Oxford University Hospitals
NHS Foundation Trust

Transplant Immunology & Immunogenetics Laboratory Handbook November 2024

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Introduction to the Transplant Immunology & Immunogenetics Laboratory

General Profile

The Transplant Immunology and Immunogenetics Laboratory is a consultant-lead specialist regional service for clinical transplantation. Transplantation currently encompasses deceased and living donor renal allografts, haematopoietic stem cell transplants, combined kidney/pancreas transplants, pancreas alone, isolated pancreatic islet transplants, small bowel, modified multi-visceral transplants, and uterine transplants. There is an active programme for antibody incompatible renal transplantation. Currently, the laboratory services include HLA typing to the DNA sequence level, HLA antibody detection and definition, and crossmatching. The laboratory provides a 24-hour on-call service for transplantation. Immunogenetics services are also provided to clinicians to define disease susceptibility genes as an aid to patient diagnosis and treatment. The laboratory is accredited by UKAS to ISO 15189 and also to the European Federation for Immunogenetics (EFI) Standards for Histocompatibility and Immunogenetics Testing and, in order to maintain the highest standards of service in a continually developing field, supports research and development in histocompatibility and immunogenetics testing and in the broader field of clinical transplantation.

Contact Numbers

Location	Number
Director of Clinical Transplant Immunology & Immunogenetics Consultant Clinical Scientist: Martin Barnardo, PhD, FRCPath.	01865 (2)26104
Deputy Director of Clinical Transplant Immunology & Immunogenetics Clinical Scientist: Mian Chen, BMed, MSc, DipRCPath.	01865 (2)25542
Head of Solid Organ Transplantation Section Quality Manager Clinical Scientist: Jeanette Ayers, MSc, DipRCPath.	01865 (2)25521
Head of Haematopoietic Stem Cell Transplantation Clinical Scientist: Maggie Sutton, BSc.	01865 (2)26165
Head of Molecular Clinical Scientist: Hannah Docker, BSc, DipRCPath.	01865 (2)25521
General Office	01865 (2)26102
Main Laboratory	01865 (2)26169
Post PCR laboratory	01865 (2)26163

Email addresses:

For haematopoietic stem cell transplants related enquiries - handi@ouh.nhs.uk

For all other enquiries – transplant.immunology@ouh.nhs.uk

Laboratory address:

Transplant Immunology & Immunogenetics Laboratory
Oxford Transplant Centre
Churchill Hospital
Oxford, OX3 7LE

Laboratory Opening Hours
Laboratory opening times: 08.00-16.30, Monday to Friday.

Advice on Requesting Assays and Interpretation of Results
Advice can be sought by contacting the laboratory on 01865 226102 where all staff members will endeavour to assist you. Consultant advice in Transplant Immunology & Immunogenetics is also available via the number above.

Out of hours Service
The Histocompatibility and Immunogenetics services in support of transplantation are provided 24 hours a day, 7 days a week. The services include HLA typing, and crossmatching and are no different from those provided during the normal working day. An on-call scientist is always available out-of-hours and can be contacted by mobile telephone through the Churchill Hospital switchboard (Tel: 01865 741841; Internal: 55000). Consultant advice in Transplant Immunology & Immunogenetics can be obtained out-of-hours through contacting the on-call scientist.

Training and Registration
The laboratory participates in the National Scientist Training Programme (STP) managed by the National School of Healthcare Sciences. In addition, the laboratory has a Training Programme approved by the British Society for Histocompatibility and Immunogenetics (BSHI). Trainee Clinical Scientists are encouraged to work towards either the STP qualification or BSHI Diploma depending on their post, and senior staff towards Fellowship of the Royal College of Pathologists. Clinical Scientists are registered with the HCPC.

Quality Assurance
The quality of services provided by the Transplant Immunology & Immunogenetics Laboratory is internally assured and assessed externally by UK NEQAS (National External Quality Assessment Scheme) for Histocompatibility and Immunogenetics.

The laboratory is registered for the following schemes:

1B	HLA-B27 testing
2B	Crossmatching by Flow Cytometry
3	HLA antibody Specificity Analysis
4A1	DNA HLA Typing at 1st Field Resolution
4A2	DNA HLA Typing at 2nd Field Resolution
6	HLA Antibody detection
7	HLA-B*57:01 Typing for Drug Hypersensitivity

The laboratory is also registered for the TPMT scheme provided by the External quality Control of diagnostic Assays and Tests (ECAT) Foundation / RfB (Reference Institute for Bioanalytics, a German proficiency testing organisation)

Copies of the participation certificates and performance levels are available upon request.

Accreditation
The laboratory is accredited by the United Kingdom Accreditation Service (UKAS) to ISO 15189:2012 [laboratory number 8758] and the European Federation of Immunogenetics (EFI).

Request Forms
Request forms can be obtained from Request for Laboratory Supplies (gp.ordering@ouh.nhs.uk) or directly ordered from our supplier: S.F. Taylor & Company Ltd, product code: OUH_RTH0020

Alternatively, we can email a PDF copy of the request card – please contact the laboratory.

Patient consent

The tests performed by the Oxford Transplant Immunology Laboratory do not require a specific consent from patients. Consent is inferred when the patient willingly submits to the sample collection procedure for example by venipuncture as part of their clinician-led pathway.

Consent for samples from deceased donors is through the SNOD (Specialist Nurse for Organ Donation) and complies with NHSBT OTDT processes and regulations.

Kidney, Kidney/Pancreas, Pancreas alone, Islet, Modified Multi-Visceral, Uterine, Small Bowel and Abdominal Wall Transplantation - Overview

We provide the following services for patients requiring kidney, kidney/pancreas, pancreas alone, islet cell, kidney/islet cell, modified multi-visceral, small bowel, uterine and abdominal wall transplantation:

HLA typing of patients, living and deceased donors and female patients' partners

All individuals are HLA typed using either PCR-SSP/NGS/qPCR methods as appropriate.

Living donor matching and selection service

Patients and their donors are HLA typed twice, on separate blood samples, prior to transplant.

Patient registration on local and national transplant list (TL) held at Organ Donation and Transplantation, NHS Blood and Transplant (ODT NHSBT)

Patients are HLA typed twice, on separate blood samples, prior to registration for a transplant.

Patient database management

Regular alloantibody specificity identification

Upon registration for transplantation, a clotted blood sample is required every 2 months until transplantation. The patient's serum is regularly investigated for the presence of antibodies that may potentially damage an allograft. This is achieved using Luminex technology; antigens to which the patient is sensitised are considered unacceptable for transplantation and registered onto the national database.

In addition to the 2-monthly samples, we request serum samples 4 weeks after each blood transfusion to identify any subsequent change in HLA antibody profile.

Living donor lymphocyte crossmatching

Early in the transplant work-up, an initial crossmatch is performed; this is usually a virtual crossmatch, although a laboratory crossmatch may be performed depending on the circumstances. Once the donor has been selected, a final laboratory crossmatch is performed using serum from no longer than 14 days prior to the planned transplant.

Virtual crossmatching

Blood is taken from the potential donor(s) for HLA typing. In addition, a serum sample is taken from the recipient for antibody detection and definition assays. A virtual crossmatch is performed; the antibody profile of the recipient is scrutinized in conjunction with the HLA type of the potential donors to establish any incompatibility.

Laboratory crossmatching

The lymphocytes of the potential living donor are crossmatched against patient serum using Flow Cytometry (FC).

Flow Cytometry Crossmatch

Serum samples for FC crossmatch are selected according to the following criteria:

- A sample taken 6 months prior to prospective transplant, or the closest sample available.
- A current sample (within 4 weeks of the crossmatch test).
- Any sample within the last 6 months that has had a marginal Luminex screen result and no antibody definition result.
- Any sample with a relevant low level unacceptable specificity.

Antibody-incompatible transplant service

The laboratory supports the antibody-incompatible transplant programme. Titration crossmatches and antibody monitoring are performed as agreed with the clinical team.

Deceased donor-specific patient selection service

The laboratory provides an advisory service for selecting patients for consideration for deceased donor transplant.

Deceased donor lymphocyte crossmatching

A crossmatch must be performed prior to transplant for all organs. This may be a virtual or laboratory crossmatch depending on the circumstances.

Laboratory Crossmatching

The lymphocytes of the potential deceased donor are crossmatched against patient serum by Flow Cytometry (FC) technique as described in the living donor section above.

Virtual Crossmatch – Deceased Donors

Recipients may proceed to transplant on the basis of a virtual crossmatch if certain criteria are met, in accordance with the BSHI and BTS guideline on the detection of alloantibodies in solid organ (and islet) transplantation Nov 2023, and BSHI/BTS Guidance on crossmatching before deceased donor kidney transplantation, 2022.

Virtual crossmatching is based on the donor centre offer HLA type, which has not yet been confirmed. Based on national audit data, there is a <1% error rate in UK donor offer types. If there is an error in this HLA type and the patient has pre-existing HLA antibodies, the transplant may be antibody incompatible. Furthermore, regardless of sensitisation, the mismatch grade may be incorrect. If there is any concern regarding the donor centre offer HLA type, the laboratory will request donor blood samples for pre-transplant confirmatory HLA typing.

In accordance with the European Federation for Immunogenetics Standards for Histocompatibility and Immunogenetics Testing, a laboratory retrospective antibody test is always performed in the deceased donor situation.

i. All Patients on the Transplant List

When an offer of an organ is received, the identified recipient is assessed for eligibility for proceeding to transplant using the results of a virtual crossmatch. The patient's antibody profile, sensitisation status, and donor HLA type are considered.

However, if the recipient has had a recent infection / blood transfusion, a current sample must be obtained for day of transplant testing.

ii. Patients requiring a Modified Multivisceral Intestine Transplant

For patients requiring a small bowel plus other related organs such as stomach, abdominal wall in the absence of either a kidney or pancreas organ, risk stratification according to the NHSBT Multi-visceral and Composite Tissue Advisory Group (MCTAG) guidelines are applied.

iii. Antibody Screening / Definition Requirements

A minimum of 2 serum samples must have been assayed by Luminex Single Antigen Beads, and a current sample * must have been tested.

* A current sample is locally defined as up to 3 months pre-transplant depending on the patient's level of sensitisation, stability of their antibody profile and presence of low-level donor specific antibodies.

iv. Pregnancy / Previous Transplants

Patients who have had pregnancies and/or previous transplants are considered for proceeding to transplant on a virtual crossmatch; however, this is taken into account when performing the crossmatch. It is documented on the virtual crossmatch report.

v. Imlifidase Enabled transplants

This is an HLA antibody incompatible transplant for patients with a very low chance of receiving an HLA compatible deceased donor kidney offer. At the time of offer, the patient's HLA antibody profile is scrutinised and discussed with the senior Clinical Scientist and the specialist imlifidase nephrologist.

A pre-transplant, pre-Imlifidase treatment, clotted blood sample may be required for additional HLA antibody testing prior to administration of Imlifidase. This depends on the date of the patient's current sample held by the laboratory and if it has been tested.

Patient clotted blood samples at 4 and 6 hours post-Imlifidase treatment are required for pre-transplant HLA antibody testing.

Post-transplant antibody monitoring

Serum samples are routinely requested according to the following protocol:

- 1, 2 and 4 weeks after transplant, at 3, 6 and 9 months and annually thereafter.
- 2 and 4 weeks following graft failure.
- Serum samples are requested for monitoring antibody-incompatible transplants as required on a patient-specific basis. For imlifidase-enabled transplantation, monitoring for DSA rebound is recommended using serum from days +2, +4, +6, +8, +10, +12, +14 and then twice weekly thereafter for 6 weeks or as clinically indicated in response to graft dysfunction.

Kidney, Kidney/Pancreas, Pancreas alone, Islet, Kidney/Islet, Modified Multi-Visceral, Small Bowel, Uterine and Abdominal Wall Transplantation - HLA typing, Crossmatching and Antibody Analysis

The following tests are performed during transplant workup.

HLA Typing

Category	Loci tested	HLA resolution
Patient	HLA-A, B, C, DR, DQ, DP	Higher
Patient confirmatory sample	HLA-A, B, DR	Low
Potential living donor (first presentation)	HLA-A, B, C, DR, DQ, DP	Higher
Potential living donor (second presentation)	HLA-A, B, DR	Low
Deceased donor	HLA-A, B, C, DR, DQ, DP	Low
Patient's partner (female patients only)	HLA-A, B, C, DR, DQ, DP	Higher

HLA Antibody Analysis and Crossmatching

Test	Assay	When performed
Antibody detection	Luminex	As indicated
Antibody specificity determination	Luminex	As indicated
Donor Specific Antibody (DSA)	Luminex	As indicated
Living donor crossmatch (virtual)	Luminex	At presentation
Living donor crossmatch	Flow cytometry	Pre-transplant [Clinic 3]
Living donor titration crossmatch	Flow cytometry	During workup and pre-transplant
Deceased donor crossmatch	Virtual crossmatch based on Luminex data	At time of transplant
	Flow cytometry	As indicated

Samples required

From patients:

For initial presentation prior to registration for transplant (deceased or living):
2 tubes (8 mls) EDTA anticoagulated blood (purple top); **EPR - HLA genotyping (patient)**
1 tube (10 mls) clotted blood (yellow/red/brown top); **EPR - HLA antibody profile**

For confirmatory HLA genotyping:
2 tubes (8 mls) EDTA anticoagulated blood (purple top); **EPR - HLA genotyping (patient)**

Following registration for transplant:
1 tube (10mls) clotted blood (yellow/red/brown top); **EPR - HLA antibody profile**
frequency: every month, and 4 weeks following blood transfusion

Autologous crossmatch
4 tubes (16 mls) EDTA anticoagulated blood (purple top); **EPR - HLA crossmatch (autologous)**
1 tube (10 mls) clotted blood (yellow/red/brown top); **EPR - HLA antibody profile**

Autologous titration crossmatch
4 tubes (16 mls) EDTA anticoagulated blood (purple top); **EPR - HLA crossmatch (autologous)**
1 tube (10 mls) clotted blood (yellow/red/brown top); **EPR - HLA antibody profile**

Desensitisation Treatment
1 tube (10mls) clotted blood (yellow/red top) immediately pre and post treatment
EPR - HLA DSA - donor specific antibodies

Donor Specific Antibody (DSA)
1 tube (10mls) clotted blood (yellow/red top)
EPR - HLA DSA - donor specific antibodies

Imlifidase enabled or other antibody removal Transplantation
1 tube (10mls) clotted blood (yellow/red top)
EPR - HLA DSA - donor specific antibodies
These samples are required at specific time points (pre- and post-antibody removal treatment) pre- and post-transplant from the recipient. The schedule is agreed with the clinicians. Samples must be correctly labelled with the date and time they are taken. Please inform the laboratory as soon as the sample is taken.

From living donors:

Living donor virtual crossmatch (clinic 1)
2 tubes (8 mls) EDTA anticoagulated blood (purple top)
EPR - HLA genotyping (live donor)

Living donor laboratory crossmatch (clinic 3/final pre-transplant)
4 tubes (20 mls) EDTA anticoagulated blood (purple top)
EPR - HLA genotyping (live donor) and HLA crossmatch (live donor)

Living donor titration crossmatch
8 tubes (32 mls) EDTA anticoagulated blood (purple top); **EPR - HLA genotyping (live donor) x2**

For confirmatory HLA genotyping
2 tubes (8 mls) EDTA anticoagulated blood (purple top); **EPR - HLA genotyping (live donor)**

From deceased donors:

2 tubes (8 mls) citrate or EDTA anticoagulated blood (pale blue or purple top) are preferred.
Spleen and / or Lymph nodes

Please note: if using EPR to request tests caresets have been devised to simplify this process.

Tests can also be requested using the following Caresets on EPR.

Careset Name	Individual Test Name	Tubes
Recipients - Listing		
Solid Organ Tx assessment	HLA genotyping (patient)	2
	HLA antibody profile (transplant)	1
Recipients – Autologous Crossmatching		
LD Clinic 1 Recipient	HLA genotyping (patient)	2
	HLA antibody profile (transplant)	1
LD Clinic 3 Recipient	HLA crossmatch (autologous)	4
	HLA antibody profile (transplant)	1
Titration crossmatch Recipient	HLA crossmatch (autologous)	4
	HLA crossmatch (autologous)	4
	HLA antibody profile (transplant)	1
Living Donors		
LD Clinic 1 Donor [Virtual crossmatch]	HLA genotyping (live donor)	2
LD Clinic 2 Donor	HLA genotyping (live donor)	2
LD Clinic 3 Donor	HLA crossmatch (live donor)	4
Titration crossmatch Donor	HLA crossmatch (live donor)	4
	HLA crossmatch (live donor)	4

Request form

Requests should be made using the electronic patient record (EPR) system. Alternatively please use the lilac HLA/Tissue Typing specimen request form (Appendix A) and include at least the following information:

Name
NHS number
Hospital number
Date of birth
Clinical details
Date and time when blood taken
Name of requestor, bleep/telephone number and address for report
For potential donors: name of patient and relationship

Please write legibly. A printed label with the relevant identifiers can be used.

Hazardous samples

Hepatitis B (HBV), Hepatitis C (HCV) and HIV:
Please clearly label **both** request form and sample tube with **BIOHAZARD**

The laboratory will NOT process samples from known HCV or HIV infected potential **donor** samples for crossmatching.

NO SPECIMENS OF ANY KIND are accepted from patients known to be infected with a transmissible spongiform encephalopathy (TSE) such as Creutzfelt-Jacob disease (CJD), variant CJD or Kuru.

Turnaround time

HLA typing	5 working days
Living donor initial crossmatch & HLA type	8 working days
Living donor final crossmatch	3 working days
Virtual Crossmatch Report	8 working days
Living donor initial titration crossmatch & HLA type	15 working days
Baseline Living donor titration crossmatch	8 working days
Living donor final titration crossmatch	24 hours
Deceased donor crossmatch	Same day
Donor Specific Antibody	7 working days

Urgent samples can be processed more quickly by prior arrangement

Key Factors known to affect the tests requested

HLA Typing	EDTA or citrate anti-coagulated blood samples MUST be kept at room temperature, do NOT store in a refrigerator.
Crossmatching	The samples MUST be received by the laboratory within 12 hours of being taken. If samples are received after this time period, the crossmatch will be performed but the cell viability may be too poor to allow valid interpretation of the test results. Repeat samples will be requested if this occurs. The EDTA or citrate anti-coagulated blood samples MUST be kept at room temperature, do NOT store in a refrigerator.
Antibody Analysis	Ideally the clotted sample should be received by the laboratory within 24 hours of it being taken. However, up to 5 days may be acceptable depending on the level of haemolysis. Samples received after 5 days will be discarded and another sample will be requested. Samples taken at dialysis centres MUST be taken prior to dialysis and MUST NOT be contaminated with heparin.

Please note that failure to meet the requirements for both the completion of the request form and the sample requirements may result in the rejection of the sample.

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Haematopoietic Stem Cell Transplantation

We provide a full HLA typing and matching service for patients requiring haematopoietic stem cell transplantation. This usually involves typing patients and their relatives in the first instance. Should suitably matched relatives be unavailable, we provide an unrelated donor selection and retyping service in conjunction with the stem cell registries.

Patients receiving grafts mismatched at major HLA loci may require screening for HLA antibodies prior to transplant, as indicated by the Haematology consultants.

Patients and related donors are HLA typed twice, from separate blood samples. The following tests are performed during stem cell transplant work-up.

Category	Loci tested	HLA resolution
Patient	HLA-A, B, C, DR, DQ, DP	High/allelic
Patient confirmatory sample	HLA-A, B, DR	Low
Potential related donor (sibling – first presentation)	HLA-A, B, C, DR, DQ, DP	High/allelic
Potential related donor (all other relatives)	HLA-A, B, C, DR, DQ, DP	High/allelic
Potential unrelated donor	HLA-A, B, C, DR, DQ, DP	High/allelic
Potential related donor confirmatory sample	HLA-A, B, C, DR, DQ, DP	Low
Cord blood unit	HLA-A, B, C, DR, DQ, DP	High/allelic
Other categories	please enquire	

Samples required for HLA typing:

- 2 tubes (20 mls) of EDTA (purple top) or citrate (pale blue top) anticoagulated blood. Please ensure patients have an adequate WBC (greater than 1 is preferable).
  EPR – HLA genotyping BMT donor or HLA genotyping BMT patient
- Material from the cord unit.
- Buccal swabs by prior arrangement with the laboratory

Sample required for HLA antibody screening:

- 1 tube (10 mls) clotted blood (yellow/red/brown top)
  EPR –HLA DSA - donor specific antibodies

Request form

Requests should be made using the electronic patient request (EPR) system. Alternatively, please use the lilac HLA/Tissue Typing specimen request form (Appendix A) and include at least the following information:

Name
NHS number
Hospital number
Date of birth
If potential donor then: name of patient and relationship
Name of requestor, bleep/telephone number and address for report
Clinical details
Date and time when blood taken

Please write legibly. A printed label with the relevant identifiers can be used.

Hazardous samples

Hepatitis B (HBV), Hepatitis C (HCV) and HIV:
Please clearly label **both** request form and sample tube with **BIOHAZARD**

NO SPECIMENS OF ANY KIND are accepted from patients known to be infected with a transmissible spongiform encephalopathy (TSE) such as Creutzfelt-Jacob disease (CJD), variant CJD or Kuru.

Turnaround time

Patient typing (intermediate resolution):	5 working days
Relative typing (intermediate resolution):	9 working days
All high-resolution typing:	10 working days

Urgent samples can be processed more quickly by prior arrangement

Key Factors known to affect the test requested

The EDTA or citrate anti-coagulated blood samples MUST be kept at room temperature, do NOT store in a refrigerator.

Please note that failure to meet the requirements for both the completion of the request form and the sample requirements may result in the rejection of the sample.

HLA and Disease/Pharmacogenetic Association

The laboratory has the capability to perform HLA-A, B, Cw, DR, DQA, DQB and DPB1 and non-HLA genotyping.

Please refer to the following table to determine which test is appropriate for your patient.

Disease/Pharmacogenetics association	Test	Associated alleles	EPR test
Abacavir hypersensitivity	HLA-B57	HLA-B*57:01	HLA-B*57:01 abacavir
Actinic prurigo/photosensitivity	HLA-DR4	HLA-DRB1*04 (inc DRB1*04:07)	HLA class II genotyping
Behçet's disease	HLA-Class I	HLA-B*51:01/B*57:01	HLA-B Behçet's
Birdshot retinopathy	HLA-Class I	HLA-A*29	HLA-A birdshot chorioretinopathy
Coeliac disease	HLA-DQA & DQB	DQA1*05/DQB1*02 or DQB1*03:02	HLA-DQ coeliac
Carbamazepine sensitivity	HLA-Class I	HLA-A*31:01, HLA-B*15:02	HLA class I genotyping
Dermatological diseases	HLA-Class I & II	Various associations	Various
Encephalitis	HLA-Class II	HLA-DRB1*07, HLA-DRB1*11	HLA genotyping
IBD/ Crohn's/ Ulcerative colitis	HLA-CII & TPMT		
Melanoma	HLA-A2	HLA-A*02	
Narcolepsy	HLA-DQB	DQB1*06:02	HLA-DQ narcolepsy
Primary sclerosing cholangitis (PSC)	HLA-Class I & II	HLA-B*08, DRB1*03, DRB1*13	HLA class II genotyping
Spondylarthropathies (HLA-B27 assoc. diseases)	HLA-B27	HLA-B*27	HLA-B27 genotyping
Thiopurine S-methyltransferase gene	TPMT	TPMT*1, *2, *3A, *3B, *3C	TPMT mutation
Uveitis (birdshot and Behcet's-related markers may also be tested)	HLA-Class I	HLA-B*27 (also A*29 and B*51/57)	HLA class I genotyping
Other conditions	please enquire		

Sample required 1 tube (4 mls) of EDTA anticoagulated blood (purple top)

Request form

Requests can be made using the electronic patient record (EPR) system. Alternatively please use the lilac HLA/Tissue Typing specimen request form (Appendix A) and include at least the following information:

Patient name
NHS number
Hospital number
Date of birth
Name of requestor, bleep/telephone number and address for report
Clinical details
Investigation required
Date and time when blood taken

Please write legibly. A printed label with the relevant identifiers can be used.

Hazardous samples

Hepatitis B (HBV), Hepatitis C (HCV) and HIV:
Please clearly label **both** request form and sample tube with **BIOHAZARD**

NO SPECIMENS OF ANY KIND are accepted from patients known to be infected with a transmissible spongiform encephalopathy (TSE) such as Creutzfelt-Jacob disease (CJD), variant CJD or Kuru.

Turnaround time

5 working days. Urgent samples can be processed more quickly by arrangement.

Key Factors known to affect the test requested

The EDTA or citrate anti-coagulated blood samples MUST be kept at room temperature, do NOT store in a refrigerator.

Please note that failure to meet the requirements for both the completion of the request form and the sample requirements may result in the rejection of the sample.

Description of Tests

Genotyping

All routine genotyping is performed by polymerase chain reaction using sequence-specific primers (PCR-SSP) or NGS

PCR-SSP. HLA genotyping using PCR-SSP to either intermediate or low levels of resolution is offered as appropriate to individual application. Higher resolution can be obtained by requesting HLA subtyping or DNA sequencing. The following table lists the tests offered:

Test name	Genotypes tested
Low resolution HLA class I & II type	HLA-A, B, Cw, DR, DQ
High resolution HLA class I & II type inc. DP	HLA-A, B, Cw, DR, DQ, DP
HLA-B27	All known HLA-B27 alleles
HLA-B5701	HLA-B*57:01 and other related alleles
TPMT	TPMT alleles *1, *2, *3A, *3B, *3C, *4
Subtyping of HLA class I & II loci	Various allele groups, please enquire
Other genes	Please enquire

The range of HLA alleles tested for is updated at least annually based on the current revision of the European Bioinformatics Institute IMGT/HLA database. This contains sequences of all HLA alleles currently officially recognized by the WHO HLA Nomenclature Committee.

An HLA result is reported either as a single allele or as a list of alleles that we know contains the allele present. For example, the allele HLA-A*03:01 may be reported as one of the following:

- HLA-A*03:01 allele group HLA-A*03:01 has been identified
- HLA-A*03:01/04/07 this means one of the alleles/allele groups – HLA-A*03:01, HLA-A*03:04 or HLA-A*03:07 is present
- HLA-A*03:01-07 meaning that one of the alleles/allele groups – HLA-A*03:01, HLA-A*03:02, HLA-A*03:03, HLA-A*03:04, HLA-A*03:05, HLA-A*03:06, or HLA-A*03:07 is present

These shortened strings comply with correct nomenclature guidelines and are designed to save space and to be more readable.

Out of hours deceased donor typing is performed using a rapid real-time qPCR assay enabling the reporting of HLA types that comply with the minimum resolution for reporting donor HLA types as required by NHS Blood and Transplant.

High resolution typing. Further characterization of HLA alleles is offered using next generation sequencing.

HLA Antibody identification and Specification

A combination of assays are used to monitor a patient's antibody profile and assign HLA specificity to antibody reactivity detected in serum samples:

LabScan 3D (Luminex FM3D). This is a sensitive technique in which microparticles coated with purified HLA proteins are used to identify and characterise allo-reactive antibodies in patient serum. Different assays are used to detect and specify the HLA antibodies.

The HLA antibody specificities obtained are used to identify unacceptable donor HLA specificities for patients. The unacceptable specificities are entered onto the local and national databases and used in the allocation of donor organs.

Laboratory crossmatching

Crossmatching donor cells with recipient serum identifies the presence of potentially graft-damaging antibodies and is performed using flow cytometry.

Flow Cytometry. The flow cytometry crossmatch is a sensitive technique performed by mixing potential donor cells with recipient serum to determine the presence of IgG donor-specific antibodies (DSA) within the recipient. A flow cytometer is used to detect the antibody binding to donor lymphocytes using fluorescence.

Crossmatching using patients' own cells and sera (autologous crossmatch) may be performed using flow cytometry, either during transplant workup or immediately prior to transplant, to assist interpretation of the allogeneic-crossmatch tests.

All crossmatch results for deceased donors are reported to the on-call Consultant transplant surgeon.
All crossmatch results for living donors are reported to the Living Donor Team.

The laboratory provides a service for both prospective laboratory-based crossmatching and virtual crossmatching.

Clinical Decision Values

The laboratory adheres to the published guidelines listed below for implementing appropriate clinical decision values:

- BSHI and BTS UK guideline on the detection of alloantibodies in solid organ (and islet) transplantation. British Transplant Society (BTS) and British Society for Histocompatibility and Immunogenetics (BSHI). https://onlinelibrary.wiley.com/doi/full/10.1111/iji.12641

- BSHI/BTS Guidance on Crossmatching before Deceased Donor Kidney Transplantation. https://onlinelibrary.wiley.com/doi/10.1111/iji.12558

- UK Guideline on Imlifidase Enabled Deceased Donor Kidney Transplantation https://bshi.org.uk/wp-content/uploads/2023/01/bts.org .uk-UK-GUIDELINE-ON-IMLIFIDASE-ENABLED-DECEASED-DONOR-KIDNEY-TRANSPLANTATION.pdf

- Guidelines for selection and HLA matching of related, adult unrelated donors and umbilical cord unit for haematopoietic progenitor cell transplantation. BTS and BSHI. https://onlinelibrary.wiley.com/doi/epdf/10.1111/iji.12527

- *Coeliac* – Translational Mini-review Series on the Immunogenetics of Gut Disease: Immunogenetics of coeliac disease. Dubois and van Heel 2008. Clinical and Experimental Immunology, 153:162-173

- *TPMT* - Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for Thiopurine Methyltransferase Genotype and Thiopurine Dosing. Also, the Supplemental Material on-line - Relling, Gardner, Sanborn et al 2011. Clinical Pharmacology & Therapeutics, http://www.pharmgkb.org/guideline/PA166104933.

- *Behçet's disease* - Mapping the HLA association in Behçet's disease: a role for tumor necrosis factor polymorphisms? Ahmad, T. et al. Arthritis Rheum. 2003 Mar; 48(3):807-13

- *Actinic Prurigo* - Actinic Prurigo. Ross G, Foley P and Baker. *Photodermatol, Photoimmunol & Photomed,* 24, 272-275

- *HLA-B27* - The ramifications of HLA-B27. Nicholas J Sheehan. J R Soc Med 2004; 97: 10–14

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- *Carbamazepine Hypersensitivity* - Clinical Pharmacogenetics Implementation Consortium Guideline for HLA Genotype and Use of Carbamazepine and Oxcarbazepine: 2017 Update. (2018). doi:10.1002/cpt.1004
- *Allopurinol Hypersensitivity* - Clinical Pharmacogenetics Implementation Consortium Guidelines for Human Leukocyte Antigen-B Genotype and Allopurinol Dosing: 2013. doi:10.1038/cpt.2012.209

Laboratory Policy on the Protection of Personal Information

All human samples and tissues are handled in accordance with the Human Tissue Act 2004, the appropriate Human Tissue Authority codes of practice and other relevant guidelines. The guidance is written in laboratory policies and standard operating procedures, which are reviewed on an annual basis. All staff members are assessed against the relevant policies and standard operating procedures; records are kept by the quality manager.

The laboratory stores information both on computer and in paper format.

All files are stored in locked offices accessible by keypad lock and access to the laboratory is limited to authorised personnel only via a swipe card.

All computers and databases are password protected requiring login onto the secure OUH Trust servers, access to the relevant areas on the server is restricted to administrators and laboratory staff. Where applicable certain documents are further password protected to limit access to authorised personnel only.

The laboratory complies with the data protection act and the Caldicott report, according to the OUH guidelines, and furthermore endeavours to implement new local (OUH Trust) and national legislation as required.

All laboratory staff members are bound by the OUH Trust's conduct policies. Including but not limited to the Confidentiality, Email Use, Equality and Diversity, Uniform and Standard Dress Code, Complaints, Counter Fraud and Reporting, Performance & Conduct, Raising Concerns (Whistle blowing) and Recruitment & Selection policies.

Specimen Collection and Handling

Proper preparation of the patient, specimen collection and handling are essential for the production of valid results by the laboratory.

In accordance with the United Kingdom Accreditation Service (UKAS), the Laboratory Management has to advise on a procedure(s) for specimen collection and handling.

a. Please ensure the request form (either electronic or paper) is fully completed, including the collection date (and time where appropriate) of the sample and any relevant clinical information.

b. Confirm the identity of the patient and check against the identifiers on the request form.

c. Please check the specimen container is correctly labelled with at least 3 identifiers.
(NOTE: printed labels are preferred to handwritten if available).

d. Please ensure the patient is appropriately prepared.

e. Please ensure the correct type and amount of sample is to be taken into the correct sample bottles. Refer to the relevant section for the particular assay being requested within this handbook to check sample requirements, or alternatively, contact the laboratory on 01865 226102.

f. Please employ procedures that minimise the risk of interchange of samples.

g. Please follow the environmental & storage conditions set down in this handbook for the particular assay being requested.

h. Please ensure the safe disposal of all materials used in the specimen collection.

i. Please ensure that high-risk samples are identified as such so that these can be processed appropriately.

j. Please ensure all spillages & breakages are dealt with correctly.

k. Please employ procedures that ensure the safety of the specimen collector, carrier, the general public and the receiving laboratory.

l. Please ensure that samples are transported to the laboratory within the appropriate timeframe as indicated in this handbook. If in doubt, seek advice from the laboratory on 01865 226102.

Transport of Samples

1. Outside the Oxford Radcliffe Hospitals NHS Trust

Clinical material may be sent by post provided that the conditions of the Post Office are met. As always, all clinical material must be considered potentially infectious, and there are strict guidelines to protect any personnel who may come into contact with it.
The following has been distilled from Post Office guidance for sending diagnostic specimens through the post:

- Use only FIRST-CLASS LETTER post or DATAPOST. Royal Mail regulations specifically forbid sending material by second class letter or parcel post.
- Specimens must be in a securely closed leak-proof, preferably unbreakable, bottle or tube (the specimen container) – these MUST comply with P650 Packaging instructions.
- The capacity of the specimen container must not exceed 50mls. Multiple packs are acceptable provided that each primary (specimen) container is separated from the next by soft absorbent tissue.
- Any documentation accompanying the specimen (e.g. request forms) must not be placed in the same bag as the specimen container. Most specimen bags have a separate pocket for this purpose. Otherwise, bag separately.
- Diagnostic specimens must always be sent in packaging that complies with Packaging instructions 650 (available from the DTI).

Packing Instructions 650
(Taken from the HSE publication: Biological agents: Managing the risks in laboratories and healthcare premises) The link is below:
https://personal.help.royalmail.com/app/answers/detail/a_id/96/~/prohibited-and-restricted-items---advice-for-personal-customers

1 The packaging shall be of good quality, strong enough to withstand the shocks and loadings normally encountered during carriage, including trans-shipment between vehicles and containers and between vehicles or containers and warehouses as well as any removal from a pallet or overpack for subsequent manual or mechanical handling. Packagings shall be constructed and closed to prevent any loss of contents that might be caused under normal conditions of carriage by vibration or by changes in temperature, humidity or pressure.

2 The packaging shall consist of three components:
(a) a primary receptacle;
(b) a secondary packaging; and
(c) an outer packaging.

3 Primary receptacles shall be packed in secondary packagings in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packagings shall be secured in outer packagings with suitable cushioning material. Any leakage of the contents shall not compromise the integrity of the cushioning material or of the outer packaging.

4 For transport, the mark illustrated in Figure 1 (page 29) shall be displayed on the external surface of the outer packaging on a background of a contrasting colour and shall be clearly visible and legible. The width of the line shall be at least 2 mm; the letters and numbers shall be at least 6 mm high.

5 The completed package shall be capable of successfully passing the drop test set out in the regulations except that the height of the drop test shall not be less than 1.2 m. The smallest external dimension of the outer packagings shall not be less than 100 mm.

The District Post Office and the sender must be notified immediately if a damaged package is received where the outside of the package may have become contaminated

Pathological specimens should only be sent by trained members of staff. Members of the public may send samples through the post IF it is at the specific request of a medical practitioner, a registered nurse or a recognised laboratory or institution. In each case, the person or organisation making the request must supply the approved or specified packaging and clear instructions on its use.

Figure 1:

2. Within the Oxford University Hospitals NHS Trust

These samples are transported by the hospital portering staff.
All samples must be placed in a specimen bag, then in a brown envelope and then in a pathology bag.

- The brown envelopes must be clearly labelled with the destination address
- More than one brown envelope can be placed in a single pathology bag.
- The samples are taken to Laboratory Medicine for sorting and distribution within the OUH

Contact the porters to request transport of samples to Laboratory Medicine.

3. Samples from outside the UK

Please check the international regulations; UK's Customs, courier company's and the country of origin's requirements and regulations on sending biological samples. Appropriate arrangements should be made before samples are taken. Inform the laboratory on when we should receive the sample and provide the tracking/packaging reference numbers and Courier's or postal company's contact details.

Sample packaging must conform to the above information on transport of samples and any additional requirements.

Model Rules for Portering, Transport & Messenger Staff Regarding the Transport of Transplant Immunology Specimens

Some of the work carried out by laboratory porters and messengers in the hospital may involve accidental contact with material that could be infectious, although precautions are taken to minimize this risk.

The following guidelines must be observed

1. Cuts or grazes on hands must be covered with a waterproof dressing.

2. Do not eat, drink or smoke while handling clinical specimens.

3. Carry all specimens in the specimen bags, trays or boxes provided, not in your hands or pockets.

4. Touch specimen containers as little as possible. If you do touch them, wash your hands as soon as practicable afterwards.

5. Please be aware of the Trust's patient confidentiality policy, patients or patient information must not be discussed with other people.

6. Always wash your hands before meal breaks and at the end of a spell of duty.

7. If a specimen leaks into a bag, tray or box, inform the laboratory reception staff on arrival at the laboratory (01865-226102).

8. If you drop and break a specimen, do not touch it or try to clear up the mess. Stay with the specimen to prevent other people touching it and send someone to the laboratory for help. If you spill the specimen onto your uniform, you must remove it at once and then wash your hands and put on a clean uniform. Report the accident to your supervisor as soon as possible.
   The incident must be reported using the OUH Trust ULYSSES system.

9. If you drive a van, make sure that you have gloves and a spillage kit with you in the vehicle. If a specimen leaks and runs out of the tray or box, put on gloves and cover the spillage with cotton wool or paper towels. Do not mop it up. Contact the laboratory for advice (01865-226102).

10. If your vehicle breaks down or you have an accident, do not let anyone touch the specimens unless they come from a hospital and know the appropriate procedure.

11. Handle specimen containers gently at all times.

Any spillage should be bought to the attention of Laboratory Staff (01865-226102) who will give appropriate advice.

If out-of-hours please contact the on-call scientist via the John Radcliffe switchboard (01865 741841; Internal: 55000)

Feedback

Complaints

We aim to provide service of the highest standard at all times. However, should we fail to meet your requirements, we welcome the opportunity to discuss matters so that we can take appropriate action.

Also refer to the OUH Complaints Policy, https://ouhnhsuk.sharepoint.com/ and website, https://www.ouh.nhs.uk/patient-guide/feedback/complaints.aspx

All complaints, both formal and informal, written or verbal, are considered seriously and are brought to the attention of the senior management of the laboratory as quickly as possible.

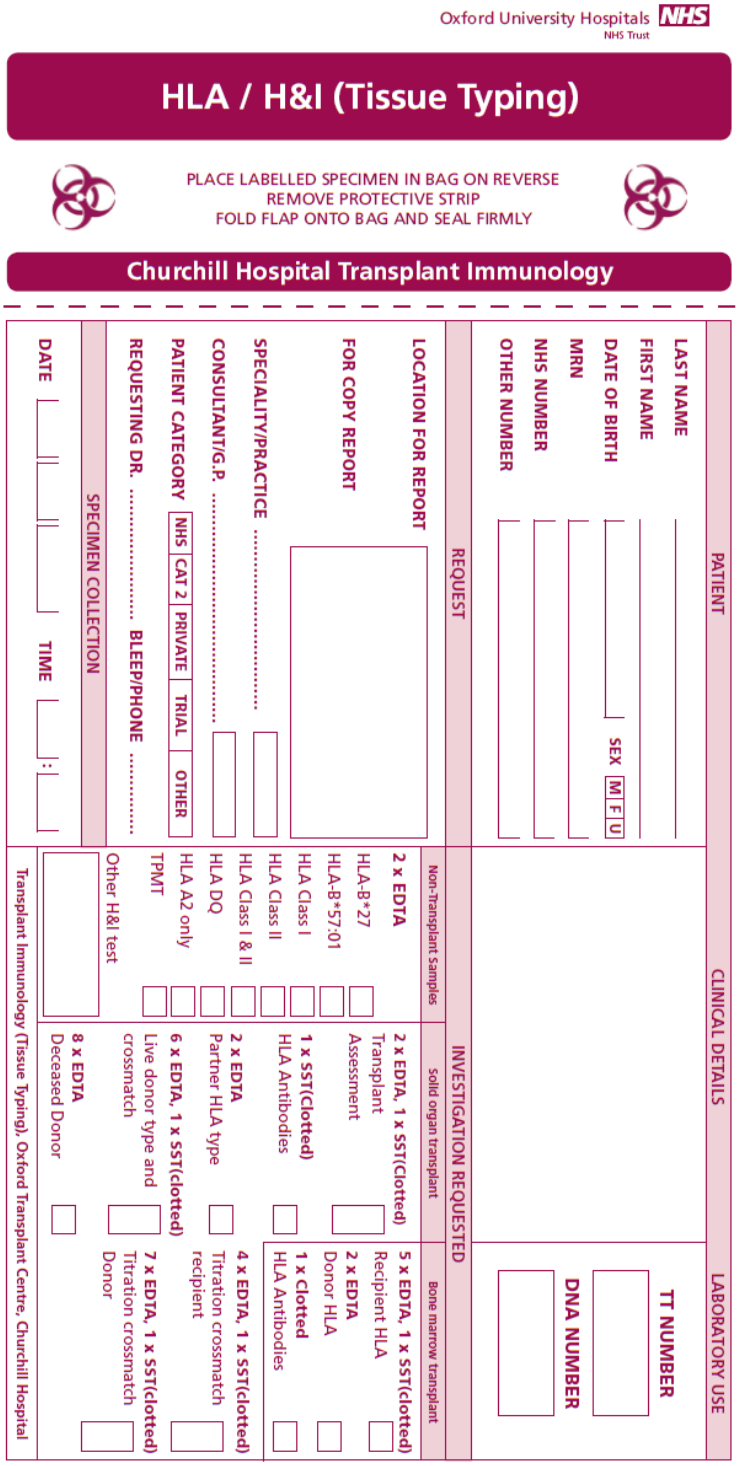
Flow chart showing the process and timeframe in resolving a complaint:

Start of process		Receipt of complaint	All laboratory staff are responsible for ensuring the Director / LMT delegate are aware of the complaint
1 week	1 week	Acknowledgement of receipt of complaint to the complainant	Director / LMT delegate
2 weeks	1 week	Confirmation complaint relates to laboratory activities	Director / LMT delegate
10 weeks	8 weeks	Investigation Completion of ICE form Corrective Action Resolution	Director / LMT delegate
14 weeks	4 weeks	Submission to and Review by Independent person	Independent person
15 weeks	1 week	Provide complainant with the outcome	Director / LMT delegate

Your comments

We welcome your comments on the services provided by the Transplant Immunology & Immunogenetics Laboratory and would be pleased to discuss our work with you. Members of staff are willing to visit satellite units to give educational talks on the role of the laboratory and the implications of our work in transplantation. Please contact Dr Martin Barnardo to arrange a visit.

Appendix A


Oxford University Hospitals NHS
NHS Trust
HLA / H&I (Tissue Typing)
PLACE LABELLED SPECIMEN IN BAG ON REVERSE
REMOVE PROTECTIVE STRIP
FOLD FLAP ONTO BAG AND SEAL FIRMLY
Churchill Hospital Transplant Immunology
PATIENT
LAST NAME
FIRST NAME
DATE OF BIRTH
SEX M F U
MRN
NHS NUMBER
OTHER NUMBER
CLINICAL DETAILS
LABORATORY USE
TT NUMBER
DNA NUMBER
REQUEST
LOCATION FOR REPORT
FOR COPY REPORT
SPECIALITY/PRACTICE .....
CONSULTANT/G.P. .....
PATIENT CATEGORY NHS CAT 2 PRIVATE TRIAL OTHER
REQUESTING DR. ..... BLEEP/PHONE .....
SPECIMEN COLLECTION
DATE
TIME :
INVESTIGATION REQUESTED
Non-Transplant Samples
2 x EDTA
HLA-B*27
HLA-B*57:01
HLA Class I
HLA Class II
HLA Class I & II
HLA DQ
HLA A2 only
TPMT
Other H&I test
solid organ transplant
2 x EDTA, 1 x SST(Clotted)
Transplant Assessment
1 x SST(Clotted)
HLA Antibodies
2 x EDTA
Partner HLA type
6 x EDTA, 1 x SST(clotted)
Live donor type and crossmatch
8 x EDTA
Deceased Donor
Bone marrow transplant
5 x EDTA, 1 x SST(clotted)
Recipient HLA
2 x EDTA
Donor HLA
1 x Clotted
HLA Antibodies
4 x EDTA, 1 x SST(clotted)
Titration crossmatch recipient
7 x EDTA, 1 x SST(clotted)
Titration crossmatch Donor
Transplant Immunology (Tissue Typing), Oxford Transplant Centre, Churchill Hospital