SOP 1023 - TRANSPLANT LABORATORY SERVICE USER MANUAL

Authors: Clare Collins & Nikita Sinha **Section: QMS** **Location of paper copy: N/A**

INDEX

1. Introduction

This User Manual is intended as a guide to the Histocompatibility and Immunogenetics (H&I) service offered by the Transplant Laboratory based at Leicester General Hospital. The Transplant Laboratory is part of the Renal Respiratory and Cardiovascular Clinical Management Group (RRCV) of University Hospitals of Leicester. The role of the Laboratory is to provide comprehensive Laboratory support for the Renal Transplantation Programme which serves the Renal Units located at Leicester General, Kettering General, Boston, Northants, Peterborough District, Lincoln County and Loughborough Hospitals. The Laboratory also provides testing for potential HLA association with hypersensitive drug reactions and autoimmune diseases.

The Transplant Service at UHL, which includes the Transplant Laboratory activity, is licensed by the Human Tissue Authority, (40054). A UKAS accredited medical laboratory No. 8100. Accredited by the European Federation for Immunogenetics (EFI no. 03-GB-010.990).

2. Contact Details:

Head of Transplant Laboratory

Clare COLLINS BSc (hons) MSc BSHI Diploma DipRCPath

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Deputy Head of Transplant Laboratory

Stephen WESTON BSc (hons) MSc BSHI Diploma DipRCPath

0116 258 4606 stephen.weston@uhl-tr.nhs.uk

Quality Manager

Nikita SINHA BSc (hons)

Laboratory staff 0116 258 4607

Relevant laboratory staff members are registered with the Health and Care Professions Council (HCPC) and undertake Continuing Professional Development. Clinical Scientists and trainees are also members of the professional body, British Society for Histocompatibility and Immunogenetics (BSHI).

Administration staff 0116 258 4603

Kirti ODEDRA Senior Administrative Assistant

The Laboratory can be contacted by email uho-tr.eastmidlandstransplantlab@nhs.net

3. Working Hours/Clinical Advice

Opening Hours

Normal routine laboratory hours:

Monday – Friday 08:30am – 5:30pm excluding bank holidays.

Out of hours service: On-call laboratory scientist available for tissue typing and crossmatching (see section 6 for service details and contact information).

The Head of the Transplant Laboratory is available to provide clinical and scientific advice to Nephrological and Surgical staff concerned with the Renal Transplant Program. This service is available throughout the year and the Head of the Transplant Laboratory can be contacted through Switchboard out of working hours. During the absence of the Head of the Transplant Laboratory, the Deputy Head of the Transplant Laboratory will be available to provide advice.

As the Head of Laboratory is currently working to become a Fellow of Royal College of Pathologists (FRCPATH), Consultant Clinical Scientist support is provided to the laboratory via a named NHS Blood and Transplant (NHSBT) Consultant Clinical Scientist for two days per month and the laboratory has 24 hour access to the NHSBT On-call Consultant Clinical Scientist rota.

The Laboratory Director's duties and responsibilities, comprise the following:-

a) Provide effective leadership of the medical laboratory service, including budget planning and financial management in accordance with institutional assignment of such responsibilities;

b) Relate and function effectively with applicable accrediting and regulatory agencies, appropriate administrative officials, the healthcare community, and the patient population served, and providers of formal agreements, when required;

c) Ensure that there are appropriate numbers of staff with the required education, training and competence to provide medical laboratory services that meet the needs and requirements of the users;

d) Ensure the implementation of the quality policy;

e) Implement a safe laboratory environment in compliance with good practice and applicable requirements;

f) Maintain a departmental risk register and implement actions to mitigate any identified risks and review effectiveness of actions.

g) Serve as a contributing member of the medical staff for those facilities served, if applicable and appropriate;

h) Ensure the provision of clinical advice with respect to the choice of examinations, use of the service and interpretation of examination results;

i) Select and monitor laboratory suppliers;

j) Select referral laboratories and monitor the quality of their service;

k) Provide professional development programmes for laboratory staff and opportunities to participate in scientific and other activities of professional laboratory organizations;

l) Define, implement and monitor standards of performance and quality improvement of the medical laboratory service or services to enhance patient care;

m) Monitor all work performed in the laboratory to determine that clinically relevant information is being generated;

n) Address any complaint, request or suggestion from staff and /or users of laboratory services;

o) Design and implement a contingency plan to ensure that essential services are available during emergency situations or other conditions when laboratory services are limited or unavailable;

p) Plan and direct research and development, where appropriate.

The Head of Laboratory delegates some duties to the Deputy Head of Laboratory and other trained clinical scientists as required. The Transplant Laboratory's Quality Manual describes which of the duties above may be delegated and to whom. Other duties are assumed by the Deputy only during the absence of the Head of Laboratory.

Protection of personal information The Laboratory abides by all UHL and National Guidelines regarding data confidentiality to ensure personal information is protected.

Complaints Procedure

Any issues/complaints/comments/compliments should be directed in the first instance to Head of the Transplant Laboratory who will review the issue and report it to the relevant committee or to the Clinical Business Unit Manager. All complaints will be dealt with according to A11/2002 Policy for the Management of Complaints, which can be found on InSite.

Any member of lab staff who receives comments from Users that could be interpreted as a complaint or a concern (see A11/2002 for definitions) will inform both the Head of Laboratory and the Quality Manager using the specific instructions for complaints and concerns described in internal document number 1234. This includes comments received in response to circulation of the User Survey.

Complaints and concerns are seen as an opportunity to review the service being offered to Users, e.g. for its appropriateness or currency, and may constitute an appropriate idea for a Quality Improvement project. They are reviewed monthly, alongside other incidents and nonconformities, by the QM and are discussed at Laboratory Management Committee (LMC) meetings. Complaints and concerns also form part of the Annual Management Review (AMR) agenda.

Contingency Planning

The laboratory has contingency planning in case of any issues that impact the laboratory's ability to provide patient care, the Head of Laboratory or Deputy Head of Laboratory in her absence, are responsible for coordinating the response.

4. Laboratory Testing Schedule

Please note: all assays carried out in a clinical laboratory have a degree of uncertainty (i.e. variability). We have estimated the uncertainty associated with each of the assays following guidelines produced by the Royal College of Pathologists ("Recommendations for the determination of measurement uncertainty for assays performed in the Histocompatibility and Immunogenetics (H&I) laboratory" August 2016). Estimates of Uncertainty are given in Appendix 2 for crossmatch and antibody analyses.

4.1 Patient Workup to be registered on the National Transplant List operated by the Organ Donation and Transplantation Directorate (ODT) of National Health Service Blood and Transplant (NHSBT).

a. HLA-A, B, C, (Bw), DRB1,3,4,5, DQB1, DPB1 type the patient to conform with NHSBT 'Minimum resolution for reporting donor and recipient HLA types' recommendations. The patient's type SHOULD be verified from a second sample. Samples should be from different phlebotomy events. *See EFI Standard E5.1.12.2 Every effort must be made to perform verification typing for recipients prior to transplantation.*b. HLA antibody screening on two separate samples (HLA-A, B, C, DR, DQ and DP specificities).

c. Collate HLA data, patient data and blood group results and submit data to the national transplant list database i.e. activate patient on the transplant list.

*The laboratory should be notified of any sensitizing events e.g. transfusion, pregnancy, nephrectomy and these should be recorded on proton.

4.2 Provide H&I support for patients registered on the National Transplant List according to National Standards (British Transplantation Society).

a. HLA antibody screening (HLA-A, B, C, DR, DQ and DP specificities) on serum samples taken at 3 monthly intervals (minimum requirement) for all patients awaiting a renal transplant. HLA antibody specificity also determined.

b. Maintain (i.e. update, suspend, reactivate) patient details on the ODT transplant list

c. Store collected serum samples in frozen state for all patients.

*The laboratory must be notified of any sensitizing events e.g. transfusion, nephrectomy and these should be recorded on proton.

4.3 Perform a crossmatch for recipients receiving a deceased donor kidney transplant. The crossmatch may be prospective or retrospective (following active assessment of histocompatibility "virtual crossmatch")

a. Provide a 24 hour 365 days per year service via an on-call rota of scientists and Consultant Clinical Scientist cover.

b. Crossmatch a selection of historic and current sera against donor T and B-cells, using the complement dependent cytotoxic (CDC) crossmatch method for all patients. CDC crossmatch is carried out with and without DTT to distinguish IgM antibodies from IgG. A Flow Cytometry crossmatch is also performed for prospective crossmatches.

c. Report results to transplant registrar/surgeon, with Consultant Clinical Scientist advice when required.

*As of August 2024 retrospective lymphocyte cross matches will be replaced by HLA antibody testing of the acute sample followed by a repeat of the virtual cross match.

4.4 Work-up potential live donor for renal transplant.

a. HLA-A, B, C, Bw, DRB1,3,4,5, DQA1/B1, DPA1/B1 typing of donor based on patient's antibody profile. This typing conforms to NHSBT 'Minimum resolution for reporting donor and recipient HLA types' recommendations. At this time an assessment of histocompatibility will be undertaken and reported ("virtual crossmatch"). The donor's type MUST be verified from a second sample. Samples should be from different phlebotomy events. *See EFI Standard E5.1.12.3 Verification typing must be performed on living donors prior to transplantation* (exception is donors in Paired Exchange who will be typed by another Centre).b. Crossmatches between patient and selected live donor will be performed using a selection of patient sera and donor T and B-cells, by flow cytometry and CDC (+DTT). A preliminary cross match is performed if the patient is sensitized and there are potential DSA. Pre-transplant cross match is performed within 2-3 weeks of scheduled transplant (as agreed annually at MDT and outlined in SOP_1016 Cross match Policy)

d. Report results to the live donor transplant coordinator and clinician requesting the tests.

Flow cytometry crossmatch clinical decision values are as follows:

- Living / PBL donor T cell XM:
    - A MCS difference of less than 64 = a Negative result

- A MCS difference of 64 or greater = a weakly positive result
- A MCS difference of 89 or greater = a positive result

- Living / PBL donor B cell XM:
  - A MCS difference of less than 75 = a Negative result
  - A MCS difference of 75 or greater = a weakly positive result
  - A MCS difference of 99 or greater = a positive result
- Deceased T cell XM performed using spleen cells
  - A MCS difference of less than 45 = a Negative result
  - A MCS difference of 45 or greater = a weakly positive result
  - A MCS difference of 69 or greater = a positive result
- Deceased B cell XM performed using spleen cells
  - A MCS difference of less than 75 = a Negative result
  - A MCS difference of 75 or greater = a weakly positive result
  - A MCS difference of 99 or greater = a positive result

Measurement Uncertainty for this assay is described in Appendix 2.

4.5 Monitor transplanted patients for the presence of donor specific (and other) HLA specific antibodies.

Currently this service is not funded. Therefore the laboratory will only monitor patients who are referred specifically for this testing due to a change in their clinical status.

a. HLA antibody specificity (HLA-A, B, C, DR, DQ and DP).

b. Report results to Renal Physician

Luminex Single Antigen test clinical decision values (normalised MFI) are as follows:

"Low Risk" = Above cut-off (sample dependent, usually 1000 MFI) but less than 2000 MFI

"Moderate Risk" = 2000 to 5000 MFI

"High Risk" (or "Specificity") = Greater than 5000 MFI

Measurement Uncertainty for this assay is described in Appendix 2.

4.6 HLA type local deceased donors

a. HLA-A, B, C, DRB1,3,4,5, DQA1/B1, DPA1/B1 type all locally referred deceased donors in compliance with NHSBT 'Minimum resolution for reporting donor and recipient HLA types'.

b. Report HLA types to NHSBT ODT.

4.7 H&I service to aid disease diagnosis

HLA typing can provide help in the diagnosis of various auto-immune disorders.

These tests may be requested by GPs or hospital physicians.

4.7.1 Ankylosing spondylarthropathies and uveitis

HLA-B27 testing to aid their diagnosis

The laboratory will test for the presence or absence of HLA-B27 and report results as positive or negative.

Please note this test is primarily referred to Immunology at LRI who carry out a flow cytometry based test. The B27 tests are referred from Immunology to the Transplant Laboratory when their results are equivocal, positive or the EQA requires confirmation.

4.7.2 Coeliac disease

HLA-DQ testing to aid diagnosis of Coeliac disease. HLA-DQA1 and DQB1 typing is performed and the results reported as according to the haplotypes identified.

4.7.3 Behcet's Disease

HLA-B testing to aid diagnosis of Behcet's disease. HLA-B51 is reported as positive or negative.

4.7.4 Birdshot Retinopathy

HLA-A testing to aid diagnosis of Birdshot retinopathy. HLA-A29 is reported as positive or negative.

4.7.5 Narcolepsy

HLA-DQ testing to aid diagnosis of Narcolepsy. HLA-DQB1*06:02 is reported as positive or negative.

4.8 HLA association with hypersensitive drug reactions

HLA-B*57:01 testing is performed to identify individuals who are at risk of hypersensitivity to the anti-HIV drug, abacavir.
The laboratory will test for the presence or absence of HLA-B*57:01 and report results as positive or negative.

Other HLA typing tests will be undertaken when referred from a recognised authority and where there is clinical evidence to support HLA typing as providing a useful parameter in diagnosis and/or patient treatment.

4.9 Immunosuppressive Drug Measurement (ISD)

The Transplant Laboratory manages the ISD monitoring service, but arranges for the analysis of Tacrolimus and Cyclosporin and Sirolimus samples received by the Transplant Laboratory to be carried out by an external referral laboratory based at the Department of Clinical Pathology, City Campus, Nottingham University Hospitals NHS Trust. The ISD levels are measured by Liquid Chromatography interfaced with Tandem Mass Spectrometry (LC-MS/MS).

In summary, the following arrangements are made:

Same day reporting of Urgent Tacrolimus tests: Samples/requests for Tacrolimus must be received in the Transplant Laboratory before 12.00pm Monday to Friday inclusive. Couriers collect samples at 12.30pm on weekdays. Results will be reported on iLab within 2 hours of completion of the test by Clinical Pathology NUH, via NPex link, and to Proton within a further 2 hours of this. There is no weekend service.

NB, The Transplant Laboratory must be informed of any sample that requires a same day service.

Next working day (routine) reporting of ISD test results: arrangements are the same as listed above, except that routine samples are all those not marked as 'urgent'.

NB, Please contact the Laboratory to discuss other urgent or out-of-hours requests, as the Transplant Laboratory does not arrange these but can provide information on how to directly refer samples from clinical areas to testing laboratories.

For all adult renal transplant requests within UHL, results will be reported on Proton within one working day of sample receipt, except urgent samples where results will be reported the same working day (i.e. before midnight).

For samples referred from non-transplant/non-renal areas of UHL, results will be reported on iLab within one working day of sample receipt. Please provide cost centre for invoicing.

For samples referred from outside UHL, results will be reported by email from the Laboratory's uho-tr.eastmidlandstransplantlab@nhs.net email account within one working day of sample receipt. A recipient email address MUST be provided and MUST be compliant with the NHS/government secure email standard (e.g. nhs.net) or results cannot be reported.

For information about NPex (the National Pathology Exchange), contact the Immunosuppressive Drug Monitoring Section Head (stephen.weston@uhl-tr.nhs.uk), or other Senior Transplant Lab Clinical Scientist staff.

It is Laboratory policy to NOT report ISD results verbally. Telephone reporting is possible by text or other secure message. Contact Head or Deputy Head of Lab to arrange.

Please note that the Transplant Laboratory ceased to offer 'routine' access to MPA/MMF/Mycophenolate testing from 12/4/2021.

Please note, in addition, that the Transplant Laboratory is currently undertaking a review of options for the longer-term delivery of the ISD monitoring service in Leicester. Please contact the Head or Deputy Head of the Transplant Laboratory for more information if required.

The NUH laboratory does not provide a Saturday or Sunday Tacrolimus service and if analysis is required over the weekend samples will need to be sent to Wythenshawe Hospital in Manchester.
Wythenshawe offer Tacrolimus analysis for **urgent** requests on a Saturday and Sunday. If an urgent analysis is required you are advised to contact the Wythenshawe laboratory by telephoning 0161 291 2699 to arrange this (ideally Friday). Please note you must arrange your own transport of the samples to the Wythenshawe laboratory and if same day analysis is required all samples must reach the Wythenshawe laboratory by **11am.** You will be able to obtain the results by telephoning the laboratory on the same number used to arrange analysis (0161 291 2699). Please note the results will not appear on the hospital (LGH) computer system until a hard copy of the results has been received by the Transplant laboratory. The Wythenshawe laboratory telephone all results greater than 15µg/L so you must provide a contact number on the request form when sending the sample. Please note the sample requirements for tacrolimus at the referral site are identical to those of NUH (EDTA whole blood) and the assay methodology is comparable to the NUH in-house service.
To re-iterate the NUH Pathology laboratory will not organise urgent analysis with Wythenshawe on behalf of the clinical teams and the laboratory will also not be able to organise transport of samples to the referral laboratory. All samples for weekend analysis must be transported directly to the laboratory at Wythenshawe.

The address for the Wythenshawe hospital is detailed below:
Clinical Science Building
Biochemistry Department
Wythenshawe Hospital

Manchester University NHS Foundation Trust

Southmoor Road

M23 9LT Tel: 0161 291 2699

5. Assay request

(a) Instructions for preparing the patient for venepuncture

All phlebotomy must be carried out in accordance with current UHL guidelines and clinical best practice. Please ensure all staff are fully trained before taking blood samples.

See current Document on Insite http://insitetogether.xuhl-tr.nhs.uk/pag/pagdocuments/Venous%20Blood%20Sample%20-%20Obtaining%20UHL%20Guideline.pdf

(Accessed ~~09/11/2021~~ ~~19/10/2022~~ 09/11/23)

All materials used in the collection of blood must be disposed of according to current UHL guidelines.

(b) Instructions for completing assay request forms

A request form must accompany every sample and may be hand-written or generated via the UHL on-line ordering system.

It is the responsibility of the requester and phlebotomist to ensure that the form and specimen tubes are correctly and fully labelled. The tubes must be dated and the time included if more than one sample sent per day. The request form should clearly identify the examination requested.

All supplies of hard copy assay request forms are available from the Transplant Laboratory, Leicester General Hospital, please contact the Laboratory , tel 0116 258 4603.

Assay request forms are available from UHL on-line Pathology test ordering system.

Patient details

The patient's full surname and forename, and two identifiers e.g., Hospital Number, NHS number, date of birth, will be required for identity purposes plus any other relevant information, (e.g. relationship with a potential recipient).

Requesting individual & consultant

Please ensure the Consultant's surname is clearly and legibly written in full Insert the requesting location, hospital and ward number, the requester's identity, print surname and sign (if using paper forms), provide contact details (either bleep number, telephone number).

If the patient is not part of the renal replacement program, i.e. not on PROTON database, please supply cost code as the assay cost must be re-claimed. If you do not provide a code the Laboratory will assign a code based on the location provided and may cause a delay in testing the sample until the correct code can be identified, (see Appendix 1 for cost). Also please ensure that the contact information is adequate to ensure that the results can be communicated back to the requestor- e.g. email address has been supplied on the request form. Requesters are reminded that the Laboratory's normal policy is NOT to accept requests for testing or provide results over the telephone to avoid transcriptional errors.

Nature of specimen

Indicate sample type, e.g. venous blood. If the sample is high risk (i.e. BBI, Blood Borne Infection), clearly mark sample and form accordingly and seal sample in Biohazard labelled sample bag &/or label with BBI stickers. Please give clinical details relevant to the request. If problems are anticipated in obtaining the required sample volume, please contact the Transplant Laboratory to ascertain minimum sample requirements for that assay.

Consent

It is the responsibility of the requester to ensure that any patient or donor has been informed of, and has consented to, the tests being requested according to the current legislation for example, the requirements of the Human Tissue Act 2004.

6. Blood sample requirements

(a) HLA Type

4-7.5 ml EDTA blood (deceased donors: total volume 6 ml EDTA blood, paediatric patients under 30 kg total volume 5 ml)

Storage: samples are fine up to 14 days at room temperature for HLA typing although lab will process sample for DNA extraction as soon as possible after arrival.

Key factors which may affect test

The tests rely on preservation of viable lymphocytes and the cell's DNA & this can be compromised by:

- storage at inappropriate temperatures - e.g. freezing,
- inadequate anti-coagulation resulting in micro-clots,
- presence of cytotoxic agents, e.g. anti-lymphocyte antibody
- anaphylaxis or infusions of other cells - e.g. bone marrow

Reference range: None

Assay frequency: As required

(b) Anti-HLA Antibody Screen Characterisation and Identification

5–9 ml clot for serum

Storage: short term storage (<48 hours) at room temperature 18-25^{o}C. Deliver to lab as soon as possible. Lab will separate clot from serum and serum stored frozen -20^{o}C.

Key factors which may affect test:

- any recent sensitising event - e.g. blood transfusion, transplant, nephrectomy, pregnancy
- treatment with antibody anti-rejection therapy
- intravenous immunoglobulin treatment

Reference range: Negative – no anti HLA Class I or II antibody detected

Reference range: Positive – anti HLA antibody detected. "Low Risk" = Above cut-off (sample dependent, usually 1000 MFI) but less than 2000 MFI, "Moderate Risk" = 2000 to 5000 MFI, "High Risk" (or "Specificity") = Greater than 5000 MFI

The presence of antibody may affect the cross-match result if this is found to be donor specific and of high titre.

Assay frequency: antibody runs are performed on a batch basis, usually twice per week

(c) Cross match

Deceased donor for crossmatch:

- Spleen tissue, approx. 2 cm^3, and/or lymph nodes.

Living donor / PBLs from a deceased donor for crossmatch / Auto crossmatch:

- Whole blood anticoagulated with EDTA, approx. 40 ml.

Crossmatch recipient:

- Clotted blood sample, 9-18 ml, plus whole blood anticoagulated with EDTA, 5-9 ml.

All of the above samples are required to be less than 24 hours old, except for retrospective CDC crossmatches carried out on the basis of a negative virtual crossmatch.

Storage: short term storage (<24 hours) at room temperature 18-25^{o}C.

This test analyses reaction of patient serum with donor lymphocytes derived from EDTA blood to assess compatibility.

Key factors which may affect test and the laboratory needs to be informed

- any recent sensitising event - e.g. blood transfusion, transplant, nephrectomy, pregnancy
- treatment with antibody anti-rejection therapy
- intravenous immunoglobulin treatment

Reference range: Negative cross match – no anti HLA Class I or II antibody detected

Reference Range: Positive cross match – anti HLA antibody detected

Assay frequency: As required

(d) Immunosuppressive drug (ISD) level

Provide only one sample per ISD drug test being requested.

Sample provided must be a full EDTA anticoagulant sample bottle of any size between 2 ml and 9ml for adults, and 0.5 ml to 1.5ml for paediatric patients.

Deliver to lab as soon as possible. Store samples at room temperature in the short term (during transport to the laboratory), or between 2^{o}C to 8^{o}C for longer periods prior to transport to the laboratory, where necessary.

Normally, only a trough level (i.e. pre-dose) sample is considered clinically relevant (although in exceptional circumstances a peak drug level may be specifically requested for absorption investigation).

Key factors which may affect test:

- insufficient mixing of blood and anticoagulant leading to inadequate anticoagulation and formation of clots,
- incorrect anticoagulant used,
- concomitant medication that induces/delays liver metabolism e.g. phenytoin induced depression of Tacrolimus blood levels

e) Sample Collection /Transport

All samples must be transported according to current UHL guidelines. Samples must be kept at room temperature for short term storage only (<48 hours), thereafter to be kept at 4^{o}C. All samples to be delivered to Transplant Laboratory as soon as possible. **If clinically urgent please phone the Laboratory to indicate this fact and provide patient details.**

Glenfield Hospital (GH):

Samples collected from dedicated fridge on Ward 37 (Transplant) by ward staff each working day. Outside these hours, please phone the Laboratory on 0116 258 4607.

Requesting Clinician to arrange transport to Laboratory via portering service or taxi.

Other locations within the UHL:

Requesting Clinician to arrange transport to Laboratory via appropriate pathology route

Refer to http://insitetogether.xuhl-tr.nhs.uk/pag/pagdocuments/Workplace%20(Site)%20Transport%20UHL%20Policy.pdf for current UHL guidance (accessed ~~09/11/2021~~ ~~19/10/2022~~ 09/11/23)

Non UHL:

Requesting Clinician to arrange transport to Laboratory via local pathology route or via first class post (samples MUST be packaged to conform to postal regulations for pathological samples) or other suitable means (e.g. taxi).

Sample transport to the Laboratory is the responsibility of the requesting unit. Packaging of the sample must comply with current postal regulations.

Samples will not be accepted by hospital reception or the Laboratory if inappropriately labelled, packaged or damaged.

All samples must be correctly labelled and accompanied by the patient's identification details and with relevant contact information to whom the result is to be reported. Samples must be sent in containers that comply with postal regulation and Post office packing instruction 650.

Refer to https://www.royalmail.com/sites/default/files/Guidance-Document-Infectious-Substances-171012.pdf for guidance on postal regulations (accessed ~~09/11/2021 19/10/2022~~ 09/11/23).

Patient Category/Test	Test method	Blood Sample type or tissue, storage	Vol (ml)	Contact Lab in advance?	Turnaround
Renal transplant patient/donor HLA typing	NGS/SSO	EDTA, RT	4-7.5 ml	No	4 weeks for activation on List (electronic/written report)
Deceased organ donor HLA type	Real Time PCR	EDTA, RT	6 ml (adult) 5 ml (paed)	Yes (SNOD)	Within 4 hours to send HLA to NHSBT (email)
HLA antibody single antigen bead	Luminex	Clot, RT	5-9 ml	Yes if urgent	4 weeks for activation on list (see above)
HLA antibody single antigen bead (DSA)	Luminex	Clot, RT	5-9 ml	Yes if urgent	1 week or next day (urgent), latter by prior discussion with Consultant
Recipient and donor cross match	CDC and Flow	Donor EDTA, RT	40 ml	Yes for live donor cross match - book date in advance	2 weeks live donor (electronic/written report), 4-6 hours deceased donor (verbal) 2 weeks
		Spleen, RT	2 cm^3 (spleen)		
		Recipient EDTA	5-9 ml		
		Recipient clot	9-18 ml		

					electronic/written
HLA type, disease association	SSO	EDTA, RT	4-7.5 ml	No	2 weeks paper and electronic report
HLA type, potential hypersensitive drug reaction	SSO	EDTA, RT	4-7.5 ml	No	2 weeks paper and electronic report
Immunosuppressive drug levels	LC-MS referred	EDTA, RT	2-9 ml (adult) 0.5-1.5 ml (paed.)	Yes if urgent	See section 7

Any samples received not as stated above will be rejected for testing as "Incorrect sample received".

7. Reporting of results

Results will be reported either solely or jointly via:

- Proton Renal Database, providing the patient can be uniquely identified upon that database
- UHL Pathology iLab/ICE system
- Hard copy to the requesting location, posted or emailed.
- Emails from nhs.net if email account is on the tested and secure list

NOTE: With the exception of pre-transplant crossmatch results, which will be reported directly to the Consultant Transplant Surgeon and Specialist Registrar; telephoning of results is discouraged. Any result given by telephone will be followed up a report.

Centres without access to Proton must provide NHS email address for transmission of results, otherwise a hard copy will be posted to the requesting location or the result reported by iLab if available.

Any unexpected delays to result reporting will be communicated as soon as possible to the requestor.

<u>Reporting turnaround times</u>

Immunosuppressive Drugs

External service East Midlands Pathology (Nottingham):

Same day for urgent samples if received in Laboratory before 12.30pm for Tacrolimus only. Other samples next working day which also applies to cyclosporin and sirolimus test requests.

All other tests see table above.

8. Sample storage and retention

After testing DNA and sera from all patients and donors are stored frozen (-20^{o}C) in case of future use. Deceased donor spleen and lymph nodes are stored for 3-4 weeks at 4^{o}C. The Transplant Service at UHL, which includes the Transplant Laboratory activity, is licensed by the Human Tissue Authority, (40054).

9. Quality Assurance

The Laboratory participates in the following External Quality Assurance schemes:

Scheme	Clinical Test and details
NEQAS 1B	HLA B27
NEQAS 2A	CDC crossmatch
NEQAS 2B	Flow cytometry crossmatch
NEQAS 3	HLA antibody specificity analysis by Luminex
NEQAS 4A1	HLA typing: first field (low) resolution DNA type A,B,C,DRB1,3/4/5,DQADQB, DPADPB
NEQAS 4A2	HLA typing: DNA second field (high) resolution DNA type A,B,C,DRB1, 3/4/5, DQADQB,DPADPB
NEQAS 7	HLA B*57:01
NEQAS 8	HLA typing for coeliac and other HLA associated diseases
iED	Interpretative educational scheme

10. Distribution List (all @UHL-tr.nhs.uk unless otherwise stated)

Bagul Atul - Transplant and Endocrine Surgeon
Banerjee Somnath- Head of Service Opthalmology
Baines Richard - Consultant Nephrologist
Buswell Lesley (Lesley.buswell@ULH.nhs.uk)
Crotty Charlotte – Transplant Co-ordinator
Duraisingham Sai S – Principal Clinical Scientist
Elwell Rosemary - Transplant Co-ordinator
Faust Guy - Oncology Consultant
Feather Penny (p.feather1@nhs.net)
Frost Danielle – General Manager and Transformation Lead Rheumatology
Jagger Caroline (caroline.jagger@ulh.nhs.uk)
Jesus-Silva Jorge – Head of Renal Service
Martinez Maria - Consultant Renal Transplant Pharmacist
Pattni Sanjeev - Consultant Gastroenterologist, Head of Service
Pringle Luke - Clinical Nurse Specialist
Reeves Melody - Renal Recipient Transplant Co-ordinator
Samanta Rajib – consultant paediatric neurologist
Sampath Raghaven – consultant opthalmologist
Sentance Zack – General Manager Opthalmology
Stephenson Iain – Consultant

Appendix 1

Test prices

Immunosuppressive drug assay charges for Leicester Renal Transplant [from 01/10/2023]:

- CyA or FK within normal working hours £12.00
- Rapamune (sirolimus) within normal working hours £12.00
- CyA/FK or rapamune outside normal working hours Contact Wythenshawe

External users – please enquire
Other assay charges [from 1/4/18]:

- Any other test Contact Head of Laboratory

Typical Assay Charge:

Recipient or Live Donor Tissue Type	£286
Cytotoxic and Flow Crossmatch Assays	£600
Serum Processing	£27
Antibody Characterisation for anti HLA Class I and Class II antibody	£240
Deceased donor tissue type	£516
Patient Registration/Maintenance @ UK Transplant	£64
Individual HLA e.g. B27, B57, B51, DQ, A	£55

Appendix 2

Measurement Uncertainty

(A) Measurement Uncertainty Report For LABScreen Assay

Intra-assay CV is a measure of the variance between data points within an assay, where sample replicates are run within the same plate and can be used to assess the variance in the sample at one time. The cut-off values set for registration of specificities at ODT are subject to an estimate of uncertainty for class I and class II based on the selection of beads. The measurement uncertainty for this assay is continually monitored and adjusted, for details contact the transplant laboratory.

Inter-assay CV is a measure of the variance between runs of sample replicates on different plates that can be used to assess run-to-run consistency. The cut-off values set for registration of specificities at ODT are subject to an estimate of uncertainty for class I and class II based on the selection of beads. The measurement uncertainty for this assay is continually monitored and adjusted, for details contact the transplant laboratory.

The numerical MFI value for positive samples tested by Single Antigen kit is used to assign specificities at ODT; in general following Scientist review all specificities above MFI 2000 are registered as unacceptable with ODT.

(B) Measurement Uncertainty in the Flow Cytometric Cross match

17 replicates of the Flow negative control serum were crossmatched against the same donor cells for both the T and B cell IgG crossmatch sets within the same assay. This was performed in October 2023 due to the imminent introduction of the new FacsLyric flow cytometer. The crossmatch was performed using SOP 1031. The standard deviation of the 17 identical sample's median channel shift (MCS) values was used to calculate the measurement uncertainty for the T and B cell flow crossmatch. An MU of +/- 2SD was chosen as advised in an article written by David Briggs entitled 'Measurement Uncertainty (or turtles all the way down?) published in the BSHI newsletter vol 107 - 2017 issue 2. The %CVs were also calculated for information only. The transplant laboratory has 2 flow cytometers, the FacsCanto and the FacsLryic, therefore the crossmatch was acquired on both machines in order to calculate the assay MU for each machine.

For a flow crossmatch assay acquired on the FacsCanto:

The measurement uncertainty for the T cell IgG flow crossmatch is +/- 14 of the reported MCS. (2.9 %CV)
The measurement uncertainty for the B cell IgG flow crossmatch is +/- 14 of the reported MCS. (2.1 %CV)

For a flow crossmatch assay acquired on the FacsLyric:

The measurement uncertainty for the T cell IgG flow crossmatch is +/- 15 of the reported MCS. (1.2 %CV)
The measurement uncertainty for the B cell IgG flow crossmatch is +/- 68 of the reported MCS. (1.1 %CV)

Please note the FacsCanto machine operates on a 4 log decade range whereas the FacsLyric operates on a 5 log decade range hence the increased variability of the MCS values for the B cell crossmatch on the Lyric. The crossmatch results from both machines have been standardised so that both machines produce the same crossmatch results.

Appendix 3

Reasons for this version:

V25 June 2024 Addition of change in retrospective cross match procedure

V24 Dec 2023 Annual Review. Change of Head of Laboratory details.
Change of turnaround time for activation on waiting list increased to 4 weeks, to allow for completion of required testing. Update staff list. ISO standards have been changed to reflect new ISO15189:2022 standards. Turnaround time for deceased donor HLA typing within 4 hours. Review and update of Appendix 2. Amendment to distribution group.

Previous versions:

V23 Oct 2022 Annual Review. Verfify type from second sample before transplant included in 4.1 and 4.4.
Inclusion of NGS in typing method (sample collection table p.12), replace SSP with Real Time PCR.
Update Quality Assurance section 9. Update Distribution List. Update staff list.

V22 Oct 2021 Update to ISD service information
Update to MU for the LABScreen assay
Minor edits throughout
No training required

V21 March 2021 Revision of wording to meet UKAS requirements
Addition of HLA-B51, HLA-A29, HLA-DQ for disease associations
Update to Flow crossmatch clinical decision values and MU for the B cell crossmatch
Update to distribution list
No training required

V20 Oct 2020: Incorporation of detail relating to complaints and delegation of laboratory director's duties
Major reformatting of document and minor edits throughout
Training required = None

V19 June 2020 Reasons for this version – annual review and amendments: 2 weeks TAT for B57 as per SLA not 1 week. Antibody DSA test TAT incorporated. Minor changes and change to staff list. Incorporation of Billboards 511 & 510 into main body of document

V18 July 2019 Reasons for this version & major changes. Incorporation of Billboard 486: amendment to Estimate of Uncertainty for the flow cytometric cross match appendix 2. Incorporation of Billboard 479: slight editing of NEQAS schemes in section 9 (Quality Assurance). Amendments in accordance with HA760.

V17 April 2018 Reasons for this version & major changes: a major re-write as a User Guide. Incorporation of Billboards 407 & 406 into main body of document. Review history now in Appendix 3.

V16 Apr 2017 Update of Laboratory and Administrative staff list. Removal of reference to high resolution typing sent to NHSBT Sheffield as this is carried out in-house. Addition of measurement of uncertainty information for users. Change accreditation from CPA to UKAS. Inclusion of billboard 329. Other minor changes. Training = 1, none required.

V15 Apr 2016 Review of sample requirements. Updating of Laboratory Staff list & qualifications. Inclusion of Billboard 253 + 254. Removal of reference to CD3 and endotoxin testing. Section 5, (d), amended to include user notification process where excess blood is received and discarded.

V 14 Mar 2015 Change in Head of Laboratory. Change in service provision – endotoxin measurement & CD3 count monitoring are no longer provided by the Transplant Laboratory. Change in sample requirements for Immunosuppressive drug monitoring – EDTA only, no clot. Updating of Laboratory Staff list & qualifications

V13 Sept 2014 Addition of further advice for users to comply with ISO 15189; removal of previous appendix 4. Removal of reference to EFI accreditation status

Ver 12 Aug 2013 Addition of further advice for users to comply with ISO 15189

Ver 11 July 2013: Annual Review. Change in sample requirements. Change in personnel.
Major change in methodology & processes for immunosuppressive drug (ISD) monitoring. Increased reporting time for non-urgent ISD results.
Change in charges for non-renal ISD assays – reduction for FK & CyA, increase for rapamune.
Change in availability of ISD assay out of hours.
Addition of appendix for recording request form allocation (lab use only).
Removal of reference to Sharepoint.
Addition of assumed consent statement for sample received without specific assay request.
Addition of List of key factors affecting results as appendix.
Addition of time limits for requesting additional tests as appendix.

V10 June 2012: Inclusion of information on Virtual Crossmatching and requirement for regular serum samples for anti HLA antibody screening and samples after potential sensitising events. See- Anti-HLA antibody screening and identification section

V9 Mar 2012: Annual review, addition of Share point Document Number
Requirement to add consultant name to all request forms
Update of index
N.B. New automated contact system in place [out of hours]
N.B. Update of sample volumes
N.B. Sample Identification requirement increased to conform to UHL recommendations [full name + ID number + DOB]
Amendment to reporting time for antibody results
Update to staff Listing
Recipient Transplant Coordinator will cover Satellite Renal Units for User input
Updated Internet links for sample packaging
N.B. Increase in charges for non-renal drug requests and tissue typing

Jan 2011: Annual review
Increase in charges for non-renal patient testing
Addressographs no longer acceptable on sample tubes
CyA/FK assays now only done three times per week
Non-urgent weekend CyA/FK assays not assayed
Replaced references to 'UKT' with 'NHSBT'
Replaced references to APEX with iLab/iCM
Removal of requirement for EDTA sample for antibody screening
Removal of requirement for clotted sample for non-renal drug requests only
Change in user communication information
Change in contact name for Proton database updating

V8 Nov 09: Annual review
Addition of Staff qualifications
Update of staffing
Addition of Apex reporting
Update of contact details
Addition of delayed reporting information

Appendix 4

Staff training table

Training required for V25 June 2024

Name of Staff	Training required Y/N	Signature	Date
Clare Collins	Y		
Judith Owusuwaah	Y		
Maureen Bloxham	Y		
David Wimbury	Y		
Pamela Charles	Y		
Nikita Sinha	Y		
Anjali Reuben	Y		
Lauren Parry	Y		
Sheila Smith	Y		
Stephen Weston	Y		
Kirti Odedra	Y		
Kunti Chonilal	Y		
Sarika Parmar	Y		
Rhea McArdle	Y		