This guide outlines the Histocompatibility and Immunogenetics (H&I) services provided by the Transplantation Laboratory, Manchester Royal Infirmary in support of the cardiothoracic transplant programme. The guide is of use to clinical and support staff in the Wythenshawe cardiothoracic transplant unit.

Next Revision due Apr 2018
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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) Solid Organ Transplantation

The Transplantation Laboratory provides Histocompatibility & Immunogenetics (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) Haemopoietic Stem Cell Transplantation

The Transplantation Laboratory provides H&I support for the haemopoietic stem cell transplantation programmes at Central Manchester Foundation Trust (MRI and RMCH). The laboratory provides both low and high resolution molecular typing of patients and their potential donors who may need a stem cell or bone marrow transplant.

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.

d) Research and Innovation

The Transplantation Laboratory participates in innovative research and innovation relevant to the clinical services provided to ensure that we continually improve our service provision in line with the clinical evidence base. Projects are closely tailored to local clinical practice to ensure the right services are provided at the right time for the right 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 nephrology, dialysis and transplantation 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.

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.

The laboratory has a well-established quality management system (QMS) 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, 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 in anticipation of 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 on 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 Histocompatibility Service.

An 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.

A 24-hour, 365-day on-call service is provided for deceased donor HLA typing and crossmatching and a Consultant Clinical Scientist is similarly 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

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

Consultant Clinical Scientists
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Consultant Clinical Scientist
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Email: natalia.diazburlinson@cmft.nhs.uk
Cardiothoracic Transplant Support Services Enquires
Mrs Amanda Robson MSc, SCRS
Principal Clinical Scientist
0161 276 6656
Email: amanda.robson@cmft.nhs.uk

Mr Patrick Flynn MSc, SCRS
Senior Clinical Scientist
0161 276 6651
Email: patrick.flynn@cmft.nhs.uk

Laboratory Operations and Quality Manager
Julie Kane MSc, SRCS
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
Histocompatibility Team – General Enquiries 0161 276 7988 / 7919 / 6651 / 6656

2.5 Essential Email Addresses
Solid Organ Transplantation Enquiries: cmm-tr.Histocompatibility@nhs.net
SCT Enquiries: cmm-tr.Transplantationlabhsct@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: 780).

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 solid organ transplant programme and the Tissue Typing 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 **3 key identifiers** that should include at least:

- Patient’s name (both 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 and 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 Genito–Urinary Medical Centres or potential bone marrow donors.

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.

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. 780. 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 Tissue Typist on-call via the hospital switchboard (0161 276 1234).

3.5 Acceptance time limit after sample drawing

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Acceptance Time Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotted blood for HLA specific antibody testing</td>
<td>Up to 48 hours</td>
</tr>
<tr>
<td>Heparinised blood for crossmatching</td>
<td>Up to 24 hours</td>
</tr>
<tr>
<td>EDTA blood for HLA typing</td>
<td>No time limit</td>
</tr>
</tbody>
</table>

Heparinised blood samples must be kept at room temperature (22°C -25°C) whilst waiting and during transport to the laboratory. Clots can be kept overnight at 4°C but sent immediately the next morning to the laboratory for testing, these cannot be tested if over 48 hours old. EDTA samples can also be kept overnight at room temperature avoiding any excessive heat exposure.

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 2 for supplier of heparin bottles
See Appendix 3 for Specimen Transportation
See Appendix 5 for List of tests and samples required
4 General Information and Notes on Tests Available

4.1 Descriptions of Standard Tests

What is HLA Typing (Tissue Typing)?
HLA (Tissue) typing refers to the series of DNA based laboratory tests whereby an individual’s HLA genes are characterised and hence the HLA molecules expressed on the surface of their cells identified. HLA molecules are on the surface of all the nucleated cells (i.e. in humans, all cells apart from red blood cells) but for ease of sampling, DNA from peripheral blood cells is routinely used.
The DNA based tests used for HLA typing in this laboratory are referred to as PCR-SSP and LABType SSO.
*The sample used for HLA typing is 5ml EDTA blood.*

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 and cause transplant rejection. It is therefore crucially important that patients awaiting transplantation are screened for the presence of antibodies and that the specificities of the antibodies are defined.
*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 event (e.g. blood transfusion).* 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.
*The sample used for antibody testing is 10ml clotted blood.*

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.
*The sample used for pregnancy related sensitisation is 5ml EDTA blood from the patient’s children or their father.*
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.

The samples used for “auto-crossmatching” are 20ml heparinised and 10ml clotted blood.

Transplant recipients can produce HLA specific antibodies associated with transplant rejection. Samples for donor specific antibody testing to support the diagnosis of rejection should be sent when clinically indicated

The sample used for donor specific antibody (DSA) testing is 10ml clotted blood.

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.

A “virtual crossmatch” can be performed pre-transplant for selected patients, whereby the donor HLA type is reviewed against the patient’s HLA antibody profile to determine whether the patient has donor-directed antibodies that would cause a positive crossmatch test result. This is the basis of the current 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 result is available. Potential recipients will be assessed for their suitability for “virtual crossmatching” and a “virtual crossmatch negative” report issued when appropriate. It must be noted that the validity of a virtual crossmatch result for a sensitised patient depends on the donor HLA type being correct. In 2013, nationally, discrepancies were detected in 0.7% of donor HLA types after the organs had been allocated.

The purpose of this approach is to reduce the cold ischaemia time without compromising the safety of transplantation. For these recipients the crossmatch test will be performed retrospectively.
Some humanised monoclonal antibodies used as treatment options may interfere with assays used in the laboratory particularly the crossmatch assays (See Appendix 1) Please ask for advice if you have any concerns

**Recipient samples used for crossmatching are:**

- **Cytotoxic crossmatching** – stored sera from the monthly clotted samples including one from the previous 4 to 6 weeks.
- **Flow cytometry crossmatching** – stored sera including one from the previous 4 to 6 weeks
- **Donor samples required for crossmatching are:**
- **Deceased donor** – lymphocytes isolated from donor spleen or lymph node
5. Requesting Tests/Samples Required

The Transplantation Laboratory has its own distinctive request cards which can be obtained available from the Admin manager. All requests should be made using the request cards following the procedure described below for the test required.

5.1 HLA (Tissue) Typing

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 second referral send 5ml EDTA blood for verification typing and 10ml clotted blood for antibody screening.

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**

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| THE TRANSPLANTATION LABORATORY, MANCHESTER ROYAL INFIRMARY |
| TEL: 0161 276 6397    FAX: 0616 276 6148 |

<table>
<thead>
<tr>
<th>Surname*</th>
<th>Forename*</th>
<th>Date of Birth*</th>
<th>Sex</th>
<th>Hospital*</th>
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</thead>
<tbody>
<tr>
<td>Hospital Number*</td>
<td>NHS Number</td>
<td>Requested By*</td>
<td>Consultant*</td>
<td>Ward</td>
</tr>
<tr>
<td>YES</td>
<td>NO</td>
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<th>No. Pregancies</th>
<th>Ethnic Origin</th>
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<tr>
<td>☐ Recipient ☐ Heart ☐ Single Lung</td>
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<table>
<thead>
<tr>
<th>Tests Required*</th>
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</thead>
<tbody>
<tr>
<td>☐ HLA Typing ☐ Send 5ml EDTA Blood</td>
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<table>
<thead>
<tr>
<th>Cytotoxic Antibodies</th>
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<tr>
<td>☐ Send 10ml Clotted Blood</td>
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<tr>
<th>Urgent Thoracic Assessment</th>
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<tbody>
<tr>
<td>☐ Send 20ml Heparin Blood + 5ml EDTA Blood + 10ml Clotted Blood</td>
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<table>
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<tr>
<th>Donor Prospective Crossmatch</th>
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</thead>
<tbody>
<tr>
<td>☐ Send 20ml Heparin Blood + 5ml EDTA Blood</td>
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</table>

* Essential Information Required In Order To Process Request*
5.2 HLA Antibody Screening

Routine monthly samples from patients on the transplant list or post transplant must be accompanied by a request card and tubes must be clearly labelled with patient name, date of birth, hospital number and date of sample collection.

Samples from new patients cannot be accepted without a request card – see an example below.

Samples can be received within 48 hours Monday to Friday and sent by 1st class post in appropriate packaging. (See Appendix 3)

Send 10ml clotted blood

THE TRANSPLANTATION LABORATORY, MANCHESTER ROYAL INFIRMARY
TEL: 0161 276 6397    FAX: 0616 276 6148

<table>
<thead>
<tr>
<th>SURNAME*</th>
<th>FORENAME*</th>
<th>DATE OF BIRTH*</th>
<th>SEX</th>
<th>HOSPITAL*</th>
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</tr>
<tr>
<td>HOSPITAL NUMBER*</td>
<td>NHS NUMBER</td>
<td>REQUESTED BY*</td>
<td>CONSULTANT*</td>
<td>WARD</td>
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<td>Rhesus___________</td>
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<td></td>
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<tr>
<td>DATE_____</td>
<td>No. UNITS_____</td>
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<tr>
<th>DIAGNOSIS*</th>
<th>CYTOTOXIC ANTIBODIES</th>
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</thead>
<tbody>
<tr>
<td>☐ RECIPIENT</td>
<td>SEND 10ml CLOTTED BLOOD</td>
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<table>
<thead>
<tr>
<th>DONOR</th>
<th>HEART/LUNG</th>
<th>DOUBLE LUNG</th>
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</thead>
<tbody>
<tr>
<td>☐</td>
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TESTS REQUIRED*:

<table>
<thead>
<tr>
<th>HLA TYPING</th>
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<tbody>
<tr>
<td>☐ SEND 5ml EDTA BLOOD</td>
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</tbody>
</table>

* ESSENTIAL INFORMATION REQUIRED IN ORDER TO PROCESS REQUEST
5.3 Crossmatching

**Deceased organ donor samples**
Donor spleen and lymph node specimens should be taken by the retrieval surgeon and sent by taxi to the Transplantation Laboratory the next working day. A time of transplant 10ml clotted blood sample from the recipient should be sent at the same time as the donor material. The on-call tissue typer must be notified of any transplant due to take place so that the samples will be expected. The on-call tissue typer is contactable by bleep via Manchester Royal Infirmary switch (0161 276 1234)

5.4 Post-transplant follow-up

**Donor specific antibody (DSA) testing**
Transplant recipients can produce HLA specific antibodies associated with transplant rejection. A post-transplant antibody monitoring service is available, including testing for donor directed antibodies when requested. Post-transplant samples for donor specific antibody testing to support the diagnosis of antibody-mediated rejection must be accompanied by a request card.

Antibody production can be monitored and a comprehensive immunological profile established should the patient require repeat transplantation.

**Send 10ml clotted blood**
6. Reporting of Results

To maintain patient confidentiality and comply with the Data Protection Act and other legal requirements all results are reported electronically only to authorised individuals. They are signed by a Consultant Clinical Scientist or named deputy, with the exception of urgent deceased donor / recipient crossmatching results that are reported verbally and in writing by the person performing the tests following verbal authorisation from the on-call Consultant Clinical Scientist. 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.

**HLA typing results** for heart and lung transplant patients. 90% of results are reported within **8 working days** of correct specimen receipt.

**HLA antibody screening results** for heart and lung transplant. 90% of results are reported within **15 working days** of correct specimen receipt. When HLA specific antibodies are identified the report will be delayed for up to 5 additional working days while further tests are performed to help identify HLA specificities.

**Unacceptable antigens.** Any HLA specificities that are identified will be reported as unacceptable antigens and should be absent from any potential donor HLA type or included within any virtual crossmatch risk assessment. The virtual crossmatch is explained on the next page under **Donor crossmatching**.

**Urgent assessments.** When a patient requires listing urgently the HLA antibody status of a patient can usually be reported within **5 hours** of correct specimen receipt. The Transplantation Laboratory must be informed of such clinically urgent patients as soon as possible.

**Deceased Donor / Recipient Crossmatch** results are reported to the transplant co-ordinator, 90% within **6 hours of receipt of crossmatch specimens**.

**Donor Specific Antibodies** results will be reported as “Cardiothoracic Transplant Recipient – Post Transplant Antibody Investigations” report usually within **4 working days of receipt** or if requested for urgent assessment within **5 hours**.

**Transplant waiting list**

Copies of the transplant waiting list held by the Transplantation Laboratory will be sent monthly to the transplant coordinators for their use. Any amendments to the list should be noted on a copy and returned to the Transplantation Laboratory. In addition, a list of patients who have been assessed for transplant but not yet listed is sent to be amended as necessary.
Donor crossmatching

A prospective crossmatch may be required when a potential recipient has HLA specific antibodies that are not clearly defined. A lymphocytotoxic crossmatch is done in this case where stored patient sera are tested against donor peripheral blood lymphocytes. If the laboratory has not received a clotted blood sample within the previous 3 months or the patient has experienced a potential sensitisation event since the date of the last serum sample tested a 10ml clotted blood sample should be sent to be included in the crossmatch.

Virtual crossmatching

Non sensitised recipients can be transplanted without a prospective crossmatch. The transplant unit must be able to confirm that no potential sensitisation events have occurred since the date of the last serum sample tested for HLA antibodies. 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 have been shown to be absent from the donor HLA type. Such patients and their unacceptable antigens will be highlighted on their antibody screening report. None of these patients should be transplanted without first contacting the Laboratory or, out of hours, the Clinical Scientist on call to confirm the suitability of the donor.

Mean Fluorescence Intensity (MFI) Risk Assessment

When donor directed HLA specific antibodies are identified, the level of risk associated with proceeding to transplant can be assessed on request. The level of risk is determined by calculating the cumulative MFI value of the defined donor directed antibodies as detected by Luminex bead assays following national CTAG guidelines. (Appendix 5) The transplant unit must be able to confirm that no potential sensitisation events have occurred since the date of the last serum sample tested for HLA antibodies.

A flow diagram outlining the process used to provide a virtual crossmatch result and a risk assessment can be seen in (Appendix 6)

All crossmatch results are reported to the on-call Transplant Co-ordinator verbally and in writing by encrypted email.
Dear Colleagues

Please can I bring to your attention the fact that Rituximab interferes in the crossmatch test. 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.

Yours sincerely

Kay Poulton

Consultant Clinical Scientist
Appendix 2

Supplier of heparin bottles:-

B.D. Vacutainer (Ref 368480)
10ml sodium heparin tubes (preservative free) 100/pack

HM& S
Brook House
4 The Lakes
Bedford Road
Northampton
NM4 7YD

Appendix 3

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.
### 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 referral)</td>
<td>5ml EDTA and 10ml clotted blood</td>
</tr>
<tr>
<td>Recipient HLA Typing (2nd set of bloods)</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 and 10ml clotted blood</td>
</tr>
<tr>
<td>Patient relative HLA Typing</td>
<td>5ml EDTA blood</td>
</tr>
<tr>
<td>Donor HLA Typing</td>
<td>20ml preservative free heparin and 5ml EDTA blood</td>
</tr>
<tr>
<td>Crossmatching</td>
<td>Donor: spleen and lymph node samples</td>
</tr>
<tr>
<td></td>
<td>Recipient: 10ml clotted blood</td>
</tr>
<tr>
<td>Recipient Post Transplant Monitoring</td>
<td>10ml clotted blood</td>
</tr>
</tbody>
</table>
### Appendix 5

**MFI risk level definitions using LABScreen Single Antigen beads as defined by national CTAG guidelines:-**

<table>
<thead>
<tr>
<th>Risk Level</th>
<th>Cumulative MFI</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>MFI = 0</td>
<td>No detectable antibody. Standard risk</td>
</tr>
<tr>
<td>Level 2</td>
<td>MFI &lt; 2,000</td>
<td>Minimum risk of hyperacute rejection but greater than standard risk of rejection</td>
</tr>
<tr>
<td>Level 3</td>
<td>MFI 2,000 – 5,000</td>
<td>Low risk of hyperacute rejection but significant risk of early rejection and antibody mediated graft damage. Immediate pre-transplant antibody reduction advised.</td>
</tr>
<tr>
<td>Level 4</td>
<td>MFI &gt; 5,000</td>
<td>Transplant veto apart from exceptional cases</td>
</tr>
</tbody>
</table>

**Patients with IgM antibodies only**

- MFI < 5000 negative
- MFI ≥ 5000 < 10000 listed as unacceptable - Risk level 2
- MFI ≥ 10000 listed as unacceptable - Risk level 3

All MFI data are based on current (most recent sample) values.
Appendix 6.

Virtual crossmatch flow chart used by the on call Clinical Scientist.

Does patient have unacceptable antigens listed?

Yes

Are the antibodies donor directed?

Yes

Calculate cumulative MFI and assign risk level.

Discuss with the Consultant Scientist.

Issue a verbal report

No

Issue a verbal report

No

Email a negative virtual crossmatch report.

Yes

Discuss with the Consultant Scientist.

Issue a verbal report

Email a risk assessment report.
Appendix 7.

Overview of the cardiothoracic organ transplant support service.

**PATIENT REFERRED FOR TRANSPLANT**

1. **1st 5ml EDTA Blood**
   - HLA-A, -B, Cw, -DR, -DQ by PCR-SSP/SSO

2. **2nd 5ml EDTA Blood**
   - HLA-A, -B, -DR, by PCR-SSP/SSO

3. **Urgent assessment only 20ml Heparinised Blood**
   - Antibody Screening
   - Stored for Auto crossmatch if needed

4. **1st 10ml Clotted Blood**
   - Antibody Screening
   - 2nd 10ml Clotted Blood

5. **10ml clotted blood every 3 months and / or 14 days following any potential sensitisation event**

**TRANSPLANT WAITING LIST**

- HLA antibody positive patient
  - Crossmatch test positive or unacceptable antigens present
  - Virtual crossmatch negative, unacceptable antigens avoided or crossmatch test negative
  - MFI risk level assessment on request

- HLA antibody negative patient
  - Potential Donor

**TRANSPLANT**

**POST TRANSPLANT MONITORING**