenarios post HPCT it is of clinical value to assess donor engraftment in more than one cell lineage in the post- transplant period. Several options exist and the assay can be tailored to a specific patient on request using the contact options supplied in section 2.3, 2.4 and 2.5.
What is Antibody Screening?
Individuals can produce antibodies directed against HLA specificities that they do not possess. This can happen following exposure to non-self HLA during pregnancy, blood transfusion or transplantation. These antibodies are detected in serum and can potentially react with a donor organ or graft and cause transplant rejection.

Sometimes it is not possible to identify a fully HLA matched donor or the patient is to undergo an haploidentical HPCT from a partially matched relative. In these scenarios it is important that the patient is screened for the presence of antibodies and that the specificities of the antibodies are defined. If a patient has HLA specific antibodies, it is best to avoid these specificities in a potential donor, as the antibodies would combine with the infused donation and reduce the cell dose available for the transplant. Not all donor directed antibodies are a veto to transplantation and a risk assessment following antibody definition would be required. This is particularly important in HPCT using umbilical cord donations due to the limited material available. **Samples for HLA antibody screening should be sent to the laboratory on initial referral prior to donor selection and as close as possible to the time of transplant.**

HLA specific antibodies are detected and defined by microbead array techniques, which are highly sensitive and specific. They are referred to as Luminex assays and are semi quantitative.

What is KIR Genotyping?
The KIR genes profile (KIR genotype) of the recipient and their donor are thought to be important in HPC transplant outcome. It may be of clinical value in some cases to perform testing for KIR genes for example if a haploidentical donor is to be considered or there are multiple HLA matched donors for a patient. KIR genotyping is available upon request from the laboratory.
Graft Information and Advisory Service (GIAS)

The laboratory offers a rapid and professional Graft information and Advisory Service (GIAS) to undertake donor selection. This is to ensure that the optimal HSC donor is selected for each patient. The team has vast expertise in the selection of adult unrelated donors, cord blood units and family members for haploidentical transplantation. This service is delivered by a highly qualified and experienced HCPC registered team of staff and directed by an RCPath qualified H&I Consultant Clinical Scientist (CCS) who is involved in national HPCT policy making within the H&I community at a national and international level. The CCS is also the Chair of the UK Cord Blood working group that assists in the selection of appropriate cord blood units for UK centres that require additional expertise and advice.

During working hours the generic email address for the HPCT team in the laboratory and the telephone lines ensure a fully qualified member of staff is always available to the transplant clinicians and coordinators to answer any enquiries.

The procedures followed by the Transplantation Laboratory for the selection of related and matched unrelated donors are illustrated in appendices 3 and 4.

- At initial referral to laboratory (1st set of bloods) send 5ml EDTA blood for HLA typing, a buccal swab and 5ml clotted blood for antibody screening (if required).
- At second referral send 5ml EDTA blood for verification typing. A further 5ml clotted blood for antibody screening if requested by the team for patient’s who are undergoing an HLA mismatched transplant. It is an essential accreditation requirement to confirm the patient and donor HLA type on a second sample.

Recipient samples should be received within 24 hours by the laboratory and by midday on Friday, to allow adequate time for processing, except by prior arrangement. They can be sent by 1st class post in appropriate packaging. (See Appendix 7.1)
5. Requesting Tests/Samples Required

The Transplantation Laboratory has its own distinctive request cards which can be obtained available from the Admin manager. Internal on site requests should be made using the ICE system using the Transplantation Laboratory tab following the CMFT standard operating procedure. All other requests should be made using the request cards following the procedure described below for the test required.

5.1 HLA (Tissue) Typing (Recipient)

As a minimum requirement, include patient surname, forename, date of birth, hospital number, referring hospital, consultant, person requesting the test and the date sample taken. It is essential that the patient is clearly identified on the card and on the specimen.

Complete the request card – an example is shown in RED below

**TRANSPLANTATION LABORATORY, MANCHESTER ROYAL INFIRMARY**
2nd FLOOR/PURPLE ZONE TEL: 0161 276 6397 FAX : 0161 276 6148
TransplantationLaboratory.HSCT@cmft.nhs.uk

<table>
<thead>
<tr>
<th>Surname (block capitals)*</th>
<th>Forenames*</th>
<th>Date of birth*</th>
<th>Sex</th>
<th>Hospital*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital number</td>
<td>NHS number</td>
<td>Sample date*</td>
<td>Consultant*</td>
<td>Ward</td>
</tr>
<tr>
<td>District number</td>
<td>Diagnosis</td>
<td></td>
<td>NHS Patient</td>
<td>Yes No</td>
</tr>
</tbody>
</table>

**Haemopoietic stem cell transplant**

- [ ] Recipient
- [ ] Relative
- [ ] Donor

- [ ] WBC count, if known
- [ ] CMV status
- [ ] ABO group

**Tests required**

- [ ] HLA typing (5 ml EDTA)
- [ ] HLA antibody screen (5 ml CLOTTED)

**Chimaerism analysis**

- [ ] PB
- [ ] BM
- [ ] Buccal

- [ ] Split cell (20 ml)

- [ ] CD3+
- [ ] CD19+
- [ ] CD33+
- [ ] CD66+

- [ ] Date of TxP
- [ ] Donor identity

*Essential information required in order to process request
5.2 HLA (Tissue) Typing (potential family donor)

As a minimum requirement include potential donor surname, forename, date of birth, referring hospital, consultant and person requesting test, date sample taken and name of potential recipient plus relationship.

Complete the request card – an example is shown in RED below

<table>
<thead>
<tr>
<th>SURNAME (BLOCK CAPITALS)*</th>
<th>FORENAMES*</th>
<th>DATE OF BIRTH*</th>
<th>SEX</th>
<th>HOSPITAL*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOSPITAL NUMBER</td>
<td>NHS NUMBER</td>
<td>SAMPLE DATE*</td>
<td>CONSULTANT*</td>
<td>WARD</td>
</tr>
<tr>
<td>DISTRICT NUMBER</td>
<td>DIAGNOSIS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DISTRICT NUMBER</th>
<th>ETHNIC ORIGIN</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>HAEMOPOIETIC STEM CELL TRANSPLANT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ ] RECIPIENT</td>
</tr>
<tr>
<td>WBC COUNT, IF KNOWN</td>
</tr>
<tr>
<td>CMV STATUS</td>
</tr>
<tr>
<td>ABO GROUP</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TESTS REQUIRED*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA TYPING (5 ml EDTA)</td>
</tr>
<tr>
<td>CHIMAERISM ANALYSIS</td>
</tr>
<tr>
<td>PB</td>
</tr>
<tr>
<td>SPLIT CELL (20 ml)</td>
</tr>
<tr>
<td>DATE OF TXP</td>
</tr>
</tbody>
</table>

*Essential information required in order to process request

HSCT REQUEST CARD
5.3 **Chimaerism monitoring whole blood**

As a minimum requirement, include patient surname, forename, date of birth, hospital number, referring hospital, consultant, person requesting the test and the date sample taken. It is essential that the patient is clearly identified on the card and on the specimen.

Complete the request card – an example is shown in RED below

<table>
<thead>
<tr>
<th>TRANSPLANTATION LABORATORY, MANCHESTER ROYAL INFIRMARY</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd FLOOR/PURPLE ZONE TEL: 0161 276 6397 FAX:0161 276 6148</td>
</tr>
<tr>
<td><a href="mailto:TransplantationLaboratory.HSCT@cmft.nhs.uk">TransplantationLaboratory.HSCT@cmft.nhs.uk</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TRANSPLANTATION LABORATORY, MANCHESTER ROYAL INFIRMARY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surname (block capitals)*</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Hospital number</td>
</tr>
<tr>
<td>District number</td>
</tr>
</tbody>
</table>

**Haemopoietic stem cell transplant**
- **Recipient**
- **Relative**
- **Relative**

- WBC count, if known
- CMV status
- ABO group

**Tests required**
- **HLA typing (5ml EDTA)**
- **HLA antibody screen (5ml CLOTTED)**

- **Chimaerism analysis**
- **PR**
- **BMC**
- **Buccal**
- **SPLT CELL (20ml)**
- **CD19**
- **CD33**
- **CD66**

- **Date of TXP**
- **Donor identity**

*Essential information required in order to process request*
5.4 Chimaerism monitoring Bone Marrow aspirate

As a minimum requirement, include patient surname, forename, date of birth, hospital number, referring hospital, consultant, person requesting the test and the date sample taken. It is essential that the patient is clearly identified on the card and on the specimen.

Complete the request card – an example is shown in RED below

<table>
<thead>
<tr>
<th>Surname (block capitals)*</th>
<th>Forenames*</th>
<th>Date of Birth*</th>
<th>Sex</th>
<th>Hospital*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOSPITAL NUMBER</td>
<td>NHS NUMBER</td>
<td>SAMPLE DATE*</td>
<td>CONSULTANT*</td>
<td>WARD</td>
</tr>
<tr>
<td>DISTRICT NUMBER</td>
<td>DIAGNOSIS</td>
<td></td>
<td></td>
<td>WARD</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Haemopoietic Stem Cell Transplant*</th>
<th>Tests Required*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient</td>
<td>Relative</td>
</tr>
<tr>
<td>WBC Count, if known</td>
<td></td>
</tr>
<tr>
<td>CMV Status</td>
<td></td>
</tr>
<tr>
<td>ABO group</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Essential information required in order to process request

HSCT Request Card
5.5 Chimaerism monitoring – Lineage specific

Complete the request card – an example is shown in RED below

As a minimum requirement, include patient surname, forename, date of birth, hospital number, referring hospital, consultant, person requesting the test and the date sample taken.

It is essential that the patient is clearly identified on the card and on the specimen.

A minimum of 3 ml is required for each cell lineage. For example if both CD3 and CD33 lineages are required send a minimum 6ml of EDTA blood and the boxes highlighted in red should be ticked. The sample must be received within 24 hours of venopuncture.

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**TRANSPANTATION LABORATORY, MANCHESTER ROYAL INFIRMARY**
2nd FLOOR/PURPLE ZONE  TEL: 0161 276 6397  FAX: 0161 276 6148
TransplantationLaboratory.HSCT@cmft.nhs.uk

<table>
<thead>
<tr>
<th>Surname (Block Capitals)*</th>
<th>Forenames*</th>
<th>Date of Birth*</th>
<th>Sex</th>
<th>Hospital*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital Number</td>
<td>NHS Number</td>
<td>Sample Date*</td>
<td>Consultant*</td>
<td>Ward</td>
</tr>
<tr>
<td>District Number</td>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**HAEMOPOIETIC STEM CELL TRANSPLANT***

- ☐ Recipient
- ☐ Relative
- ☐ WBC Count, if known
- ☐ CMV Status
- ☐ ABO Group

**Tests Required***

- HLA Typing (5ml EDTA)
- Chimaerism Analysis
- PB
- BM
- Buccal
- SPLIT CELL (20ml)
- CD3
- CD19
- CD33
- CD66
- Date of TXP
- Donor Identity

---

*Essential information required in order to process request
6. Reporting of Results

To maintain patient confidentiality and comply with the Data Protection Act and other legal requirements all results are reported via encrypted email or in writing only to an authorised individual. They are signed by a Consultant Clinical Scientist or named deputy. Other results are only reported by telephone after agreement by a Consultant Clinical Scientist. Provision of non-urgent results by fax is available on request during office hours and Consultant Clinical Scientist advice is available on a 24hr basis.

All times are quoted as working days from the receipt of the sample in the Transplantation Laboratory

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Turnaround Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA typing of HPCT patients and potential donors</td>
<td>5 days</td>
</tr>
<tr>
<td>Single Locus Screening of related donors</td>
<td>3 days</td>
</tr>
<tr>
<td>High resolution typing of patients and donors</td>
<td>10 days</td>
</tr>
<tr>
<td>HLA antibody screening for HPCT patients</td>
<td>2 days</td>
</tr>
</tbody>
</table>

When new antibody specificities are identified a HPCT Patient Antibody Profile will be issued within **10 working days**. Urgent screening requests can be processed within 24 hours if discussed with the HPCT team.

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Turnaround Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIR typing</td>
<td>5 days</td>
</tr>
<tr>
<td>Post-Transplant Engraftment Chimaerism monitoring routine</td>
<td>5 days</td>
</tr>
</tbody>
</table>

**Urgent Post-Transplant Chimaerism Engraftment monitoring** within 24 hours (wherever possible)

All our tests can be performed with urgency upon request, subject to the technical limitations of the assays. The turnaround times quoted are supported by audit data. Some stages of our search for donors are dependent upon external agencies (national and international donor registries) and may introduce delays in the process which are outside the laboratory’s control.

All aspects of the HPCT service are compliant with the relevant standards for HPCT.


UK NEQAS Chimaerism monitoring guidelines (2014).

7. Appendix

7.1 Requirements for sending specimens by post:

In order to comply with UN code number UN3373 there should be three layers of packaging.

1. The primary container containing the specimen

2. Secondary packaging e.g. a sealable plastic bag that contains enough absorbent material to contain the entire contents of the primary container without leakage occurring.

3. Outer packaging, to be labelled with the destination address, the name of the sending department and address, and be clearly marked “Diagnostic Specimen”

Appropriate packaging is available from suppliers including the Royal Mail, Royal Mail Safebox, FREEPOST, SWC1 143, Ross-on-Wye, HR9 7ZB.
<table>
<thead>
<tr>
<th>Test</th>
<th>Sample Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient HLA Typing (Initial HLA type)</td>
<td>5ml EDTA plus a buccal swab and 5ml clotted blood</td>
</tr>
<tr>
<td>Recipient HLA Typing (Verification HLA type)</td>
<td>5 ml EDTA</td>
</tr>
<tr>
<td>Recipient HLA-Specific Antibody Screen/definition</td>
<td>10ml clotted blood</td>
</tr>
<tr>
<td>Chimaerism Analysis Whole blood</td>
<td>5 ml EDTA</td>
</tr>
<tr>
<td>Chimaerism analysis Bone Marrow Aspirate</td>
<td>Minimum 100ul</td>
</tr>
<tr>
<td>Lineage specific Chimaerism analysis</td>
<td>3 ml of EDTA per lineage</td>
</tr>
<tr>
<td>KIR genotyping</td>
<td>5 ml EDTA</td>
</tr>
</tbody>
</table>
7.3 HPCT Donor selection procedure- Related Transplantation

New Patient Referral

Samples received from related individuals who may be potential donors.

Family Members screened for HLA identity. HLA-B first, then HLA-A and DRB1 if single locus screening is uninformative

Sample Stored until HLA identity of family members is confirmed by an accredited centre.

Confirmatory typing of HLA compatible family members performed as necessary to cover the following loci: HLA-A, B, C, DRB1, DQB1, DPB1.

Patient and donor will be typed at high resolution when the HLA-identical family member selected as the donor is not a sibling or if identity by descent cannot be proven. Siblings who are HLA identical at all loci tested will be considered to be HLA identical by descent.

If a haploidentical transplant is to be performed using a parent/sibling/offspring as donor, Class I and II high resolution typing will be performed. HLA specific antibody status of the patient must be ascertained and mismatches avoided whenever possible in all HLA mismatched HSCT transplants.

Confirmation of Identity

Duplicated HLA typing reports received from an accredited centre may be accepted as confirmation of identity for either donor or recipient. Alternatively, a repeat sample will be requested for confirmation of HLA identity as necessary.

HPCT Matching Report Generated
New Patient Referral
Patient has no potential related donors

HLA Typing by Next Generation Sequencing (NGS, Illumina)
Allele level resolution: HLA-A, B, C, DRB3/4/5, DRB1, DQB1, DPB1

Initiate searches of Aligned registry and BMDW

Discuss strategy with transplant consultant

Ascertain Blood Group & CMV serostatus of patient. HLA specific antibody status is also required for a mismatched donor

Verification Typing of Patient (HLA-A, B and DRB1 by LABType® SSO)

Request expanded HLA typing on donors to be performed locally or by registry as necessary.

Verification Typing of Donor
Duplicated HLA typing reports received from a registry (or an accredited centre) may be accepted as confirmation of identity for the donor.

High resolution typing of donor of choice by NGS

HPCT Matching Report Generated