A Guide to the Histocompatibility and Immunogenetics Services Provided to Support Kidney, Pancreas and Islet Cell Transplantation
This guide outlines the Histocompatibility and Immunogenetics (H&I) services provided by the Transplantation Laboratory, Manchester Royal Infirmary in support of renal, pancreas and islet cell transplant programmes. The guide is of use to clinical and support staff in renal dialysis units, diabetes centre and renal transplant units.

Revised in July 2019 by Dr Judith Worthington,
Prof Kay Poulton & Julie Johnson.

Next review due June 2020
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Introduction

The Transplantation Laboratory is a regional specialty pathology service and as such offers a wide range of high quality, efficient and cost-effective services using state of the art technologies to Manchester University NHS Foundation Trust, other regional Trusts, and healthcare providers. The main services provided by the Transplantation Laboratory are described below:

a) Solid Organ Transplantation

The 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.

b) Haematopoietic Progenitor Stem Cell Transplantation

The Transplantation Laboratory provides Histocompatibility & Immunogenetics (H&I) support for the haematopoietic progenitor stem cell transplantation programmes at Manchester University NHS 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 progenitor 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 registered staff and is led by RCPath qualified H&I Consultant Clinical Scientists.

c) Immunogenetics testing

The Transplantation Laboratory provides testing to support disease diagnosis and management for the Manchester University NHS Foundation Trust, Primary Care Centres and hospitals. A range of tests is provided, including HLA-B*27 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 is a UKAS accredited medical laboratory No.7878 and has European Federation of Immunogenetics accreditation (EFI 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.

Any test performed in the laboratory is subject to a variety of factors that may influence the outcome of the result. Some of these factors include the sample itself, the test method, reagents used and different operators carrying out the same process. Variations can also be caused by procedures that involve the measurement of analytes and reagents whereby environmental factors such as temperature and humidity may affect results. Any equipment used in the process will further introduce the opportunity for variation. To provide a measure of confidence in results produced it is necessary to identify all factors which may contribute to variation in a process and assess their potential to influence uncertainty. Once identified these factors must be reduced or controlled to an acceptable level and a value for the range of acceptable uncertainty assigned where possible.

The Transplantation Laboratory has chosen, where possible, to utilise internal Quality Control material and data to establish Uncertainty of Measurement where applicable. Upon request the laboratory shall make its estimates of measurement of uncertainty available to laboratory users.
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.johnson2@mft.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 Multidisciplinary 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.johnson2@mft.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 Cardiothoracic Transplant Service provision.

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.


I) Confidentiality and Personal Information

The Transplantation Laboratory adheres to Manchester University NHS Foundation Trust's policies on data protection and disclosure.
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: 08.30 – 17.00 hrs

Out of hours, weekends and Bank holidays:
On call staff & Consultant Clinical Scientist can be paged via MFT switch Tel: 0161 276 1234

2.3 Laboratory Key Personnel

Laboratory Director
Prof Kay Poulton PhD, FRCPATH
Consultant Clinical Scientist,
0161 276 6397
Email: kay.poulton@mft.nhs.uk

Consultant Clinical Scientists
Mr Stephen Sheldon, FRCPATH
Tel: 0161 276 6397
Email: stephen.sheldon@mft.nhs.uk
Ms Natalia Diaz Burlinson, FRCPATH
Tel: 0161 276 6397
Email: Natalia.DiazBurlinson@mft.nhs.uk

Kidney Pancreas Support Services Enquires
Dr Judith Worthington PhD
Principal Clinical Scientist
0161 276 7988
Email: judith.worthington@mft.nhs.uk
Laboratory Operations and Quality Manager
Julie Johnson MSc. HCPC
Principal Clinical Scientist
0161 276 6424
Email: julie.johnson2@mft.nhs.uk

General Enquiries
Business and Administration Manager
Judith Spencer
Tel: 0161 276 6397
Fax: 0161 276 6148
Email: judith.spencer@mft.nhs.uk

2.4 Essential Telephone Numbers
Specimen Reception: 0161 276 6471
Admin office: 0161 276 6397
Histocompatibility Team – General Enquiries 0161 276 7988 / 7919 / 6651 / 6656

2.5 Essential Email Addresses
Transplantation Laboratory (general enquiries) : TransplantLab@mft.nhs.uk
Cardiothoracic Patient Listings : OnCall.TLab@mft.nhs.uk
Solid Organ Enquiries : cmm-tr.Histocompatibility@nhs.net

2.6 Internet page
https://mft.nhs.uk/mri/services/transplantation-laboratory
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.

The on-call team can be contacted through the paging service provided by PageOne (Page One User Guide (www.pageone.co.uk/support/downloads) or directly via the MRI 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 are insufficient unique identifiers for the patient as specified.
- Incorrect sample type or tube
- Incorrect transportation conditions mean that the sample is not viable for testing
- Sample is received in a hazardous condition e.g. leaking or sharps attached.
• 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 2)

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 the porter’s 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) or via the paging service provided by PageOne (Page One User Guide (www.pageone.co.uk/support/downloads).
3.5 Acceptance time limit after sample drawing

Time limits and storage temperature requirements are imposed to maintain the integrity of the sample, to ensure accuracy and reliability of the testing and reduce the need for repeat samples. For all tests complete the request card or an ICE request for users within MFT. See Appendix 4 for a full list of test and samples required.

**HLA genotyping**
- **Whole blood** – minimum 3 ml EDTA blood
  - No time limit
- **Buccal swab**
  - No time limit
- **Dried Blood Spot**
  - No time limit

(See pages 13-14 for instruction on how to take buccal swabs/ dried blood spot samples).

**HLA antibody/ donor specific antibody (DSA) testing**
- 5 ml Clotted blood (no anticoagulant)
  - Up to 48 hours

**Crossmatching**
- EDTA/ Heparinised blood for crossmatching
  - Up to 24 hours

**Storage conditions prior to sending**

Clotted samples can be kept overnight at 4°C but sent immediately the next morning to the laboratory for testing.

EDTA blood samples should be kept at room temperature whilst waiting and during transport to the laboratory, avoiding any excessive heat exposure.

Request cards can be obtained from the Transplantation Laboratory, please call on 0161 276 6397 or email TransplantLab@mft.nhs.uk. These request cards are also available in electronic format upon request.
3.6 Instructions for the collection of a Sample using a Buccal Swab

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.
3.7 Instructions for the collection of a Sample using a Blood Spot

Transplantation Laboratory
Manchester Royal Infirmary, Oxford Road, Manchester, M13 9WL
Tel: 0161 276 6397, Fax: 0161 276 6148

COLLECTION OF BLOOD SPOT SAMPLES USING THE ACCU-CHEK®
SAFE-T-PRO PLUS STERILE SINGLE-USE LANCING DEVICE

Penetration depth adjustor
Sterility cap
Release button
Penetration depth markers

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 regarding 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 progenitor 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.

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 intermediate resolution technique (LinkSēq™) 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 for routine testing. All routine cardiothoracic patients also receive extensive next generation sequence (NGS) based typing analysis to provide a high resolution HLA type. The Transplantation Laboratory employs state of the art NGS HLA typing technology to assist in interpretation of HLA antibodies and thus permit suitable donor identification.

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.
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.

It is important that cardiothoracic patients are screened for the presence of HLA antibodies and that the specificities of any antibodies detected are defined prior to transplantation. **Samples for antibody screening should be sent to the laboratory every three months from patients on the transplant list and approximately two weeks after a known sensitising events (e.g. blood transfusions).** When a patient is known to have antibodies against a particular HLA specificity, that specificity is listed as an unacceptable donor antigen. When donor directed HLA specific antibodies are identified, the level of risk associated with proceeding to transplant can be assessed on request.

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.

For sensitised parous female patients being listed for transplantation, it is our policy to HLA type the father of the children or the children themselves in order to fully define pregnancy-related sensitisation.

Some patients have non graft damaging “autoantibodies” that can cause false positive donor crossmatches, the laboratory will request samples to specifically test for these as necessary.

What is Crossmatching?
Crossmatching is a pre-transplant test in which donor lymphocytes are tested against serum samples from the potential recipient(s) to ascertain whether any donor–reactive antibodies are present that would cause transplant rejection. Donor-reactive antibodies that cause a positive crossmatch test are normally a contraindication to transplantation.

- The **cytotoxic crossmatch** is a cell killing test. It is carried out for all potential recipients.
- The **flow cytometry crossmatch** is a more sensitive test that uses fluorescence to detect antibody binding to donor cells and is used for “high-risk” sensitised recipients.
Virtual Crossmatching
A “virtual crossmatch” assessment is performed pre-transplant for solid organ patients, whereby the donor HLA type is reviewed against the patient’s HLA antibody profile (antibodies are listed as unacceptable antigens) to determine whether the patient has any donor-directed antibodies that could cause a positive crossmatch test result. In cases where all unacceptable antigens have been clearly defined, sensitised patients can be transplanted without the need for a prospective crossmatch as long as all unacceptable antigens are absent from the donor HLA type. This is the basis of the current solid organ allocation process in the UK and the low frequency of unexpected positive crossmatches for patients suggests that patients who have been rigorously assessed and defined as negative for donor-directed antibodies can safely proceed to transplant before the crossmatch test result is available. The purpose of this approach is to reduce the cold ischaemia time without compromising the safety of transplantation. For these recipients the crossmatch test is performed retrospectively.

Potential recipients will be assessed for their suitability for a “virtual crossmatch” against a particular donor and a “virtual crossmatch negative” report issued if appropriate. The transplant unit must be able to confirm that no potential sensitisation events have occurred since the date of the last patient serum sample tested for HLA antibodies.

Risk Assessment Crossmatching
For patients with donor-directed antibodies, a pre-transplant “risk assessment crossmatch” can be performed on request. In a “risk assessment crossmatch” the level of immunological risk associated with proceeding to transplant with a particular donor is determined by performing a cytotoxic and flow cytometry crossmatch using both current and historic serum samples for the recipient. Immunological risk is then assigned using the BSHI/BTS guidelines for Detection and Characterisation of Clinically Relevant Antibodies in Allotransplantation.

It must be noted that the validity of virtual and risk assessment crossmatch results for antibody positive patients is dependent on the donor HLA type being correct. In 2018, nationally, discrepancies were detected in 0.27% of donor HLA types after the organs had been allocated.

Post-transplant Donor Specific Antibody (DSA) testing
Post-transplant recipients can produce specific antibodies associated with transplant rejection. A post-transplant antibody monitoring service is available, including testing for donor-directed antibodies. Post-transplant samples for DSA testing to support the diagnosis of rejection should be sent when clinically indicated to support the diagnosis of antibody-mediated rejection.
Overview of Kidney / Pancreas Support Service for Patients with Chronic Renal Failure

- 5ml EDTA blood
- Initial HLA type
- Verification HLA type
- Initial Antibody Screen
- Verification Screen
- Monthly Bloods

PATIENT LISTED FOR KIDNEY/PANCREAS TRANSPLANT

- Potential Deceased Donor
  - Deceased Donor Crossmatch Test
    - Positive Crossmatch
      - No Transplant
      - Investigation of Positive Crossmatch
    - Negative Crossmatch
      - Post Transplant Monitoring
      - Transplanted

- Potential Living Donor (LD)
  - HLA type Donor
    - LD Crossmatch Test
      - Positive Crossmatch
        - Recommend Options Eg: NLDKSS / HLAi
      - Negative Crossmatch
        - Transplanted
5. Requesting Tests/Samples Required

The Transplantation Laboratory has its own distinctive request cards which can be obtained from the Transplantation Laboratory (TransplantLab@mft.nhs.uk). Internal on site requests should be made using the ICE system using the Transplantation Laboratory tab following the MFT standard operating procedure. All other requests should be made using the request cards shown below following the procedure described below for the test required.
5.1 HLA (Tissue) Typing (Recipient)

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.

- At initial referral to laboratory (1st set of bloods) send 5ml EDTA blood for HLA typing and 10ml clotted blood for antibody screening.
- At surgical referral send 5ml EDTA blood for **verification typing** and 10ml clotted blood for antibody screening.

*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.

**Send 5ml EDTA blood and 10ml clotted blood**
5.2 HLA (Tissue) Typing (Potential Living Donor)

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

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. Wherever possible attach a copy of the potential donor’s ABO report to the request card.

<table>
<thead>
<tr>
<th>Surname*</th>
<th>Forename*</th>
<th>Date of Birth*</th>
<th>Sex</th>
<th>Hospital*</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hospital Number*</th>
<th>NHS Number</th>
<th>Requested By* (Block Capital)</th>
<th>Ward</th>
<th>Consultant*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NHS Patient</th>
<th>Blood Group</th>
<th>Blood Transfusion</th>
<th>No Pregnancies</th>
<th>Sample Draw Date*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
<td>ABO____ Rh____</td>
<td>Date____</td>
<td>No Units____</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnosis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient*</td>
</tr>
<tr>
<td>Kidney</td>
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<table>
<thead>
<tr>
<th>Tests Required*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient HLA Typing</td>
</tr>
<tr>
<td>Living Donor HLA Typing</td>
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<tr>
<td>5ml EDTA</td>
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<table>
<thead>
<tr>
<th>Potential Recipient</th>
<th>Relationship of Donor to Recipient</th>
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<tr>
<th>Pregnancy Related Unacceptable Antigens</th>
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<tbody>
<tr>
<td>5ml EDTA</td>
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<thead>
<tr>
<th>Post TX Donor Specific Antibodies</th>
<th>Auto Crossmatch</th>
</tr>
</thead>
<tbody>
<tr>
<td>10ml CLOTTED BLOOD</td>
<td>20ml Heparin or EDTA</td>
</tr>
</tbody>
</table>

*Essential information required in order to process request
### 5.3 HLA (Tissue) Typing Pregnancy Related Bloods

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

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

<table>
<thead>
<tr>
<th>SURNAME*</th>
<th>FORENAME*</th>
<th>DATE OF BIRTH*</th>
<th>SEX</th>
<th>HOSPITAL*</th>
</tr>
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<table>
<thead>
<tr>
<th>HOSPITAL NUMBER*</th>
<th>NHS NUMBER</th>
<th>REQUESTED BY* (Block Captas)</th>
<th>WARD</th>
<th>CONSULTANT*</th>
</tr>
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<thead>
<tr>
<th>NHS PATIENT</th>
<th>BLOOD GROUP</th>
<th>BLOOD TRANSFUSION</th>
<th>No PREGNANCIES</th>
<th>SAMPLE DRAW DATE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>NO</td>
<td>ABO___ Rh___</td>
<td>DATE____</td>
<td>No UNITS____</td>
</tr>
</tbody>
</table>

**DIAGNOSIS***

**RECIPIENT**

- [ ] KIDNEY
- [ ] PANCREAS
- [ ] COMBINED KP
- [ ] ISLETS

**DONOR**

**OTHER**

**TESTS REQUIRED***:

- [ ] HLA TYPING (5ml EDTA)
- [ ] LIVING DONOR HLA TYPING
- [ ] 5ml EDTA

**RECIPIENT HLA SPECIFIC ANTIBODIES**

- [ ] HLA SPECIFIC ANTIBODIES (10ml CLOTTED BLOOD)
- [ ] LIVING DONOR CROSSMATCH
- [ ] 40ml HEPARIN or EDTA (DONOR), 10ml CLOTTED (RECIPIENT)

**POTENTIAL RECIPIENT**

**RECIPIENT**

**RELATIONSHIP OF DONOR TO RECIPIENT**

**PREGNANCY RELATED UNACCEPTABLE ANTIGENS**

- [ ] 5ml EDTA

**POST TTX DONOR SPECIFIC ANTIBODIES**

- [ ] 10ml CLOTTED BLOOD
- [ ] 20ml HEPARIN or EDTA

* ESSENTIAL INFORMATION REQUIRED IN ORDER TO PROCESS REQUEST
5.4 HLA (Tissue) Typing (Deceased Donor)

The on-call Clinical Scientist should be notified by the Specialist Nurse for Organ Donation (SN-OD) as soon as a potential donor has been identified. Samples must be accompanied by an NHSBT HLA Typing Request form (FRM4279/1) and should be delivered to the Transplant Unit (MRI Ward 10) or the Accident & Emergency reception at MRI. Instructions on how to contact the laboratory on-call staff are printed on the specimen box lid. The on-call Clinical Scientist should immediately be notified of their arrival by the reception staff in A&E via switchboard so that collection and delivery to the laboratory can be arranged.

Send 10ml EDTA blood
5.5 HLA Antibody Screening

Routine monthly samples from patients on the transplant list or post-transplant need not be accompanied by a request card although tubes must be clearly labelled with patient name, date of birth, hospital number and date of sample collection.

Samples can be received within 48 hours Monday to Friday and sent by 1st class post in appropriate packaging. (See Appendix 2)

Send 10ml clotted blood

| THE TRANSPLANTATION LABORATORY, MANCHESTER ROYAL INFIRMARY |
| TEL: 0161 276 0397  FAX: 0161 276 0140 |

<table>
<thead>
<tr>
<th>SURNAME*</th>
<th>FORENAME*</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>REQUESTED BY* (Block capitals)</td>
<td>WARD</td>
<td>CONSULTANT*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NHS PATIENT</th>
<th>BLOOD GROUP</th>
<th>BLOOD TRANSFUSION</th>
<th>No PREGNANCIES</th>
<th>SAMPLE DRAW DATE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>NO</td>
<td>ABO</td>
<td>Rh</td>
<td>DATE</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DIAGNOSIS*</th>
<th>RECIPIENT</th>
<th>DONOR</th>
<th>OTHER</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIDNEY</td>
<td>PANCREAS</td>
<td>COMBINED KIDNEY</td>
<td>ISLETS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TESTS REQUIRED*</th>
<th>RECIPIENT HLA TYPING</th>
<th>RECIPIENT HLA SPECIFIC ANTIBODIES</th>
<th>LIVING DONOR CROSSMATCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>RECIPIENT HLA TYPING</td>
<td>HLA TYPING (5ml EDTA)</td>
<td>HLA SPECIFIC ANTIBODIES (10ml CLOTTED BLOOD)</td>
<td>LIVING DONOR CROSSMATCH</td>
</tr>
<tr>
<td>LIVING DONOR HLA TYPING</td>
<td>5ml EDTA</td>
<td>40ml HEPARIN or EDTA (DONOR); 10ml CLOTTED (RECIPIENT)</td>
<td>RELATIONSHIP OF DONOR TO RECIPIENT</td>
</tr>
<tr>
<td>PREGNANCY RELATED UNACCEPTABLE ANTIGENS</td>
<td>5ml EDTA</td>
<td>RECIPIENT</td>
<td></td>
</tr>
<tr>
<td>POST TPX DONOR SPECIFIC ANTIBODIES</td>
<td>10ml CLOTTED BLOOD</td>
<td>AUTO CROSSMATCH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20ml HEPARIN or EDTA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* ESSENTIAL INFORMATION REQUIRED IN ORDER TO PROCESS REQUEST
5.6 Crossmatching (Deceased Donor)

**Deceased organ donor samples**
Donor spleen and lymph node specimens should be taken by the organ retrieval team and despatched to the Transplant Unit (MRI Ward 10). The on-call Clinical Scientist must be immediately notified of their arrival by the ward staff so that collection and delivery to the laboratory can be arranged.

**Recipient samples**
If a sample is requested by the laboratory for crossmatching against a potential kidney donor, send 10ml clotted blood as a matter of urgency.

**Islet Cell Viability Testing**
Islet viability is affected by temperature. Islets are transported with a data logger, which produces output files that can be analysed to ensure that islets have been transported at a stable temperature.
Islet viability is assessed by staining islets with fluorescein diacetate and propidium iodide. Living cells are permeated by fluorescein diacetate. Living cells convert fluorescein diacetate into a fluorescent green dye. Dead cells have compromised cell membranes. Propidium iodide enters cells via their compromised cell membranes and stains nucleic acid fluorescent red. The viability of an islet is determined by the ratio of green:red fluorescent cells in the islet.

The Tissue Typer On Call can be contacted through the paging service provided by PageOne (Page One User Guide [www.pageone.co.uk/support/downloads]) or directly via MRI Switchboard (0161 276 1234). Alternatively it may be necessary to contact via their On Call mobile phones.
5.7 Crossmatching (Living Donor)

These tests must be pre-arranged with the laboratory, are time sensitive and must be accompanied by a request card – see an example below.

**From the recipient** send 10ml clotted blood.

**From the donor** send 40ml heparinised and 5ml EDTA blood (Where heparinised bottles are not available send 40ml EDTA)

---

<table>
<thead>
<tr>
<th>Surname*</th>
<th>Forename*</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>Requested by*</td>
<td>Ward</td>
<td>Consultant*</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>NHS Patient</th>
<th>Blood Group</th>
<th>Blood Transfusion</th>
<th>No Pregnancies</th>
<th>Sample Draw Date*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
<td>ABO___ Rh___</td>
<td>Date____ No Units___</td>
<td></td>
</tr>
</tbody>
</table>

**Diagnosis***

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>Pancreas</td>
<td>Combined KP</td>
</tr>
</tbody>
</table>

**Tests Required***

<table>
<thead>
<tr>
<th>Recipient HLA Typing</th>
<th>Donor HLA Specific Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA Typing (5ml EDTA)</td>
<td>HLA Specific Antibodies (10ml Clotted Blood)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Living Donor HLA Typing</th>
<th>Living Donor Crossmatch</th>
</tr>
</thead>
<tbody>
<tr>
<td>5ml EDTA</td>
<td>40ml Heparin or EDTA (Donor) 10ml Clotted (Recipient)</td>
</tr>
</tbody>
</table>

**Potential Recipient**

**Relationship of Donor to Recipient**

**Pregnancy Related Unacceptable Antigens***

<table>
<thead>
<tr>
<th>Post Tpx Donor Specific Antibodies</th>
<th>Auto Crossmatch</th>
</tr>
</thead>
<tbody>
<tr>
<td>5ml EDTA</td>
<td>20ml Heparin or EDTA</td>
</tr>
</tbody>
</table>

* Essential information required in order to process request.
5.8 Donor Specific Antibodies

Post-transplant samples for donor specific antibody testing to support the diagnosis of antibody-mediated rejection must be accompanied by a request card. See example below. Guidelines for the frequency of testing are shown in Appendix 8

Send 10ml clotted blood.

---

<table>
<thead>
<tr>
<th>Surname</th>
<th>Forename</th>
<th>Date of Birth</th>
<th>Sex</th>
<th>Hospital</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Hospital Number</th>
<th>NHS Number</th>
<th>Requested By* (Block Capitals)</th>
<th>Ward</th>
<th>Consultant*</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NHS Patient</th>
<th>Blood Group</th>
<th>Blood Transfusion</th>
<th>No Pregnancies</th>
<th>Sample Draw Date*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
<td>ABO Rh</td>
<td>Date</td>
<td>No Units</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnosis*</th>
<th>Recipient</th>
<th>Donor</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>Pancreas</td>
<td>Combination K/P</td>
<td>Islets</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tests Required*</th>
<th>Recipient HLA Typing</th>
<th>Recipient HLA Specific Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HLA Typing (5ml EDTA)</td>
<td>HLA Specific Antibodies (10ml Clotted Blood)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Living Donor HLA Typing</th>
<th>Living Donor Crossmatch</th>
</tr>
</thead>
<tbody>
<tr>
<td>5ml EDTA</td>
<td>40ml Heparin or EDTA (Donor) 10ml Clotted (Recipient)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Potential Recipient</th>
<th>Relationship of Donor to Recipient</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Pregnancy Related</th>
<th>Unacceptable Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>5ml EDTA</td>
<td>Recipient</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Post TPX Donor Specific Antibodies</th>
<th>Auto Crossmatch</th>
</tr>
</thead>
<tbody>
<tr>
<td>10ml Clotted Blood</td>
<td>20ml Heparin or EDTA</td>
</tr>
</tbody>
</table>

* Essential Information Required in Order to Process Request
6. Reporting of Results

To maintain patient confidentiality and comply with General Data Protection Regulations (GDPR) 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 email is available on request during office hours and Consultant Clinical Scientist advice is available on a 24hr basis. The Measurement of uncertainty (MoU) shall be considered for all examinations which include a measurement step where it has influence on the reported result. Estimates of the MoU will be made available to users upon request.

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

**HLA typing results** for renal patients and potential living donors 90% will usually be issued **within 15 working days** of correct specimen receipt

**HLA typing results** for deceased donors will be reported by emailed to NHSBT-ODT, normally **within 4 hours of specimen receipt**.

**HLA antibody screening results** for new patients will be reported when the patient is listed for transplantation, unless specifically requested. For patients on the transplant list data on current antibody status is included on the circulated monthly transplant list. When there is a change in the antibody profile of a listed patient a **Renal Patient Antibody Profile** will be issued.

**Unacceptable antigens** resulting from pregnancy related sensitisation will be reported as an HLA Report: *HLA type performed to determine pregnancy-related sensitisation*.

**The Transplant Chance** gives a measure of the chance of a suitably HLA matched donor becoming available for the patient - the higher the value, the better the chance. It is calculated on the basis of the patient’s ABO blood group, HLA type and unacceptable antigens with reference to the ABO blood groups and HLA types of all organ donors recorded locally (over 2000). The Transplant Chance is included on the patient HLA profile for newly listed patients, the Renal Patient Antibody Profile Report and on the monthly transplant list. (See Appendix 5)

**Potential Living Donor Matching Reports** will be issued when all essential information and samples have been received by the laboratory usually **within 15 days of receipt**

**Deceased Donor / Recipient Crossmatch** results are reported to the transplant team, usually **within 5 hours of receipt of crossmatch specimens**.

**Immediate pre-transplant Living Donor / Recipient Crossmatch**. A report will be issued by the day preceding the transplant date.

**Donor Specific Antibodies** results will be reported as “Renal Transplant Recipient – Post Transplant Antibody Investigations” report usually **within 5 working days of**
receipt. Copy reports are available to view via the Renal Unit IT system Clinical Vision.

Islet cell viability results will be reported as “Islet Viability Report” to the transplant team, usually within 3 hours of receipt

Turnaround Time (TAT)

Over 90% of the results are reported within the specified working days from receipt of samples. Special arrangements requiring a shorter turnaround may be established on a user-specific basis, by arrangement with the Consultant Clinical Scientist and subject to the technical limitations of the assays. The turnaround times quoted are supported by audit data.
7. Patient Registration on the Kidney/Pancreas Transplant List

Essential Tests -

• All patients
  – ABO blood group report
  – HLA type (tested on two separate occasions)
  – HLA antibody screen

• IgM alloantibody positive patients
  – Autoantibody test

Recommended Tests -

• Parous female patients
  – HLA type of partner/father(s) of their children, or
  – HLA type of each child

Listing Process -

Refer patients to Transplant Surgeons. The referral letter must be accompanied by a completed listing proforma signed by the referring physician, plus a copy of the ABO blood group report. When the patient has been assessed and/or discussed at MDT, the form will then be countersigned by a Consultant Transplant Surgeon and then sent to the Transplantation Laboratory.

The Transplantation Laboratory will acknowledge receipt of the listing proforma and request any outstanding samples. When all necessary testing is complete the patient will be registered on the local and national (NHSBT-ODT) lists.

Each month the laboratory will issue a report to each referring centre to show which patients are pending entry to the list and the outstanding specimens/data/tests.

As soon as the patient is registered on the transplant list a “Notification of Entry to the List” will be issued to the referring physician stating the patient’s HLA type, antibody status, unacceptable antigens, transplant chance and transplant category. A letter is sent to the patient to notify them when they are first active on the list.
8. Maintenance on the Transplant List

Laboratory Tests

10ml clotted blood samples from each patient on the transplant waiting list must be sent to the Transplantation Laboratory each month for HLA antibody screening and use in crossmatching.

10ml clotted samples must be sent two weeks post blood transfusion accompanied by a request card which includes transfusion information.

Any other tests will be specifically requested in writing by the laboratory as necessary.

Administration

A copy of the transplant list will be circulated monthly to each referring centre, the transplant unit and other individuals as agreed.

A monthly list will be circulated of all patients from whom clotted samples have not been received within the previous three months. A sample from each patient on the list should be urgently sent to the laboratory.

Requests to amend a patient’s status on the transplant list must be notified to the Transplantation Laboratory in writing by FAX (forms available from the Business and Administration Manager) or email (cmm-tr.Histocompatibility@nhs.net) as soon as patient’s suitability for transplantation changes. Local and national lists will be amended Monday to Friday.

9. Transfer of Patients to Other UK Transplant Units

NHSBT-ODT issued new guidelines in April 2006 designed to prevent patients being disadvantaged when transferring from one unit to another in terms of their registration on the national list.

As part of the maintenance of the transplant list the Transplantation Laboratory will notify NHSBT-ODT when a patient is transferring to another transplant centre. In order to do this the referring dialysis centre must supply in writing to the Transplantation Laboratory the name of the patient transferring, the unit to which the patient is transferring and if possible the name of the clinician who will assume responsibility at the receiving unit. In all cases a date from which the transfer is to be effective must be included.

**Until the transfer process is completed the patient remains on the referring unit’s transplant list.** This is to ensure that the patient does not lose any accrued wait time points on the national list.
10. Deceased Donor Crossmatching and Transplantation

Potential recipients for a kidney/pancreas/islets from a deceased donor are identified by NHSBT-ODT under the National Allocation Schemes.

Crossmatching
This is an essential pre-transplant procedure which must be carried out to ensure that the recipient has no donor-directed antibodies that would result in early transplant failure.

Potential recipients will be assessed for their suitability for “virtual crossmatching” and a “Virtual Crossmatch Negative” report issued when appropriate.

Potential recipients are crossmatched using the lymphocytotoxicity crossmatch by which stored patient sera are tested against donor lymphocytes. If the laboratory has not received a clotted blood sample within the previous 4 to 6 weeks then a sample will be requested before the crossmatch proceeds. Regular clotted blood samples should therefore be sent to the laboratory in order to avoid crossmatch delays.

Unsensitised transplant recipients, or patients with low levels of sensitisation will be transplanted on the basis of a negative cytotoxic crossmatch. For recipients with high levels of sensitisation a flow cytometry crossmatch is also required. This test is performed in parallel with the cytotoxic crossmatch.

For patients with a previous failed graft a day of transplant will be requested at the time of organ offer.

If a current recipient sample is requested by the laboratory for crossmatching against a potential donor, a 10ml clotted blood sample should be sent to the laboratory as a matter of urgency.

All crossmatch results are reported to the transplant team verbally and in writing by email.
11. Living Donor Programme

Initial donor selection
Laboratory tests: patients
ABO blood group – Copy results must be sent to the Transplantation Laboratory
HLA type
HLA specific antibody test
For parous female patients - HLA type father(s) of children or HLA type of each child.

Laboratory tests: donor
ABO blood group – Copy results must be sent to the Transplantation Laboratory
HLA Type

Matching Reports
For unsensitised recipients, potential donors will be typed for HLA-A, B, DR. For sensitised recipients, potential donors will be typed for HLA-A, B, C, DR, DQ (DP). An HLA report and a Living Donor Matching Report will be issued within three weeks of specimen receipt. The Living Donor HLA Matching report will indicate the risk factors associated with each potential donor.

Crossmatching
HLA antibody positive patients: Samples for crossmatching may be requested to assess the clinical significance of donor directed antibodies. They will be crossmatched by flow cytometry and cytotoxicity if indicated.
If the crossmatch is negative, then work up can proceed, an immediate pre-transplant crossmatch and antibody screen is also performed.
If the crossmatch is positive, a positive crossmatch report will be issued and the pair may be referred for paired/pooled registration or HLA antibody reduction.
HLA antibody negative patients or patients with fully defined antibody profiles: If patients fulfil the criteria for a “virtual crossmatch” as described previously, then a Virtual Crossmatch Report will be issued when the preliminary crossmatch test is requested. For patients who do not fulfil the criteria a flow cytometry crossmatch and antibody screen will be carried out. A final flow cytometry crossmatch test will be carried out in the week preceding the transplant.

National Living Donor Kidney Sharing Scheme (NLDKSS) Registration Process
ABO incompatible and / or HLA incompatible donor / recipient pairs can be registered with NHSBT-ODT for Paired / Pooled donation.
The forms are completed by the Living Donor Co-ordinator at the referring centre and are then sent to the Transplantation Laboratory for the completion of the H&I data.

Essential tests and information for donor registration
- Donor HLA type (tested on 2 separate occasions)
- Donor ABO blood group
- Surgical review of donor and recipient
- Recipient registration with NHSBT-ODT on deceased donor list

Once all tests are completed and information available the forms are emailed, by the Transplantation Laboratory, to NHSBT-ODT and copied to the Living Donor Co-ordinator for their records.
12. Post-Transplant Follow-up

Transplant recipients may produce HLA specific antibodies associated with transplant rejection.

10ml clotted blood samples should be sent from kidney/pancreas transplant recipients to the Transplantation Laboratory weekly while the patient is on the Transplant Unit and then when a transplant patient attends the follow-up clinic**. Antibody production can then be monitored to identify humoral rejection. Also, a comprehensive immunological profile will be established should the patient require repeat transplantation.

** 10ml clotted blood samples accompanied by a request card must also be sent to the Transplantation Laboratory whenever a diagnostic transplant biopsy is performed.

Islet cell transplant recipients receive Basiliximab as part of the immunosuppressive protocol. Serum samples should be sent to the Laboratory 48 hours, 7, 14 and 28 days post first infusion.
13. Standards

All aspects of the services provided for the cardiothoracic transplant programme are compliant with the relevant standards/guideline.

*BSHI BTS: Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation.*

*HLA specific antibodies in cardiothoracic transplantation: Standardisation of testing, reporting and crossmatch protocols in the UK. (CTAG (13) S9) Sensitised Patients. September 2013.*

*Standards for Histocompatibility Testing Version 7.0 European Federation for Immunogenetics (EFI). January 2018*
As you will be aware, Rituximab is an IgG monoclonal antibody directed against CD20 which is expressed on B lymphocytes. It is therefore an anti-B cell agent and one of the main routes of action is thought to be by complement activation.

In the cytotoxic crossmatch test, donor lymphocytes are incubated with recipient serum. If donor directed HLA specific antibodies are present then the donor cells are killed and this positive result is a contraindication to transplantation.

If a patient is treated with Rituximab then that will be present in their serum and will also cause cell killing in the crossmatch test. That false positive result will deny a patient a transplant that might actually have been compatible. This situation arose recently when a patient’s current serum sample was unexpectedly crossmatch positive. In the absence of information to identify this as a false result, transplantation had to be denied pending further investigation. It transpired that there were no HLA specific antibodies in that sample but the patient had been treated with Rituximab four months previously.

With this in mind, please notify the Transplantation Laboratory when a patient is treated with Rituximab. The solid phase HLA antibody detection and definition tests we employ are not affected by Rituximab. It is possible to increase the frequency of these tests and to use the results alongside information on the patient’s treatment in the interpretation of a crossmatch result. In that way, patients will not be denied a compatible transplant.

Many thanks for your support,

Kay Poulton

Consultant Clinical Scientist
Appendix 2

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.
## Appendix 3  List of Tests and Samples Required

<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 and 10ml clotted blood</td>
</tr>
<tr>
<td>Recipient HLA Typing (Verification HLA type)</td>
<td>5 ml EDTA and 10ml clotted blood</td>
</tr>
<tr>
<td>Recipient HLA-Specific Antibody Screen</td>
<td>10ml clotted blood</td>
</tr>
<tr>
<td>Recipient Autoantibody test</td>
<td>20ml preservative-free heparin or EDTA and 10ml clotted blood</td>
</tr>
<tr>
<td>Potential Living Donor HLA Typing</td>
<td>5ml EDTA blood</td>
</tr>
<tr>
<td>Deceased Donor HLA Typing</td>
<td>10ml EDTA blood</td>
</tr>
</tbody>
</table>
| Crossmatching (Deceased Donor)            | **Donor:** spleen and lymph node samples  
**Recipient:** 10ml clotted blood |
| Crossmatching (Living Donor)              | **Donor:** 40ml preservative free heparin or EDTA and 5ml EDTA blood  
**Recipient:** 10ml clotted blood |
| Recipient Post-Transplant Monitoring      | 10ml clotted blood                                    |
Appendix 4  Renal Transplant Chance Calculation and Estimated Wait Times

The Transplant Chance calculation was introduced to give an indication of the percentage of deceased donors on our database that would fulfil matching criteria for a particular patient. The criteria include ABO identity and 3 or fewer mismatches at HLA-A and HLA-B and no mismatch at HLA-DR. The number of patients on the transplant list continues to increase and consequently there has been an increase in the average wait times.

A review of the transplant list in April 2014 showed that:

<table>
<thead>
<tr>
<th>Median % Chance</th>
<th>Predicted Waiting time (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 – 0</td>
<td>&gt;10</td>
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<tr>
<td>0.2 – 0.1</td>
<td>Up to 10</td>
</tr>
<tr>
<td>0.2 – 0.4</td>
<td>Up to 9</td>
</tr>
<tr>
<td>0.4 – 0.8</td>
<td>Up to 7</td>
</tr>
<tr>
<td>0.8 – 1.0</td>
<td>Up to 5</td>
</tr>
<tr>
<td>&gt; 1.0</td>
<td>Up to 4</td>
</tr>
</tbody>
</table>
Appendix 5  
**Crossmatching for Deceased Donor Renal Transplantation**

The purpose of the crossmatch test is to determine whether a patient has antibodies which indicate sensitisation against the specific donor. The cytotoxic crossmatch test has long been established as the definitive pre-transplantation test to prevent hyperacute rejection.

**Cytotoxic crossmatching**

Patient serum samples are added to donor lymphocytes and incubated. If the serum contains donor-specific HLA antibodies these bind to the donor lymphocytes and cause cell death. This can be visualised using a microscope. All selected potential recipients of deceased donor kidneys and/or pancreata are crossmatched using the cytotoxicity method.

**Flow cytometry crossmatching**

Flow cytometry crossmatching is a more sensitive method of crossmatching and is used as an additional test for patients deemed to be at “higher risk” ie: those with an HLA specific antibody reaction frequency of >50%.

A flow cytometer detects antibody binding to donor lymphocytes using fluorescence. Software is used to calculate whether the result is positive or negative. This test, when required, is conducted in parallel with the cytotoxic crossmatch test.

Patient serum samples are chosen carefully to represent the patient’s screening history. A clotted blood sample from the patient taken within 6 weeks of the crossmatch test must be included and a day of transplant sample.

These tests take on average 3 hours from receipt of donor material or, if required, a clotted sample from the recipient.

**Virtual crossmatching**

A virtual crossmatch report may be issued before crossmatch testing takes place for some eligible patients. This is followed up by a retrospective cytotoxic crossmatch test which is subsequently also reported.

Patients eligible for a virtual crossmatch are those who have been rigorously assessed and defined as having no donor-directed sensitisation.

In some instances HLA antibody positive patients may be considered as suitable for virtual crossmatch. However, these patients are assessed on a case by case basis and may require HLA antibody specificity definition testing prior to a report being issued.

The virtual crossmatch report states that it is essential that the patient has experienced no sensitising events (e.g. transfusion of blood products, infection or pregnancy) since the date of the most recent HLA antibody test result. It is also noted that in 2018, nationally, discrepancies were detected in 0.25% of donor HLA types after organs had been allocated. This poses a risk for sensitised patients progressing to transplant on the basis of a negative virtual crossmatch.

**Samples required for deceased donation crossmatching**

**Recipient**

Serum samples stored by the laboratory are used for crossmatching. A clotted blood sample taken from the recipient taken on the day of transplant is required for the crossmatch test.

**Deceased Donors**

Spleen (or lymph node) for crossmatch and confirmatory HLA type.
Appendix 6

Transplantation Laboratory
Guidelines for Email Communication with the Transplantation Laboratory

Request to List Forms
Judith Spencer
judith.spencer@mft.nhs.uk
Donna Whiteoak
donna.whiteoak@mft.nhs.uk
Gemma Jawando
gemma.jawando@mft.nhs.uk
Marcus Lowe
marcus.lowe@mft.nhs.uk

Letters Relating to Patient Listings
TransplantLab@mft.nhs.uk

Activations/Suspensions
Histo Team Generic email
cmm-tr.histocompatibility@nhs.net

Specific Patient Queries
(Re: samples required / DSA's / status on list etc)
Histo Team Generic email
cmm-tr.histocompatibility@nhs.net
or telephone
67988/67919/6656/6651

Compiled by Judith Spencer/Judith Worthington April 2018
Appendix 7
Guidelines for DSA testing

Background
Donor-Specific HLA-Antibodies (DSA) are associated with allograft rejection and graft failure of transplanted organs. By systematic post-transplant monitoring in kidney, or simultaneous pancreas and kidney transplant recipients, it is possible not only to identify patients at highest risk of immune-mediated graft loss, but also to initiate early intervention strategies to prevent this. In this policy, we aim to provide guidelines for monitoring all recipients post-transplant for the presence of DSA, with recommended additional measures should the clinical post-transplant profile indicate that the patient is at high risk of graft loss. These recommendations have been adapted from Tait et al, 2013 Transplantation Volume 95, Number 1, January 15, 2013 and BSHI BTS: Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation

1. During the First 12 months
   a. Standard Risk Patients (No pre-transplant donor directed antibody)
      Screen for the presence of DSA under the following circumstances:
      i. 12 months after transplantation, unless there is cause for concern. eg
         1. Whenever a significant change in immunosuppression is considered (eg reduction/withdrawal/conversion)
         2. Suspected non-compliance
         3. Graft dysfunction
         4. Before transfer of care to another transplant centre.

   b. Standard Risk Patients (Pre-transplant donor directed antibody detectable by Luminex only)
      i. Monitor for DSA at one month, unless there is cause for concern. eg
         1. Whenever a significant change in immunosuppression is considered (eg reduction/withdrawal/conversion)
         2. Suspected non-compliance
         3. Graft dysfunction
         4. Before transfer of care to another transplant centre.

   c. Intermediate risk Patients (Pre-transplant donor directed antibody detectable by flow cytometry crossmatch)
      i. Monitor DSA weekly for first month. If there is additional cause for concern:
         1. Send samples to the Transplant Laboratory daily during period of concern for accurate monitoring of DSA fluctuations. All samples will be stored and testing will be performed as appropriate after discussion with the laboratory and clinical teams to assess appropriate frequency of testing.
         ii. Monitor monthly if all is well until Month 6

   d. High Risk Patients (Pre-transplant donor directed antibody detectable by complement dependent cytotoxicity)
      These patients are recognised to be at very high risk of early clinical rejection and AMR in the first three months.
      i. Monitor daily for the first three weeks
      ii. Monitor weekly after that until Month 2
      iii. Monitor monthly if all is well until Month 6

2. After 12 months
Monitor annually upon the anniversary of the transplant, unless there is cause for concern as outlined above.