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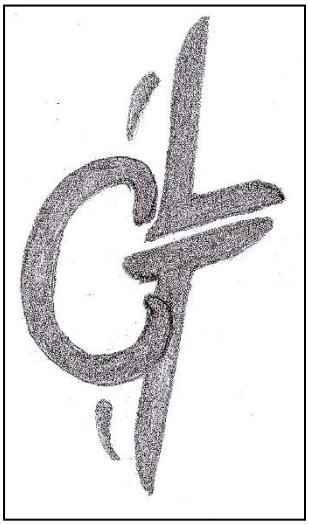
Histocompatibility and Immunogenetics

User's Manual



Contents

1. History of the Laboratory

A Tissue Typing Laboratory has been based at Guy's Hospital for over forty years. It was perhaps presaged by the work of Peter Gorer, who both trained at Guy's and spent most of his working life from 1940 there. It is widely recognised that Peter Gorer was the first person to describe transplantation antigens and formulate an immunological theory of transplantation. He has been described as "undoubtedly the father of histocompatibility antigens."[1]

The Tissue Typing Laboratory was established by Prof Richard Batchelor and stemmed from his pioneering work in the characterisation of HLA antisera. The first renal transplantation in the South Thames region was performed at Guy's Hospital in May 1967. It is sad therefore to relate that serology has almost entirely disappeared from today's laboratory. HLA typing is now based on the detection of variation in the genes that encode HLA antigens. Antibodies that are specific for HLA are now detected using flow cytometry and capture assays using purified antigen.

With Standards an increasingly important consideration, the laboratory takes part in National and International quality control exercises, such as UK NEQAS for H&I, to ensure our results are of the highest possible standard. The laboratory has been accredited by the United Kingdom Accreditation Service (UKAS) to ISO15189 since 2018, laboratory number 9568, and the European Federation of Immunogenetics since 2008 and is approved as a teaching laboratory for Histocompatibility and Immunogenetics by the Royal College of Pathologists.

As tissue typing has become a rather old-fashioned term it was decided to change the name of the laboratory to the Clinical Transplantation Laboratory. This change took place in September 2002, but the function, siting and contact numbers of the laboratory have remained the same.

The laboratory supports a variety of Solid Organ (Renal, Pancreas, Liver, Small Bowel/Multivisceral, Islet and Cardiothoracic) and Stem cell transplant programs, alongside a comprehensive companion HLA typing service for disease association and pharmacogenomics.

The laboratory is open from Monday to Friday 9am to 5pm. Out of hours, weekends and bank holidays, the laboratory operates an on-call system for emergency work.

2. Laboratory Contacts

Postal address:

Clinical Transplantation Laboratory
3rd Floor Borough Wing
Guy's Hospital
Great Maze Pond
London SE1 9RT

Website:

Clinical Transplantation Department | Synnovis

Laboratory Director and Consultant Clinical Scientist

Dr Olivia Shaw, PhD FRCPath email: olivia.shaw@viapath.co.uk
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Deputy Director and Consultant Clinical Scientist

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Principal Clinical Scientists

Dr Louise Howe, PhD FRCPath email: louise.howe@viapath.co.uk
020 718 81540
Operations Manager

Emma Lougee email: emma.lougee@viapath.co.uk
020 718 81534
DNA Section Head

Kamla Reddi, DipRCPath email: kamla.reddi@viapath.co.uk
020 718 81540
Serology Section Head

Laboratory queries	0207 188 1540
Laboratory email	gst-tr.CTL@nhs.net
On-call pretyper	07889 925 647
On-call crossmatcher	07850 814 971
On-call H&I for Cardiothoracic	07973296913

The department has a number of staff dedicated to the routine workload supplemented by Trainee Clinical & Biomedical Scientists, Associate Practitioners and Medical Laboratory Assistants.

3. Laboratory Tests

The laboratory performs a number of tests designed to facilitate successful transplantation and aid clinical diagnosis. These include:

- HLA typing - for solid organ transplantation, haematopoietic stem cell transplantation (HSCT) and as companion testing for disease association and pharmacogenetics.
- HLA-specific antibody screening and identification – routine and urgent
- Crossmatching – routine and urgent
- Post-Transplant monitoring – Donor specific antibody and CD3/19 counts for immunosuppression monitoring.

We also take an active role in research projects.

The turn-around times for each test can be found in the table in section 12.

4. HLA Typing

HLA typing is now performed using a combination of molecular techniques, PCR-SSP, Luminex PCR-SSOP, qPCR and next generation sequencing (NGS). The procedure is performed on 10ml EDTA anticoagulated blood. The laboratory can offer advice on HLA typing samples that have been taken using different anticoagulants. **In particular, it is important to inform us if the sample is likely to contain heparin.**

Routine HLA typing is carried out to a low, medium or high resolution for the definition of HLA-A, -B, -C, -DRB1, -3, -4, -5, -DQA1, -DQB1, –DPA1 and -DPB1 to support solid organ transplantation, HSCT and study work.

HLA Typing requests can be made via the EPIC system (within our partner Trusts) or using our paper request form. Samples for HLA typing should be labelled with at least our minimum requirements (detailed in section 15), and, stored and transported at ambient temperature. Ideally these should be delivered within 7 days of being taken. The laboratory will endeavour to extract DNA from samples received after 7 days but cannot guarantee sufficient quantity or quality. If samples do not arrive within this timeframe and there are issues with DNA extraction, the samples may be discarded. If this occurs the sender will be informed.

HLA typing results will be reported either via EPIC or as a PDF report via secure NHS.net email depending on where the request originated.

All reports will include patient identifiers, sample type tested, date sample received, local lab sample identifier, and the HLA typing result in both a molecular and serological type equivalent. The report will also include clinically relevant comments dependant on the information provided. For HLA typing reports for requests for HLA and disease association or pharmacogenomics this will include whether the result supports a particular clinical diagnosis or adverse drug reaction association. For HLA typing reports for solid organ transplantation or haematopoietic stem cell transplantation (HSCT), information will be supplied regarding the HLA mismatch with any relevant donors. For HSCT patients and donors, the request for a confirmatory HLA typing sample if transplantation is to proceed is also included in the report.

5. HLA Antibody Screening

The accurate definition of antibodies specific for HLA antigens plays an important role in the selection of suitable patients for both solid organ and some stem cell transplants.

BSHI/BTS guidelines for solid organ transplantation require that a fresh sample of patient's serum is screened for the presence of HLA specific antibody at least every three months whilst awaiting transplant[2]. This means all potential solid organ transplant recipients who are registered with Organ and Tissue Donation and Transplantation (OTDT) for a transplant should supply the laboratory with a serum sample at least every three months, and ideally provide a monthly sample whenever possible. The aim of this protocol is to provide a prompt crossmatching service, without the need to call patients every time they are considered for transplantation.

Up to 2ml of serum from each sample is frozen and stored at -20°C. In line with Royal College of Pathologists recommendations, these historic samples are stored for a minimum of 30 years or the lifetime of the patient.

The laboratory must be informed of any potentially sensitising events (i.e. transfusion of blood products; infection; pregnancy; skin-grafting; previous transplantation or reduction in immunosuppression following previous transplant) in any patient likely to require a transplant. It is important that a serum sample (7 ml clotted blood) is taken at 10 - 14 days and at one month after a potential sensitising event. This sample should be sent to the

laboratory for the detection of any changes in the level of HLA specific antibody. For Cardiothoracic patients active on the waiting list who receive blood transfusions, samples should be provided twice weekly for the first three weeks post transfusion.

Our routine test for antibody detection utilises Luminex technology. This method is based on antibody binding to purified HLA Class I or Class II antigens attached to Luminex microspheres. The antigens are selected to cover the gamut of HLA specificities. Antibody bound to the beads is detected using the Luminex multiplex instrument, which is based on the principles of flow cytometry. A positive signal indicates whether IgG antibodies to HLA Class I and/or II antigens are present, but does not give information on specificity. It is a technique that is both rapid and sensitive.

Sera which have a positive Luminex antibody screen are subsequently characterised using a combination of Luminex microspheres with known HLA class I (HLA-A, -B and –Cw), and HLA class II (HLA-DR, -DQ and –DP) which form antibody identification panels. Antibody specificity is confirmed on a subsequent sample using a different Luminex antibody identification kit.

We utilise a variety of commercially produced Luminex based kits, all CE marked, which provide a range of testing strategies and is not reliant on one production technique. Wherever possible we will only confirm an antibody as 'real' when it is detected using methods from more than one manufacturer to avoid the detection of antibody to denatured antigen known to be an artefact of the bead production process, and there for not clinically significant.

HLA Antibody testing can be provided for patients both awaiting a transplant and post transplant for the detection of donor HLA specific antibody, to aid in the diagnosis of antibody mediated rejection.

HLA Antibody Screening requests can be made on EPIC (within our host Trusts) or using our paper request form.

These samples should be labelled with at least our minimum requirements (detailed in section 15), be stored at ambient temperature and be delivered to the laboratory as soon as possible, certainly within 10 days of being bled. If they are received after this time period they will be automatically discarded and the sender informed. Failure to store and transport the samples in this way may lead to unacceptable haemolysis of the sample, requiring discard.

Sample, storage and transport requirements are listed in section 12.

Antibody tests may be requested for routine or urgent testing depending on how quickly the result is required. Requests for urgent testing should be clearly noted on the request form, all samples will be treated as routine unless notified otherwise.

Antibody test results will be reported either via EPIC or as a PDF report via secure NHS.net email depending on where the request originated.

All reports will include patient identifiers, sample type tested, date of sample collection, local lab sample identifier, a positive or negative result for both Class I and II and a % Calculated Reaction Frequency (%cRF) for each sample tested. For patients awaiting cardiothoracic transplantation multiple %cRF values will be reported in line with the antibody screening policy to reflect antibody levels and risk. In renal patients who are pre-transplant we will additionally report the Match points. Match points and %cRF are calculated using the latest available version of tools provided by OTDT and can be found on their website -

https://www.odt.nhs.uk/transplantation/tools-policies-and-guidance/calculators/

Match points are a value from 1 – 10 which give an indication as to the 'matchability' of a patient, or how likely they are to receive a favourably matched graft. A score of 1 – 3 indicates the patient is 'easy' to match, 4 – 7 indicates they are 'moderate' to match and 8 – 10 indicates they are 'hard' to

match. The score takes into account the patient blood group, HLA type and HLA specific antibody profile. Where a blood group has not been provided a default of blood group O will be used.

%cRF is the % calculated reaction frequency. This is based on the HLA antibody a patient produces in relation to the HLA types of a pool of 10000 UK deceased donors. A 0% cRF indicates that a patient produces no significant HLA specific antibody and would be expected to have a negative crossmatch with all blood group compatible donors in the pool. Conversely a cRF of 100% indicates that the patient produces antibody that would result in a positive crossmatch with all blood group compatible donors in the pool.

Additionally, non-routine antibody testing can be performed in a guidance and/or research capacity. To date the tests described below are not part of the CTL UKAS accredited scope, and any tests requested, and results received, should be treated as being of research level significance.

'Non-HLA' specific antibody testing can be undertaken. Targets on the donor organ endothelium beyond known mismatched HLA antigens are becoming an increasingly evident cause of antibody mediated rejection episodes in all solid organ groups[2]. Evidence as to which targets are important is currently sparse however, although some are clearer than others. CTL perform antibody screening for detection of Human Neutrophil Antigen – 3a/b (HNA) specific antibodies as part of the work up for solid organ transplantation. Currently the results of these tests are used as a guide to assist in crossmatch analysis within the lab, where an unexpected positive crossmatch result could be due to mismatches at HNA-3. The presence of this antibody has been associated with renal transplant rejection episodes in presented case series[2]. Where a positive crossmatch is thought to be due to the presence of these antibodies the case and potential implications will be discussed with the clinical team involved.

Luminex based kits for other so called 'Non-HLA antibody' or 'Auto-Antibody' targets are also available. The clinical implications of these antibodies are largely unknown, however testing can be performed on request to assist in cases where AMR is detected on biopsy with no HLA DSA detected.

Complement binding (C1q) or Complement fixing (C3d) HLA specific antibodies can also be tested for on request. The functional ability of an antibody to interact with, and initiate, the complement cascade has been linked with being of higher risk to transplant outcome[2]. These antibodies are largely those of a higher titre and all specificities will be detected within a patient profile as part of the routine antibody screening methods. However, for highly sensitised patients, with few transplant options, these tests can be used to assess which antibodies are likely to be more clinically relevant to the outcome and provide assurances in the ability to disregard the presence of non-complement fixing antibodies as part of a higher risk strategy to achieve transplantation in this patient group.

Please contact the laboratory directly if further information is required.

6. Crossmatching

The crossmatch is a critical compatibility test prior to renal transplantation. Donor cells are mixed with recipient serum to check for the presence of antibody in the recipient to the proposed donor's HLA antigens. A positive crossmatch is a contraindication to the transplant.

Crossmatching using flow cytometry has superseded the traditional technique of CDC crossmatching, because it is a more sensitive and objective test, and enables us to identify antibodies directed at T cells and B cells and thereby infer whether the antibodies are directed at HLA class I and/or II.

The serum samples selected for crossmatching are important. Generally we choose the most recent serum sample plus the most positive historic sample(s). This selection depends on antibody screening and knowledge of sensitising events (see appendix 1).

Donor lymphocytes are separated from peripheral blood for living donor transplants, and we may use cells from peripheral blood, spleen or lymph nodes for deceased donor transplantation.

All routine (living donor) crossmatch tests must be pre-booked with the laboratory via telephone or email, detailed in section 15. Samples taken for crossmatching should be labelled with at least our minimum requirements (detailed in section 15), and, stored and transported at ambient temperature, arriving for testing within 24 hours of being taken. If they are received beyond this time they will be automatically discarded and the sender informed.

Crossmatch test results will be reported as a PDF report via secure NHS.net email.

All reports will include patient and donor identifiers, sample type tested, date of the most recently tested sample, local lab sample identifier, a positive or negative result for both T and B cells, the match grade between donor and recipient and a current and peak % Calculated Reaction Frequency (%cRF) for the recipient. There will be additional interpretive comments to explain the result achieved and recommendations on immunological risk where relevant.

Please note - It is known that some mono- and poly-clonal antibody treatments, such as Rituximab, ATG or Campath can cause unexpected positive reactions in the crossmatch. It is therefore very important to inform the laboratory if a patient has received any antibody based therapy to ensure this information is available at the time of a potential crossmatch to aid sample selection and result analysis. Failure to do this could lead to a patient being denied an otherwise suitable transplant.

7. CD3 Counts – Post-Transplant Monitoring

The Clinical Transplantation Laboratory provides a service for T cell (CD3 positive) monitoring. This gives an estimation of the patient's absolute CD3 positive cell count.

For renal and SPK recipients - patients that have an aggressive rejection or who are highly sensitised, may require immunosuppression with a monoclonal or polyclonal reagent directed at T lymphocytes (e.g. ATG). The aim is to maintain a level of no more than 30 T cells per µl of peripheral blood during the 10 day treatment course. Retrospective studies have shown this to be the optimal level for reversing rejection whilst retaining an adequate resistance to infection. This data is available on request in CTL-REC-123.

For Cardiac recipients – Patients who receive heart transplants often receive induction therapy of ATG at the time of transplant. CD3 cell counts at day 2 and 5 post transplant can guide the clinical team around the timing of additional immunosuppression requirements.

In addition, an estimation of absolute CD19 positive cell counts (B cells) can be determined pre and post Rituximab therapy to monitor the effectiveness of the drug.

CD3/19 count requests can be made on EPIC (within our host Trusts) – request 'ATG Monitoring' - or using our paper request form. If testing is required outside of routine laboratory hours the laboratory should be contacted in advance to organise for testing to be undertaken.

Samples taken for CD3/19 counts should be labelled with at least our minimum requirements (detailed in section 15), be stored and transported at ambient temperature, arriving for testing within 1 day of being taken. If they are received beyond this time they will be automatically discarded and the sender informed.

CD3/19 test results will be reported either via EPIC or as a PDF report via secure NHS.net email depending on where the request originated.

All reports will include patient identifiers, sample type tested, date of the sample tested, local lab sample identifier and an absolute CD3 and CD19 cell count per µl. There will be additional interpretive comments to explain the result achieved and recommendations on ATG dose required where relevant.

8. Deceased Donor Procedure

The Clinical Transplantation Laboratory operates three on-call rotas:

- The first for HLA typing of potential donors before organ retrieval ('Pretyper');
- The second for the crossmatching of potential non-cardiothoracic solid organ recipients against an offered donor. ('Crossmatcher')
- The third is for assessment of Cardiothoracic organ donor offers for recipients on the waiting list. ('Cardiothoracic')

The Pretyper, Crossmatcher or Cardiothoracic on call teams may be contacted by phone (numbers listed above) or via Guy's switchboard (020 7188 7188).

Additionally, there is a Consultant Clinical Scientist available 24/7 to provide advice and support as needed. They can also be contacted by phone or via Guy's switchboard (020 7188 7188).

8.1 Pretyping

The regional SNOD's provide 5 - 10ml EDTA donor blood for pretyping, which is sent to Richard Bright Ward at Guy's and collected from there by the Pretyper.

A rapid typing technique allows us to report a donor HLA type within four hours. The donor HLA type is reported to the OTDT Duty Office, who then perform a matching run for the selection of suitable recipients.

8.2 Crossmatching – Renal and other non-Cardiothoracic organ recipients:

Donor lymphocytes are isolated from the peripheral blood or donor spleen and incubated with a selection of potential recipient sera (minimum 2), depending on the sensitisation status of the recipient. The advantage of peripheral blood crossmatching is that it will often allow a crossmatch result to be available before the donor organs have been retrieved, thus removing all impact of the crossmatch on the cold ischaemia time.

Results are reported to the transplant surgeon or recipient co-ordinator responsible for the selected recipient(s).

A small volume of donor cells isolated from the spleen is frozen and stored.

DNA is also extracted from the donor cells for confirmatory HLA typing.

It is possible to omit a prospective crossmatch prior to deceased donor organ transplant if the intended recipient meets a set of criteria as agreed between the local the transplant team and CTL. This is known as a virtual crossmatch. The decision to issue a virtual crossmatch report is based on it being a first organ transplant, recipient antibody screening history, absence of any potential donor specific antibody, frequency of testing and donor HLA type. The decision to issue a virtual crossmatch report is made exclusively by the on-call crossmatcher or consultant clinical scientist.

If a transplant proceeds, the donor spleen/lymph node material must be provided so a retrospective crossmatch and confirmatory HLA typing can be performed.

8.3 Cardiothoracic assessment -

For cardiothoracic transplants, where extended cold ischaemia time has an unacceptable influence on transplant outcome the physical crossmatch is performed retrospectively. Patients and potential donors undergo a virtual crossmatch assessment prior to transplantation. Utilising the donor HLA type, provided by OTDT, the patients current and historical antibody profile will be assessed to determine if the recipient currently, or has previously, produced antibodies specific to any mismatched donor HLA antigens which could adversely affect the transplant. In some cases the interpretation is 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 OTDT 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.

If a transplant proceeds, the donor crossmatch material must be provided so a retrospective crossmatch and confirmatory HLA typing can be performed.

On occasion, for highly complex patients, it is possible for the laboratory to perform a prospective physical crossmatch using donor peripheral blood, the results of which can be available before the organs are retrieved. This should be discussed on a case by case basis with the Consultant Clinical Scientist on call.

9. Living Donor Transplants

The quality of living donor transplantation and the shortage of deceased donors have led to growth and an increased interest in living donor transplantation across a number of solid organ transplant groups. Whilst living kidney donor transplantation is the most common there is also activity in living liver, small bowel and lung lobe transplantation. Although still important, there is evidence that the influence of HLA matching on graft survival may not be so profound in living donor kidneys.

In order to facilitate living donor transplant programmes we have developed a separate work up protocol, including HLA typing, antibody screening and crossmatching, and we liaise closely with individual transplant centres to provide a service tailored to their needs. Based on a variety of factors, including previous transplant, sensitisation status and relationship of donor to recipient, we may request to do a flow cytometry crossmatch early in the living donor work-up to assess the suitability of a potential live donor, an initial crossmatch. In accordance with accreditation standards a 'final' flow cytometry crossmatch is always performed shortly before the transplant.

10. Clinical Advice and Staff Availability

All results provided by CTL contain some level of interpretation. For any further information or clinical advice relating to the results the laboratory can be contacted as shown:

General advice

Working hours only (09:00 – 17:00 weekdays): 0207 188 1540

Advice or information provided by a HCPC registered scientist

Detailed Patient Advice

Working hours (09:00 – 17:00 weekdays): 0207 188 1540

Advice provided by a HCPC registered scientist, or, where required, by a Consultant Clinical Scientist.

Out of Hours*: 07850 814971

Advice provided by the on call crossmatcher (HCPC registered), or, where required, the query will be forwarded to the on call Consultant Clinical Scientist.

**Please note, the out of hours service should only be used for urgent queries that cannot wait until the next working day. General queries relating to routine results should not be put through this route.*

11. Patient Registration, Activation and Suspension from the Transplant Waiting List

All transplants must by law be registered with Organ Donation and Transplant (OTDT). No patient will be offered an organ transplant until OTDT have received a copy of their registration form.

Registration on to the waiting list for patients who require transplants that do not involve a kidney and/or small bowel and/or islets do not require HLA related information and therefore do not involve CTL in the registration process. However if HLA related activity is involved in the decision to accept a donor organ then the lab should be informed once the patient is registered and ready to be activated.

For transplants involving a Kidney or Small Bowel or pancreatic islets - Demographic data should be completed on the online OTDT registration form on NTxD by a senior nurse or a doctor. The form should then be sent to the Clinical Transplantation Laboratory for completion of the immunological data. **To avoid errors a copy of the patient's blood group should accompany the registration form.**

Patients may be registered as *active* (fit for transplantation) or *suspended* (e.g. with an acute illness or on holiday).

For patients registered for a renal or SPK transplant, once a patient is registered, notification of changes to patient status (activation/suspension/removal), and the reason why, should be made in writing via CTL email and CTL staff will access the NTxD database to make the change.

Patients returning to the transplant waiting list after a failed transplant, and those transferring from another centre, require a new registration form.

For patients for whom immunological data is required pre-transplant – Renal, SPK, Islets, Small Bowel, Cardiothoracic - Prior to activation on the waiting list patients must have completed a minimum set of tests including HLA typing on two separate samples and HLA antibody screening on two separate samples. Further tests may be required to resolve ambiguous results prior to activation.

OTDT needs to be informed of all living kidney transplants that occur following the transplant. CTL are responsible for completing a Living Kidney Donor HLA Report form and the appropriate transplant unit are responsible for completing The Human Tissue Authority-Form B. Once both forms are received by OTDT the patient status can change to transplanted.

12. Sample, storage and transport requirements.

The table below lists the sample type, volume and container required for the tests performed by this laboratory. The storage requirements of the samples prior to dispatch and the time frame within which they must be delivered to the laboratory are also listed. Failure to provide the correct sample may mean we are unable to perform the test requested. Samples which have been stored in inappropriate conditions, or their arrival in the Laboratory has been delayed may show evidence of haemolysis or low cell counts. In such an instance we will discard the sample. Our Users will be informed why the sample was discarded and asked to provide a fresh sample. We regularly monitor our sample discards, if trends relating to a particular unit are identified we will contact the unit to discuss a solution. Samples must also be labelled as per guidance in section 15.

Test Requested	Sample Required	Sample Storage and Transport Requirements	Time to report from sample receipt
HLA typing	10ml EDTA blood	Ambient temperature Stable at least ~7 days	2 weeks
HLA typing for disease association (eg B*27, B*51)	10ml EDTA blood	Ambient temperature Stable at least ~7 days	2 weeks
HLA typing for drug hypersensitivity (eg B*57:01)	10ml EDTA blood	Ambient temperature Stable at least ~7 days	5 working days
Deceased donor pretyping	5 - 10ml EDTA blood	Ambient temperature Urgent transport	4 hours
Deceased donor PBL crossmatching	30ml EDTA blood	Ambient temperature Urgent transport Must arrive within 1 day of being bled	3 hours
HLA antibody screening (pre-or post-transplant)	7ml blood (no anticoagulant)	Ambient temperature Stable up to 10 days	Routine – 10 working days Urgent – 1 day
Crossmatch (recipient)	7ml blood (no anticoagulant)	Ambient temperature Stable up to 10 days	Initial xm – 10 working days Final xm - 3 working days
Crossmatch (living donor and autologous)	20ml EDTA blood	Ambient temperature Must arrive within 1 day of being bled	Initial xm – 10 working days Final xm - 3 working days
CD3 / CD19 counts (transplant monitoring)	10ml heparinised blood or 10ml EDTA blood	4°C fridge or Ambient temperature Must arrive within 1 day of being bled	Same day

13. Sample Dispatch

This laboratory receives samples from a wide variety of sources ranging from GP practices and satellite renal units through to in-patient hospital wards. Delivery of these samples may be in-person, via the portering system or via dedicated couriers and Royal Mail. It is the responsibility of the user/sender to collect, package and send specimens according to these guidelines and the relevant legislation in force, and ensure that the samples are transported in suitable and safe conditions.

Clinical specimens should be handled and stored in a manner that best preserves its integrity within the likely timeframe before delivery to the laboratory.

Unless ordering within EPIC, dedicated sample request form/sample bags are available from the Laboratory. This form should be completed in full, to include all relevant patient information, and also include an address for report destination.

The transport of blood samples by post or air must comply with UN 602 packaging requirements. This is a basic triple packaging system designed to protect all personnel along the delivery chain. 602 packaging is re-usable. Please refer to Appendix 2 for a list of UN 602 packaging suppliers.

If samples are received packaged incorrectly and are deemed to have had their integrity compromised, or are packaged in such a way that could have jeopardized the safety of the carrier or general public, the original sender will be contacted immediately to ensure such an occurrence is avoided in the future.

Routine samples can be delivered directly to the laboratory during our normal working hours. Samples, routine or urgent, that are delivered outside our normal working hours should be clearly labelled for the attention of the Clinical Transplant Laboratory and sent to Richard Bright Ward, 6th Floor Borough Wing, Guys Hospital. Upon delivery the staff on the ward will notify the on call team of the samples arrival.

Transport of organs, solid organ donor material or peripheral blood for pre transplant crossmatching of deceased donors is arranged by OTDT.

14. Booking in tests.

The Clinical Transplantation Laboratory provides a service for many Transplant Units. Routine crossmatching will generally only be performed between the hours of 9 – 5 Monday to Thursday and all such tests **MUST** be pre-booked with the laboratory prior to bleeding and sending of samples. Samples for crossmatching are required to arrive in the laboratory for testing within 1 day of the patient being bled. Pre-booking of these tests ensures that prior to the samples arriving we have sufficient information regarding the patient and donor, as well as sufficient capacity to perform the test. In addition it allows us to follow up on samples which have not arrived by lunchtime on the day they are due giving sufficient time for these samples to be located and tested before they become too old.

HLA typing blood and clotted blood for antibody screening do not have to be booked with the Laboratory, and can be sent at any time, provided they conform to the guidelines in the table above.

15. Minimum Labelling Standards for Testing

The Laboratory will not accept specimens that do not meet minimum labelling standards.

- It must be possible to interpret handwritten specimens (with the aid of request form)
- Specimens will be accepted with clearly printed sticky labels if that is the policy/process of the patient unit.
- Specimens must be labelled with the patient's forename and surname. (An initial in place of the first name is acceptable if there is a hospital number and / or a date of birth)
- Specimens must be labelled with the patient's hospital number and / or date of birth
- Details on the specimen must match the details on the request form
- Sample labels must not have been tampered with, overwritten, corrected or obscured by additional labels, and must relate to the individual from whom the contents has been obtained.
- The request form must include an indication of the test required or a diagnosis
- EPIC requests will be accepted without paperwork, though the laboratory may not be able to comment on the clinical significance of results if there is no diagnosis or clinical details provided

16. Quality Assurance and Quality Control

The laboratory takes part in all the UK NEQAS schemes relevant to service delivery for its external quality control. We have established an internal quality control process, to monitor our routine procedures. We have ISO15189 accreditation with UKAS and this is subject to re-inspection every year across a 4 year cycle. We have full accreditation with European Federation of Immunogenetics and this is subject to full re-inspection every three years, with annual self-inspection in the intervening years.

17. 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 Director of Service.

18. 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.

Appendix 1.

CROSSMATCHING POLICY FOR POTENTIAL RENAL TRANSPLANT RECIPIENTS

All potential transplant recipients require a flow cytometry crossmatch (FCXM), whether they are to have a deceased donor transplant or a living donor transplant. For deceased donors the crossmatch must be performed on receipt of the donor crossmatching material. A virtual crossmatch may be authorised by the on-call crossmatcher if appropriate for urgent deceased donor transplants, but a retrospective crossmatch must be performed after the transplant. For living donor transplants the crossmatch must be performed within 10 days prior to the transplant date. In exceptional circumstances the period between the final crossmatch and day of transplant may be extended but this must be discussed with CTL and authorised by the Consultant Clinical Scientist.

If a patient does not have a stored serum sample taken within the last 3 months the patient will not be crossmatched unless a fresh serum sample is obtained.

Recipients awaiting transplants may be classified as either unsensitised or sensitised, depending on the level of HLA sensitisation:

Unsensitised:

HLA antibody screening is negative and has had no previous transplants

Sensitised:

HLA specific antibody has been detected

All patients who have had a previous kidney transplant are classified as sensitised whether or not HLA-specific antibody has been detected

Users of the CTL services have requested that all recipients of living donor kidneys should be classified as sensitised, whether or not HLA specific antibody has been detected.

It is not possible to have one absolute rule for serum sample selection for all patients due to the individual nature of each patient's sample frequency and screening history. For all patients however a minimum of 2 serum samples must be selected for crossmatching.

The following samples should be used in a crossmatch for an unsensitised patient

- The most recent serum sample (no more than 3 months old) must be tested
- If the most recent serum sample is more than 3 months old the patient cannot be crossmatched with any donor unless a fresh serum sample is obtained.
- The most recently screened sample, where this is different to the most recently received sample.
- An additional sample/s may be tested at the discretion of the crossmatcher
- A positive flow crossmatch is when the RMF (relative median channel shift) is ≥ 4.0

The following samples should be used in a crossmatch for a sensitised patient

- The most recent serum sample must be tested (no more than 3 months old) must be tested
- If the most recent serum sample is more than 3 months old the patient cannot be crossmatched with any donor unless a fresh serum sample is obtained.
- The most recently screened sample, where this is different to the most recently received sample.

- The sample with the highest calculated reactivity during the last 2 years must also be tested
- Additional samples may be tested at the discretion of the crossmatcher based on the patient's screening history
- A positive flow crossmatch is when the RMF (relative median channel shift) is ≥ 2.3

It is not always necessary to carry out an initial crossmatch (prior to the final crossmatch) for living donor transplants, though we will perform an initial crossmatch whenever our Users specifically request one

An initial crossmatch must be performed if:

- The patient has at any time produced HLA-specific antibody
- The patient has had a previous transplant whether or not HLA-specific antibody has been detected
- The recipient is the mother or wife/female partner of the potential donor, or
- The User requests an initial crossmatch

There is no need to perform an initial crossmatch if:

- The patient has not had a previous transplant and has been shown to be consistently negative for HLA specific antibody, testing a minimum of two serum samples, unless the recipient is planned to have a transplant from her partner or children

The following samples should be used in a living donor crossmatch, and must include a minimum of two samples for testing -

- The most recently received sample
- The most recently screened sample, where this is different to the most recently received sample.
- The sample with most HLA specific antibody in the past two years
- Any samples in which potential donor specific antibody has been detected.
- At least one sample that has been used in a previous crossmatch (if it has been necessary to perform an initial crossmatch)
- A positive flow crossmatch is when the RMF (relative median channel shift) is ≥ 2.3

Appendix 2. UN Approved 602 Packaging Suppliers

Dangerous Goods International
Unit C8
Heathrow Corporate Park
Green Lane
London
TW4 6ER
Tel: 020 88140404

Mega-Pak Ltd
Unit 12 Banbury Avenue
Slough Trading Estate
Slough
SL1 4LH
Tel: 08003283378

19. References

[1] Leslie Brent (1997) In: "A History of Transplantation Immunology" Academic Press. p 177

[2] BSHI AND BTS UK GUIDELINE ON THE DETECTION OF ALLOANTIBODIES IN SOLID ORGAN (AND ISLET) TRANSPLANTATION - British Transplantation Society