Royal Brompton and Harefield hospitals

NHS

Laboratory Medicine

Tissue Typing Laboratory

TISSUE TYPING LABORATORY

USER GUIDE

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Histocompatibility & Immunogenetics

Contents

1 Introduction

1.1 This Guide

This guide outlines the Histocompatibility & Immunogenetics (H&I) services provided by the Tissue Typing Laboratory of the Royal Brompton & Harefield Hospitals, part of Guy's & St Thomas' NHS Foundation Trust. The guide is of use to medical and scientific staff throughout the Trust, particularly within the Transplant unit. The guide contains information about the organisation of services and contact details for key members of staff.

1.2 Service Overview

The Tissue Typing Laboratory is located on the Ground Floor of the Heart Science Centre, at Harefield Hospital. Laboratory opening hours are from 9.00am to 5.00pm Monday to Friday.
The Tissue Typing Laboratory is committed to providing a service of the highest quality in line with standards outlined by ISO 15189:2012 and the European Federation for Immunogenetics (EFI). All work is carried out within the framework of a documented quality system, according to good laboratory and good manufacturing practice (GLP and GMP, respectively). Techniques and procedures are validated, described in standard operating procedures (SOP), and conducted by staff whose proficiency is regularly monitored. The laboratory participates in external quality assurance schemes such as UK NEQAS for H&I.

1.3 Service Agreements

The laboratory does not have any formal contractual agreements. The contractual arrangement between the laboratory and its users is defined by the laboratory request form that is completed.

Each request form, together with its relevant primary samples, is checked for conformity with the laboratory's labelling requirements detailed later in this guide. If the form or samples do not meet these requirements then the request will be rejected, the user notified of this and a repeat sample requested.

1.4 Complaints/Compliments

The laboratory is committed to continuously improving the quality and range of services provided and welcomes any comments or suggestions from the service users. There is always the risk of failures in any service delivery and it is essential that these be reported to decrease the chance of recurrence, for improving the service and for compliance with clinical governance policy. Please do not hesitate to discuss complaints with the Head of Laboratory.

1.5 Data Protection

The H&I laboratory is supported by in house computer systems which store all relevant patient information. The laboratory and its staff comply with the General Data Protection Regulation (GDPR) 2018, information is given on a need to know basis for clinical care purposes only and confidentiality is respected at all times.

1.6 Consent

It is the responsibility of the requester to ensure that any samples sent to the laboratory have been taken with full informed consent for the tests being requested. Patients/donors should be informed that any residual material of a sample may be stored as part of required archiving protocols or to enable further investigation for the benefit of the individual. They also must be informed that excess

surplus material may be used anonymously for quality control purposes, service development or education, and / or ethics committee approved research projects.

2 Background

2.1 HLA Typing

Tissue typing, or HLA typing, is the process which identifies the HLA molecules expressed on the surface of cells in the human body. HLA genes are extremely variable and this results in many different HLA antigens, or molecules. The HLA antigens are divided up into groups denoted by letters, for example HLA-A, -B and –C are HLA class I molecules and are found on all nucleated cells including T lymphocytes. Class II molecules (HLA-DR, -DQ and –DP) are distributed on fewer cell types, for example B lymphocytes, macrophages and endothelial cells. The subdivisions of each group (the individual gene specificity) are denoted by numbers e.g. HLA-A 1, or HLA-DR4 and it is the combination of molecules on the surface of the cells, determined genetically, which gives an HLA type. We inherit one HLA antigen from each group, from each of our parents, so an individual HLA type might be for example, HLA-A 1, A25; B 8, B44; Cw5, Cw7; DR 4, DR 17; DQ 2, DQ7.

These HLA molecules enable the cells of the immune system to differentiate between 'self' and 'non-self' so that an immune response can be specifically directed against 'non-self' i.e. foreign organisms, in the case of infection. In the transplant situation the graft will display donor HLA molecules which are recognised as being foreign, ie 'non-self'. Cells of the immune system will react against them as if they were an infecting agent, which could lead to rejection of the graft. HLA typing of recipient and donor cells is therefore carried out to identify how well the HLA molecules are matched, in theory giving an indication of the severity of possible rejection processes. In thoracic organ transplantation HLA matching has been shown to improve graft survival, with most importance being placed on matching for HLA-DR antigens (ref: Smith *et al*. Lancet 1995; 346: 1318).

2.2 HLA Antibody Screening

Any exposure to non-self HLA antigens, such as through transplantation, transfusion or pregnancy, can stimulate the production of HLA-specific antibodies. These can vary in their potency and persistence depending on the nature and number of stimulating events but represent a significant risk of graft failure. All patients on a transplant waiting list should therefore be monitored regularly for the presence of HLA-specific antibodies. HLA-specific sensitisation is best investigated by serological analysis for antibodies.

Much of the work in this laboratory therefore involves testing patient serum for the presence of any HLA specific antibodies and it is our policy always to determine if a patient has preformed HLA antibodies prior to transplantation which could lead to early rejection of a graft (Smith *et al* Transplant Immunology 1993; 1: 60-65, Smith et al American Journal of Transplantation 2007 7:2809-2815). For prospective cardiothoracic transplant patients, the recommendation is that each patient should be tested for HLA antibodies on the first assessment visit. A second sample must be tested before the patient can be listed for transplant. Whilst on the waiting list patients should be tested at least three monthly and after each potential sensitising event (BSHI/BTS Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation, 2014). The clinical teams must inform the laboratory of potential sensitisation events such as previous transplantation, transfusion of blood products and pregnancy. Patient serum samples must be obtained following transfusion of blood products, this will optimally be between two and four weeks after the transfusion. If the patient is already on the waiting list it may be necessary to test twice a week for the first 3 weeks after the sensitising event. All antibody positive sera will be characterised for specificity for known HLA A, B, C, DR , DQ and DP antigens. For some sera (i.e. those from highly sensitised patients, reacting with over 80% of the donor population) this may require successive testing by increasingly sensitive and specific techniques. In such cases the completion of testing may

take significantly longer than for less complex cases.

Patient serum is also monitored for HLA specific antibodies after transplant. For any transplant, if rejection is suspected a test for donor-specific antibodies can aid a diagnosis of rejection and indicate a course of management. Such testing can be performed on demand during normal working hours and by prior arrangement with the laboratory. It has been agreed with the transplant unit that routine HLA antibody testing should be performed post-transplant at 1-month, 3-months, 6-months, 12-months and annually thereafter. Additional or more frequent testing should also be specifically requested for cases of suspected rejection or other clinical indications.

2.3 Crossmatching

If present at a high concentration, patient antibodies corresponding to donor mismatched HLA will cause immediate and irreversible rejection of all forms of organ transplants, with the exception of the liver. Performing a prospective serological crossmatch between donor and recipient obviates the risk of such hyperacute rejection. For cardiothoracic transplants, where extended cold ischaemia time has an unacceptable influence on transplant outcome the crossmatch is performed retrospectively. For sensitised patients it is essential that either a prospective crossmatch or a 'virtual crossmatch' is performed prior to transplantation. The latter requires that the laboratory obtain the donor HLA type from the Organ Donation and Transplantation Directorate of NHSBT, Tissue Typing staff can then determine if the recipient has previously produced HLA antibodies to those donor HLA antigens which could adversely affect the transplant. In some cases the results of crossmatch tests are complex, particularly in patients with historically high levels of antibodies which have since decreased. Specialised interpretation of these results is necessary to determine their clinical significance. Advice on specific cases can be provided by senior scientists within the laboratory, as required.

Note: It is possible, although unlikely that there may be inaccuracies within a donor HLA type provided by NHSBT-ODT which could clearly affect any virtual crossmatching procedure. All UK laboratories perform well in EQA schemes and numerous checks are made throughout the donor HLA typing process to try and ensure that the potential for errors is minimised but this is still a possibility which cannot be overlooked.

2.4 Desensitisation

Post-transplant antibody removal is termed desensitisation. Desensitisation is achieved by extracorporeal antibody removal using various techniques, such as Plasma Exchange or Immunoadsorption. During the desensitisation process, antibody removal should be monitored so that the effectiveness of the process can be assessed.

3 Laboratory Details

3.1 General

Laboratory extension: 85774

Office extension: 85863

Direct line: 01895 828774

Normal working hours: Monday to Friday: 9.00 - 17.00

24-hour emergency service:

An out of hour's service is provided for thoracic organ transplantation. Contact the Tissue typing mobile telephone via switchboard.

3.2 Minimum requirements for sample acceptance:

The Trust places great emphasis on accurate patient identification and correct specimen labelling and request form completion. The attention of users is therefore drawn to the requirements set out in the Trust-wide Laboratory Medicine Policy POLA0004 - Policy for Request form completion, patient identification, and labelling of Lab Med specimens' which can be found on the Trust intranet. If the form or samples do not meet these requirements then the request will be rejected, the user notified of this and a repeat sample requested.

The minimum requirements are detailed below:

Sample tube:
- Name
- Hospital Number
- Date of Birth
- Date of Sample

Request form: Name
- Hospital number
- Date of Birth
- Ward of Origin
- Consultant to whom report should be addressed
- Date of sample collection
- Sensitisation details (pregnancies, transfusions & surgery)

Routine samples are generally accepted 9.00 to 16.00 Monday to Thursday and 9.00 to 15.00 on Friday.

NB. It is laboratory policy not to process unlabelled samples.

3.3 Clinical Advice and Result Interpretation

Advice on the interpretation of patient results may be obtained weekdays 9:00 am to 5:00 pm from Dr Paul Brookes (Ext 85886) or Ms Anna Danskine (Ext 83255). At all other times, advice is obtainable from the on-call clinical Tissue Typist (contact via Switchboard).

4 Services Provided

4.1 For Heart and/or Lung Transplantation:

1. HLA Antibody profiles prior to patient being accepted onto the transplant waiting list and regular antibody screening whilst on the waiting list.
2. HLA -A, -B, -DR typing of patients accepted onto the waiting list.
3. HLA typing of sensitised patients for the relevant HLA loci
4. Prospective or virtual crossmatching with potential donors for patients with detectable HLA antibodies.
5. Retrospective HLA-A, -B, -C, -DR, -DQ , -DP typing of donors and HLA-C, -DQ and -DP typing of recipients.
6. Retrospective crossmatching for all patients with their donors
7. Post-transplant anti-HLA antibody profiles.

4.2 For Lung Lobe Transplantation using a Live Donor:

1. Antibody profiles and regular screening are provided as above.
2. Prospective HLA class I and class II typing is performed for the patient and all potential donors.
3. Prospective crossmatching with suitable donor(s) using peripheral blood lymphocytes.
4. Post-transplant antibody monitoring is provided as above.

5 Techniques

5.1 HLA Typing

DNA based typing of HLA class I (A, B & C) and HLA class II (DRB1, DRB345, DQA, DQB, DPA & DPB) specificities is carried out using reverse Sequence Specific Oligonucleotide probes (SSO). HLA Typing is performed to determine the HLA-A, -B, -C, -DR, -DQ and -DP types of recipients and donors for thoracic organ transplantation. Molecular techniques used have been fully validated as part of the participation in national quality assurance schemes.

Sample contaminants including heparin and ethanol are known inhibitors and can interfere with the PCR reaction.

5.2 Screening for HLA class I and class II Antibodies

HLA antibody screening is performed to determine the level of sensitisation and specificity of class I and class II reactive antibodies present in a patient's circulation. This provides a guide to the probability of a recipient being sensitised to potential donor HLA antigens in the UK donor population and is therefore a requirement prior to transplantation. The presence of HLA antibodies with a clearly defined specificity, or combination of specificities, in the sera will exclude patients from receiving a donor expressing the HLA specificities with which the antibodies are reactive. This is determined by performing a virtual crossmatch prior to the transplant. Patients with antibodies which are poorly defined will require a prospective or virtual crossmatch prior to transplantation.

The presence of antibodies reactive with HLA class I (A, B & C) and class II (DR, DQ & DP) antigens is primarily determined using a Luminex based system for IgG antibodies. If the screening is positive, further tests are carried out to identify the specificity and/or Ig class of the antibodies.

Some patients, particularly patients with in situ mechanical circulatory support such as left ventricular assist devices (LVAD) can have high levels of antibodies to albumin or other targets which can cause high backgrounds in Luminex assays. In these cases patient sera must be pre-treated which will increase the reporting time.

5.3 Donor Crossmatching

The lymphocytotoxic crossmatch is a complement dependent cytotoxicity assay that detects complement-fixing antibodies reactive with donor lymphocytes, in the patient's sera. A positive crossmatch will therefore indicate that the patient has pre-formed cytotoxic HLA antibodies directed against the donor HLA antigens and which are known to be associated with poor graft outcome in thoracic organ transplantation. A negative crossmatch will indicate that there are no such antibodies present. The flow crossmatch is a more sensitive test than the lymphocytotoxic crossmatch detecting antibodies binding to donor T and B cells. This test may be carried out for patients who have preformed antibodies prior to transplantation. Furthermore, prior to transplantation, when preformed HLA antibodies have a clearly defined specificity, the on-call tissue typist will ascertain if a donor is suitable by liaising with the ODT prior to transplantation. A virtual or prospective donor crossmatch will be carried out as necessary. If antibody screening results are not in the patient notes or on EPR prior to transplant, these are available from the on-call tissue typist.

It is laboratory policy to always perform a retrospective crossmatch with recipient sera against donor T, B and mixed lymphocytes. Crossmatching is performed on the next working day following transplant and historical sera, including a sample taken within the 24 hours prior to transplant, are used in the procedure.

It is known that some antibody treatments such as Rituximab or preparations containing antibodies including ATG can cause positive reactions in the CDC and/or flow cytometry assays. Rituximab will cause positive IgG B cell reactions and ATG positive IgG T cell reactions. Other monoclonal antibody preparations may also cause interferences in both assays.

6 Sample Requirements

Please make sure all samples are clearly labelled.

Patient status	Tests required	Sample type
New Tx Assessment	HLA Typing Antibody Screening	4 x 4.5ml Cit.Na tube (pale blue top) 2 x 6ml CAT tube (red top
Repeat Assessment	Antibody Screening	2 x 6ml CAT tube (red top
Transplant Recipient (Taken within 24hrs prior to transplant)	Crossmatching and Antibody Screening Auto-Crossmatching	2 x 6ml CAT tube (red top 4 x 4.5ml Cit.Na tube (pale blue top) Note: keep at room temperature
Live Lung Recipient	Crossmatching and Antibody Screening HLA typing and Auto Crossmatching	2 x 6ml CAT tube (red top 4 x 4.5ml Cit.Na tube (pale blue top)
Live Lung Donor	HLA typing and Crossmatching	4 x 4.5ml Cit.Na tube (pale blue top)
Post-Transplant Monitoring	Antibody Screening	2 x 6ml CAT tube (red top

Urgent HLA antibody screening can be performed, pre- or post-transplant only by prior arrangement with the laboratory.

7 Specimen Transport

Internal samples from wards are collected from the wards and require no special packaging/transportation.

For packaging and transport to the laboratory of external specimens please refer to the Trust –Wide Laboratory Medicine Policy POLA0005 - Policy and Procedure for Transport of Specimens by Porter, Van, courier or by Post, which can be found on the Trust intranet.

Please note: It is the responsibility of the sender to ensure that all samples are packaged in accordance with the current European Agreement concerning The Carriage of Dangerous Goods and Use of Transportable Pressure Equipment Regulations 2009, in order to prevent breakage or spillage in transit.

8 Results

HLA screening and post-transplant crossmatching results are uploaded to EPR on authorisation. If reports are to be sent elsewhere please mark this on the request form and detail clearly the address to which they should be sent.

HLA antibody screening reports will usually be sent out within 10 days following receipt of the sample. Transplant crossmatch results will be sent out within 5 days of transplant although they should be available on EPR within 48 hours following the crossmatch. It is sometimes necessary to perform further analysis on certain samples and these may therefore take longer. If more urgent results are required please contact the laboratory.

8.1 Target Turnaround Times

HLA antibody screening – 90% of reports to be sent out within 14 days of sample receipt.

Donor Crossmatch – 90% of reports to be sent out within 5 days of transplant. A verbal result will be available within 48 hours of transplant.

Donor HLA typing report – 90% of reports to be sent out within 21 working-days .

These targets are audited on a monthly basis and reviewed annually.

It is laboratory policy that every effort will be made to provide accurate and informative results as quickly as possible.

9 Enquiries

General enquiries should be directed to the laboratory on extension 85774.

If you have any further queries or comments regarding the service for Histocompatibility and Immunogenetics at Harefield Hospital, please contact:

Dr. Paul Brookes on extension 85886 or direct line 01895 828886.