



SCOTTISH NATIONAL BLOOD TRANSFUSION SERVICE

HISTOCOMPATIBILITY & IMMUNOGENETICS LABORATORY USER MANUAL

EDINBURGH

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CONTACT DETAILS

Histocompatibility and Immunogenetics Laboratory
Edinburgh and South East Scotland Blood Transfusion Service
Royal Infirmary of Edinburgh
51 Little France Crescent
Edinburgh EH16 4SA
Tel: 0131-242 7528
Fax: 0131-242 7530

CONSULTANTS (HISTOCOMPATIBILITY & IMMUNOGENETICS)

Prof Phil Dyer (Consultant Clinical Scientist)
Tel: 0131-242-7521/7533 Mob: 07970-987710
Email: phil.dyer@nhs.net

Dr David Turner (Consultant Clinical Scientist)
Tel: 0131-242-7521/7534 Mob: 07788-431401
Email: david.turner2@nhs.net

CONSULTANT (PLATELET IMMUNOHAEMATOLOGY)

Dr Lynn Manson (Consultant Haematologist)
Tel: 0131-242-7522/7527
Email: lynnmanson@nhs.net

H&I LABORATORY TECHNICAL HEAD

Karen Stewart (BMS 3)
Tel: 0131-242-7528
Email: karenm.stewart@nhs.net

OUT OF HOURS REQUESTS

Platelets: (Platelet antibody screening and HIT tests only)

Please phone the Blood Transfusion Service on 0131-242-7501 and ask for the BTS/Haematology Specialist Registrar

Solid Organ: There is an on-call technician for solid organ transplant related work. It is the transplant co-ordinator's responsibility to call out the technician if needed.

A Consultant Clinical Scientist is also available 24/7 for clinical advice relating to any results generated within the H&I laboratory.

AIMS OF THE LABORATORY

1. To provide a comprehensive Histocompatibility Service in support of the East of Scotland Renal Transplant Unit, The Scottish Pancreas Transplant Unit and the Scottish Liver Transplant Unit.
2. To provide low and high resolution HLA typing for potential haematopoietic stem cell transplant recipients and donors.
3. To provide a comprehensive Platelet Immunohaematology service to support patients who are refractory to platelet transfusions, suspected cases of neonatal alloimmune thrombocytopenia and suspected cases of heparin-induced thrombocytopenia.
4. To maintain and improve the service in response to our users' needs and therefore enhance our position as a centre of excellence in the provision of Histocompatibility and Platelet Immunohaematology services within Scotland.

QUALITY ASSURANCE

A Quality Management System monitors and audits all aspects of the service. All laboratory investigations and clerical procedures are governed and maintained by compliance with the SEBTS Quality Manual, Management Procedures and relevant Standard Operating Procedures (SOPs).

Standards of testing are maintained by the rigorous use of internal quality assurance protocols and through participation in appropriate UK National External Quality Assessment Schemes (UK NEQAS).

QUALITY ASSESSMENT AND EXTERNAL AUDIT

A copy of last year's participation certificate and results summary is available, on request, for each of the following:

UK NEQAS for Histocompatibility and Immunogenetics

BBTS – Platelet Serology Working Group (Organised by NIBSC)

ACCREDITATION

All SEBTS laboratories are accredited through Clinical Pathology Accreditation (UK) Ltd (CPA).

An application has been made (May 2009) for the H&I laboratory to become European Federation for Immunogenetics (EFI) accredited.

HUMAN TISSUE ACT (SCOTLAND) 2006

From April 2007 the laboratory has been in compliance with the Human Tissue Act (Scotland) 2006.

CURRENT RESEARCH/DEVELOPMENT PROJECTS

Current R&D projects include: development of antibody identification techniques to support HLA incompatible transplantation and post transplant monitoring, development of Flow cytometry based assays for monitoring antibody levels to support ABO incompatible transplantation and evaluation of high resolution HLA typing by PCR-SBT.

GENERAL LABORATORY INFORMATION

LABORATORY HOURS

Routine	Monday to Thursday	08.30 to 17.00.
	Friday	08.30 to 16.30.

Last receipt for samples is 17.00 on Thursday as some tests require fresh samples and so cannot be left from Friday to Monday. Samples can be accepted on a Friday for some tests with the prior agreement of the laboratory.

SAMPLE REFERRALS

Samples for disease association testing should be sent to the Dundee H&I Laboratory, East Scotland Blood Donor Centre, Dundee, DD1 9SY. Any samples received into the Edinburgh H&I lab are referred to Dundee for testing.

Samples for MAIPA testing are sent to the Aberdeen H&I Laboratory, North East Scotland Blood Donor Centre, Aberdeen, AB25 2ZW.

SAMPLE LABELLING

Please note failure to adhere to the sample labelling and packaging requirements may result in sample rejection

It is preferable that specimen details are filled in by hand. The following table shows the minimum information required on the sample request form and sample tube

Minimum Data Set Required (In line with LUHND mandatory dataset)	
Forms	Specimens
Surname	Surname
Forename(s)	Forename(s)
Date Of Birth	Date Of Birth
Location	Location
Unique Hospital/CHI/A&E/or Major Incident Number (if not available first line of address and postcode)	Sample date and time
Gender	

Other important additional information:

1. Name of Clinician/Consultant requesting the test
2. Tests requested
3. Clinical details
4. Date of sample

This information is on the reverse of the request form.

If addressograph labels are used for sample request forms/sample tubes the responsibility is with the clinician/responsible person taking the sample. However, please note addressograph labels are NOT acceptable on SAMPLE TUBES for transfusion related work.

REQUIREMENTS FOR SENDING SAMPLES BY ROYAL MAIL

Samples must be packaged in accordance with Packaging Instruction P650 (UN3373, Diagnostic Specimens) www.izvg.co.uk/regulations.pdf. Briefly this states:

The packaging shall consist of three components

- a) a primary receptacle;
- b) a secondary packaging; and
- c) an outer packing.

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

For carriage, the mark illustrated below 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 2mm; the letters and numbers shall be at least 6mm high.



REPORTING TIMES

The laboratory aims to meet the following targets in reporting results. Any urgent requests should be discussed with the laboratory.

HLA Type (HLA-A,B,C,DR,DQ and DP, where applicable)	1-2 weeks
HLA Antibody Screening	1-2 weeks
HLA Type of local donor	4-5 hours
Crossmatch result (Renal cadaver donor, from receipt of tissue samples)	4-5 hours
Crossmatch result (Live Renal Donor)	1-2 days
Crossmatch Result (Liver cadaver donor)	1-2 days
Platelet testing (initial screen and HPA type)	1-2 days
Platelet testing (MAIPA)	2 –3 weeks

SECTION I

HISTOCOMPATIBILITY TESTING FOR SOLID ORGAN TRANSPLANTATION

A full H&I service is provided for the renal and liver transplant units.

Potential renal, pancreas and liver recipients, local multi-organ deceased donors, deceased donors referred from Organ Donation and Transplant (ODT, formerly UK Transplant, UKT) and potential living donors are HLA typed. HLA typing is performed by both serological and PCR based assays.

Crossmatching for living or deceased donor transplants is performed by Complement Dependent Cytotoxicity (CDC) and Flow Cytometry, depending on the situation.

HLA antibody screening is performed by Luminex technology, which is a bead based immunoassay used to qualitatively detect HLA IgG antibodies.

RENAL PATIENTS

New renal patients have a full HLA type, antibody screen and an auto crossmatch performed (to identify autoantibodies). Samples required are:

10mls Lithium Heparin	for serology class I typing
10mls EDTA	for PCR class I & II typing
10mls Clotted Sample	for HLA antibody screening

Prior to listing on the Renal Transplant Waiting List a confirmatory HLA type needs to be performed as well as an up to date antibody screen. Samples required are;

5mls EDTA	for PCR class I & II typing
10mls Clotted Sample	for HLA antibody screening

When the confirmatory type and antibody screen have been completed and the transplant co-ordinators request that the patient is listed, the laboratory will enter the information into ODT NTN website (Aberdeen, Inverness, Dundee patients) or the MRU's Proton computer system (Edinburgh patients). Once on the Waiting List, a 10ml clotted sample should be sent to the laboratory every month. The serum is frozen and stored for use in future crossmatches. Patients on the waiting list will be screened for HLA antibodies every three months.

LOCAL DECEASED MULTI-ORGAN DONOR (DONATION AFTER BRAIN DEATH, DBD)

It is the Transplant Coordinator's responsibility to contact the laboratory, or if out of hours the on-call scientist, when a local DBD donor is identified. The following samples need to be sent:

10mls EDTA	for HLA typing, and CDC crossmatching of renal/ pancreas patients
10mls Lithium Heparin	for serological class I & II typing.

Results of the HLA typing and crossmatch results for the kidney/pancreas patients will be faxed to ODT.

LOCAL DECEASED MULTI-ORGAN DONOR (DONATION AFTER CARDIAC DEATH, DCD)

It is the Donor Transplant Coordinator's responsibility to contact the laboratory, or if out of hours the on-call scientist, when a local DCD donor is identified. The following samples need to be sent:

20mls EDTA	for HLA typing and CDC crossmatching
60mls Lithium Heparin	for serological HLA typing and Flow cytometry crossmatching

Results of the HLA typing will be faxed to ODT who will perform a local matching run. The decision on who to crossmatch will be made between laboratory on call staff, the H&I Consultant Clinical Scientist and the transplant coordinator. The initial CDC and Flow crossmatch will be performed using current serum samples and PBLs.

A CDC crossmatch and a Flow crossmatch will also be performed retrospectively. For this the following samples are required:

Donor	Samples obtained from the lymph node and spleen, for confirmatory crossmatching and confirmatory HLA typing of the donor. These samples must be fully labelled and accompanied by a correctly filled in request form.
Recipient	10 ml clotted sample pre-transplant 10mls EDTA

REFERRED DECEASED DBD OR DCD DONOR

It is the Transplant Co-ordinator's responsibility to contact the laboratory or if out of hours the on-call technician, when ODT allocates a kidney, pancreas, or both to a patient on the local waiting list.

Samples required are:

Donor	Samples obtained from the lymph node and spleen, for crossmatching and confirmatory HLA typing of the donor. These samples must be fully labelled and accompanied by a correctly filled in request form.
Recipient	10mls clotted sample pre-transplant 10mls EDTA

VIRTUAL CROSSMATCHING

For referred kidney or kidney/pancreas cases, patients will be eligible for a virtual crossmatch if

1. they have not received a previous graft
2. they have been shown to be consistently negative for HLA antibodies by Luminex testing over the course of at least one year
3. they have sent a recent (<3 months) sample to the laboratory

The Recipient Transplant Coordinator will hold a list of these patients and they will confirm with the Consultant Clinical Scientist that no pre-transplant crossmatch is indicated. Donor and recipient samples still need to be sent to the lab for a retrospective crossmatch.

REPORTING OF CROSSMATCH RESULTS

Results of the compatibility testing will be phoned to the Recipient Transplant Co-ordinator as soon as they are available.

A report of the crossmatch results will be generated and faxed to the Recipient Transplant Co-ordinator.

Reports will show the crossmatch results for the CDC and Flow assays, where applicable. A comment may also be included to interpret the data generated. Any unexpected positive crossmatch result will be discussed between the on call laboratory staff and the on call Consultant Clinical Scientist who will also be available to discuss any findings with the Clinical Team.

LIVING DONOR TRANSPLANTS

If a potential live donor has been identified for a renal patient then compatibility testing must be performed. The Transplant co-ordinator will discuss with the laboratory when the samples are being taken. Copies of the ABO blood group must also be sent to the laboratory.

Samples required for initial testing are:

Donor	10mls EDTA for CDC crossmatching and donor typing 10mls Lithium Heparin for serological typing of the donor and Flow crossmatching.
Recipient	10mls Serum sample for CDC and Flow crossmatching.

If the recipient has not been HLA typed then the following samples are also needed:

10mls Lithium Heparin	for serology class I typing
10mls EDTA	for PCR class I & II typing
10mls Clotted Sample	for HLA antibody screening auto crossmatch.

The crossmatch can then be repeated as necessary in the lead up to transplantation with a final crossmatch performed a few days before the operation.

SECTION II

HLA TYPING FOR HAEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS AND POTENTIAL DONORS

The laboratory provides HLA typing for patients requiring haematopoietic stem cell transplant. Related family members who are being considered as potential donors are also HLA typed. The exact relationship between the potential donor and patient should be noted on the request form. On the request form for the potential donor it should be noted who the patient is.

HLA typing is performed by both serological assays and PCR based assays depending on the level of resolution required.

Samples required are:

Patient 10mls EDTA for PCR class I and II typing

Potential Donor 10mls EDTA for PCR class I and II typing

Confirmation Of HLA Type

If a potential match is found, the HLA type of the potential donor and the patient are confirmed for both Class I and II; samples required are:

Patient and donor 10mls EDTA for PCR class I and II typing.

15mls Lithium Heparin for serological class I and II typing.

SECTION III

PLATELET IMMUNOHAEMATOLOGY

The H&I laboratory provides both platelet antibody screening and platelet antigen typing to aid in the investigation, diagnosis and possible treatment of a variety of thrombocytopenias.

The main platelet investigations undertaken are cases of platelet refractoriness, suspected cases of neonatal alloimmune thrombocytopenia (NAIT) and suspected cases of heparin induced thrombocytopenia (HIT). Any request for platelet investigations should be discussed with the BTS/Haematology duty specialist registrar. The duty specialist registrar can be contacted by phoning the laboratory and giving the patient's details, the requesting doctor's name and phone number so that the duty specialist registrar can phone them.

Platelet antibody screening is performed by a qualitative solid phase ELISA technique designed to detect IgG antibodies to HLA class I antigens and to epitopes on the platelet glycoproteins IIb/IIIa, Ia/IIa and Ib/IX. Because the current ELISA method for determining HPA antibodies cannot identify HPA-15a/15b antibodies, all NAIT cases are sent for further testing by MAIPA to the SNBTS Platelet Reference laboratory in Aberdeen. Further testing by additional techniques can also identify specific HLA class I antibodies.

Platelet typing is performed by PCR-SSP, which types for HPA-1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b, 5a, 5b, 15a, and 15b antigens.

HLA/HPA MATCHED PLATELETS FOR PLATELET REFRACTORINESS

Platelet Transfusion Refractoriness may result from immune or non-immune platelet destruction. The following can be targets for clinically relevant platelet allo-antibodies that can cause immune platelet refractoriness.

1. the ABO blood system
2. HLA class I antigens.
3. Human Platelet Antigens (HPA)

The H&I Laboratory investigates the presence of allo-antibodies against HLA class I antigens and/or antibodies against HPA. It should be noted that HPA antibodies in the absence of HLA class I antibodies are a rare cause of poor increments. The exception is if the HPA antibody is against a high frequency HPA alloantigens (e.g. HPA-1a) when it might cause poor increments with platelets from nearly all random donors.

Samples Required: **10ml clotted sample** for HLA and HPA antibody screen
 10mls EDTA for PCR based HLA and HPA typing

Depending on the antibody and typing results, HLA class I and/or HPA compatible platelets can be provided. For the provision of HLA/HPA matched platelets the following criteria should be met:

1. exclusion of non-immune causes of platelet refractoriness
2. platelet refractoriness to ABO compatible platelets on two or more occasions
3. a positive result when screening the patient for HLA class I antibodies.

A search is performed on all blood donors suitable to donate apheresis platelets who have been HLA class I typed (and HPA typed where relevant). If compatible HLA/HPA platelets are required the H&I laboratory and the BTS/Haematology duty specialist registrar should be informed and given as much notice as possible. This is because it will take time to call specific donors in and perform mandatory donor testing before platelets can be released.

NEONATAL ALLOIMMUNE THROMBOCYTOPENIA (NAIT)

NAIT is caused by maternal IgG alloantibodies directed against HPA present in the fetus/neonate and absent in the mother. Whilst numerous alloantigens have been described, HPA-1a is the most immunogenic and accounts for approximately 80% of severe cases. The next most common is HPA-5b.

Investigation of NAIT by the H&I laboratory will be undertaken after the requesting doctor discusses the case with the BTS/Haematology duty specialist registrar. The ELISA platelet antibody screen will detect antibodies against HPA-1a, 1b, 3a, 3b, 5a, 5b antigens, epitope GPIb/IIa and HLA class I. A sample will also be sent to the SNBTS Platelet Reference laboratory for MAIPA to detect HPA15a/15b.

If specific platelet antibodies are found the parents can be HPA typed and the phenotype of mother and father compared. This will help to identify any HPA antigens to which the mother might have developed antibodies.

If specific platelet antibodies are not identified the parents are HPA typed and the phenotype of mother and father compared. An indirect platelet immunofluorescence test (IPIFT) can be performed between the mother's serum and father's platelets. This more sensitive test will detect any weak antibodies in the mother not detected by ELISA. It will also detect any antibodies against antigens that are not included in the ELISA test.

Samples Required: 10mls clotted sample from mother, for antibody screening and IPIFT
 5mls EDTA from mother, for HPA typing.
 10mls EDTA from the father, for HPA typing and IPIFTHeparin

INDUCED THROMBOCYTOPENIA (HIT)

Heparin induced thrombocytopenia (HIT) occurs when patients receiving heparin develop antibodies that recognise sites on a platelet protein, platelet factor 4 (PF4), that are created when PF4 forms a complex with heparin or other linear polyanionic compounds. This leads to thrombocytopenia and if heparin continues to be administered, there is a risk that the thrombocytopenia will become more severe with the risk of arterial or venous thrombosis.

The laboratory tests for the presence of antibodies against PF4 complex by using a solid phase ELISA microwell test that utilises a PF4/polyvinyl sulfonate (PVS) complex. Repeating any positive results and diluting the patient's serum with a buffer containing an excess amount of heparin that the patient has received allows the determination that antibodies against heparin are present. The antibodies will bind with the free heparin rather than the PF4/PVS complex immobilised in the microwells of the ELISA tray.

Samples Required: 10mls clotted sample
 Sample of Heparin that the patient has received

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The Scottish National Blood Transfusion Service
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