NHS Blood and Transplant

HISTOCOMPATIBILITY AND IMMUNOGENETICS DIAGNOSTIC SERVICES

USER GUIDE

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Author(s) Julia Mahesan

User Guide for Histocompatibility and Immunogenetics Diagnostics Services

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The image on cover page is a compilation of images of Next Generation Sequencing (NGS) being carried out at H&I Colindale. For details of how this can help stem cell registries and cord blood banks, please visit:
https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/14473/nhsbt-ngs-brochure.pdf or
https://hospital.blood.co.uk/diagnostic-services/histocompatibility-and-immunogenetics/next-generation-sequencing/

User Guide for Histocompatibility and Immunogenetics Diagnostics Services

CHAPTER 1: GENERAL INFORMATION

1.1 THIS GUIDE

The NHSBT Histocompatibility & Immunogenetics (H&I) Function offers an integrated package of services, testing and clinical advice across the H&I field from a network of six laboratories. Working as an integral part of NHSBT we offer hospitals an unrivalled portfolio of testing, advice and support in transplantation, supply of selected blood products and immunogenetics.

This guide outlines the H&I services provided by NHSBT and will be of use to consultants and other medical, nursing and scientific staff in transfusion laboratories, haematology departments, transplant units and other healthcare environments requiring our services. The guide contains information about the organisation of services, contact details for key members of staff and other information to enable healthcare staff to access services on behalf of their patients.

1.2 QUALITY STATEMENT

The H&I Function, in common with other NHSBT services, is committed to the quality of our work, as outlined in our Quality Policy document (POL148). All work is carried out within the framework of a documented quality system, according to good laboratory and good manufacturing practice (GLP, GMP), in compliance with the Blood Safety and Quality Regulations, Human Tissue (Quality and Safety for Human Application) Regulations (TQSR), EU Organ Donation Directive (EUODD), the Data Protection and Freedom of Information Acts. Techniques and procedures are validated, described in standard operating procedures (SOP's), and conducted by staff whose proficiency and competency are regularly monitored.

NHSBT Quality managers carry out regular audits to establish and improve the level of GLP and GMP compliance. These complement external licensing and accreditation inspections by the Medicines and Healthcare Products Regulatory Agency (MHRA), United Kingdom Accreditation Service (UKAS), European Federation of Immunogenetics (EFI) Human Tissue Authority (HTA), Care Quality Commission (CQC) and other relevant accreditation bodies.

The Head of Function, Heads of Laboratories, laboratory and support staff have continued to standardise practice and strive for a consistent and high-quality service. Procedures are developed to work according to the principles of clinical governance.

All laboratories within the Function participate in external quality assessment (EQA) schemes such as United Kingdom National External Quality Assessment Service (UK NEQAS) and where appropriate in international workshops. In some instances, this participation extends to the provision of source material, devising the exercises or acting as a reference laboratory.

As part of NHSBT H&I's commitment to quality, and in accordance with ISO15189, Measurement of Uncertainty (MoU) values are available from the laboratories upon request.

1.3 COMPLIMENTS and COMPLAINTS

NHSBT 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 ensure the causes can be fully investigated to, reduce the risk of recurrence, help improve the service and ensure compliance with clinical governance policies (specific forms have been made available to every service user for this and can be found on the NHSBT "Hospital & Science" website at:

https://hospital.blood.co.uk/commercial-and-customer-service/complaints-compliments-and-feedback/

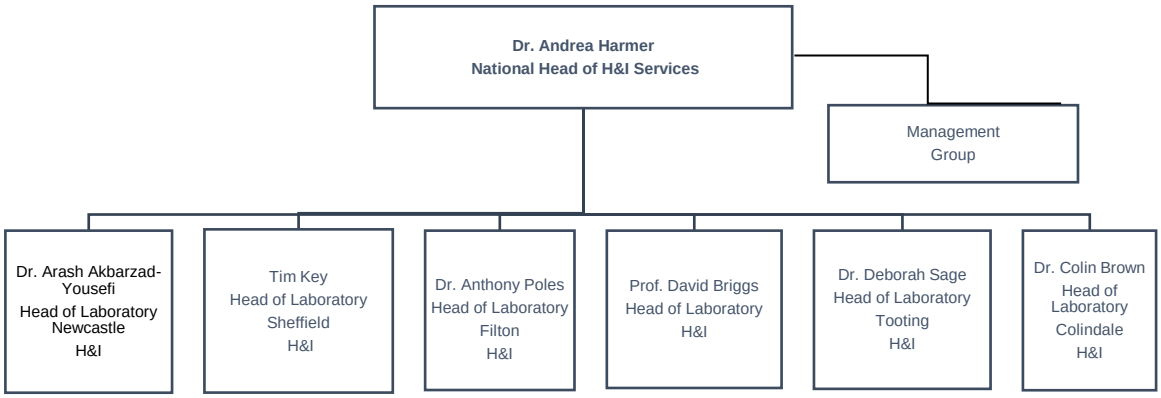
Please do not hesitate to discuss complaints with either your Customer Services Manager or the relevant Head of Laboratory. We always strive to provide a satisfactory response to any complaint. However, if you are unhappy with the handling of your complaint, then please contact the Head of Service Delivery or the Lead Quality Specialist for H&I.

Complaints must be clearly separated from communication about Serious Hazards of Transfusion (SHOT) or near misses, which have or could have affected the quality of patient care. Such incidents and near misses often require immediate action and you are advised to discuss these with an NHSBT medical consultant or a senior laboratory scientist at your local blood centre. Serious events must be reported to the SABRE scheme.

CHAPTER 2: H&I LABORATORIES AND MANAGEMENT

There are six laboratories in the H&I Function with approximately 190 members of staff. The laboratories are located at NHSBT Birmingham, Filton (Bristol), Colindale (North London), Tooting (South London), Newcastle and Sheffield, and each is directed by a consultant clinical scientist. Figure 1 highlights the management structure of the H&I Function.

Figure 1: Diagram of the management structure of the H&I Function



NHSBT H&I laboratories support haematopoietic stem cell and solid organ transplant programmes at hospitals throughout England. The H&I laboratory at Filton provides platelet immunology and granulocyte immunology services nationally.

The H&I laboratory at Colindale works in co-operation with the British Bone Marrow Registry (BBMR) carrying out high throughput HLA typing of NHSBT blood donors who have volunteered to become stem cell donors. The H&I laboratory at Colindale also performs the typing and registration of donated cord blood units collected by the NHS-Cord Blood Bank (NHS-CBB). The HLA data is then submitted to Netcord and to Bone Marrow Donors Worldwide (BMDW).

All NHSBT H&I laboratories are accredited by the European Federation for Immunogenetics for the clinical services they provide. Our laboratories are also accredited to ISO 15189:2012 by the United Kingdom Accreditation Service (UKAS).

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Table 1: License / accreditation numbers

Site	UKAS	EFI
Birmingham	9239 *	03-GB-020.980
Bristol - Filton		03-GB-015.985
Colindale		03-GB-002.998
Tooting		03-GB-003.997
Newcastle		03-GB-004.996
Sheffield		03-GB-006.994

* The scope of our accreditation can be found at the following location:
https://www.ukas.com/wp-content/uploads/schedule_uploads/00007/9239%20Medical%20Multiple.pdf

2.1 LABORATORIES 'OPENING HOURS'

The core working hours of the laboratories are between 09.00 and 17.00 from Monday to Friday, excluding bank holidays. Clinical and scientific advice from an H&I consultant clinical scientist regarding ordering of tests and on the interpretation of results is always available during these hours.

2.1.1 OUT OF HOURS

2.1.1.1 Solid Organ Transplant programmes

Out of hours on-call, is provided by H&I laboratories supporting solid organ transplant programmes, 24 hours a day, 365 days a year. A consultant clinical scientist can be contacted on your request in case of clinical emergency by contacting your local NHSBT Hospital Services. NHSBT Consultant clinical scientist will be available to **medical staff for transplantation and** transfusion advice from 23:00 to 06:00.

2.1.1.2 Out of hours ordering of HLA selected platelets

Please place **routine** orders on OBOS during normal laboratory hours (Monday - Friday 09:00 to 17:00) at least 24 hours before delivery or collection (not including weekends).

HLA selected platelets required for early delivery on weekdays and for transfusion at weekends should always be ordered within normal laboratory Monday-Friday working hours and with at least **24 hours' notice**. It may not possible to provide optimally HLA selected platelets at short notice and it may incur an ad hoc transport charge.

The out of hours' service is for **clinical emergency** use only for patients already receiving selected platelets. The out of hours service must not be used for routine orders.

If there is an urgent clinical need for out of hours transfusion in patients already receiving HLA selected platelets, orders should be placed on OBOS (Online Blood Ordering System) which is available through your Blood Transfusion department.

All out of hours / emergency orders must be followed up with a telephone call to your local Hospital Services department. The H&I consultant on call will need to approve the request and call in a member of the laboratory staff to process the request. Selection and provision of units is likely to take a minimum of 6 hours and may take longer. Whilst NHSBT will endeavour to provide a well selected unit, finding a suitably selected unit at short notice may not always be possible.

The emergency service is available weekdays from 06:00 to 09:00 and 17:00 to 23:00 and weekends and bank holidays from 06:00 to 23:00. A NHSBT consultant clinical scientist will be available to **medical staff** for transfusion advice from 23:00 to 06:00.

The process for ordering HLA selected products is described on the NHSBT "Hospital & Science" website:https://hospital.blood.co.uk/diagnostic-services/histocompatibility-and-immunogenetics/ordering-hla/.

2.2 Business Continuity Strategy

The specific strategy for managing any critical incident will depend largely upon the circumstances surrounding the incident, and strategic objectives may change during the course of an incident. However, the initial strategic objectives for managing a critical incident within H&I are:

- To protect as far as is reasonably practicable the delivery of key products and services to hospitals and patients
- To manage the incident within the constraints of regulatory and legislative requirements
- To strive for a recovery to business as usual in the shortest possible time
- Ensures that NHSBT remains compliant with legal and regulatory requirements e.g. H&S requirements

CHAPTER 3: SAMPLE REQUIREMENTS & REPORTING

3.1 SAMPLE REQUIREMENTS

For sample requirements refer to Table 5.

3.2 REQUEST FORMS

There are five request forms available which are shown in Table 2.

Table 2: Request forms

Request form			Version active
3A	H&I Diagnostic laboratory	FRM745	3
3B	H&I Organ Transplant recipients and donors	FRM1008	3
3C	H&I Haematopoietic Stem Cell Transplantation	FRM1010	3
3D	H&I Platelet Immunology	FRM999	3
3E	H&I Granulocyte Immunology	FRM1001	3.1

Request forms can be ordered directly from your **local H&I laboratory** and are also available to download from the NHSBT "Hospital & Science" website along with guidance on their correct completion (see document INF1182) at: http://tinyurl.com/h-i-forms.

Requesters are advised to ensure that the correct versions of these forms are used, as the use of out of date paperwork may cause errors in sample distribution, testing and reporting.

3.3 SAMPLE COLLECTION AND LABELLING

No specific patient preparation is needed for sample collection for H&I testing.

A request form must accompany every investigation request. Samples with different collection dates, or different sample types (e.g. bone marrow aspirate or peripheral blood) or from different individuals (including family members) must each be accompanied by their own test request forms. Request forms are the basis to establish the correct identification of the patient. Schemes, such as the SHOT scheme, have shown that serious incidents are often caused by errors of a clerical nature. Every sample needs to be dated, as this information can be significant in determining the advice we will issue; in addition, the outcome of some tests may be influenced by the age of the sample.

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The points of identification provided on the request form must match the information provided on the sample. Due to the increased risk of mislabelling when using pre-printed labels (addressographs), samples for transfusion investigations cannot be accepted when the sample is labelled with an addressograph. For our full sample labelling policy see MPD1108. MPD1108 details NHSBT H&I laboratories sample labelling and request form completion and can be accessed via https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/14477/mpd110812-hi-laboratories-repuirements-for-sample-labelling-request-form-completion.pdf .

MPD1108 section 1.4
"Only labels that are printed 'on demand' and attached to the sample tube next to the patient at the time of phlebotomy are acceptable. Since it is not possible to distinguish reliably between these and *addressograph* labels, they can be accepted only from referring organisations which have informed NHSBT, in writing, that their sample labels are generated in an audited system and are demand printed at the time of phlebotomy. Bedside generated labels need to have positive, traceable identification of the sample taker, but do not require a signature."

The laboratories may not accept referrals with inadequately completed request forms or incomplete sample labelling or where sample and request details do not match.
In case of a clinical emergency, NHSBT may agree with the requesting consultant or laboratory scientist to proceed with the requested investigations. However, in such cases the issue of blood products and laboratory reports will carry an explicit warning that the sample and/or request form did not meet the minimum acceptance criteria and responsibility for correct identification of the sample and patient lies with the requester, such sample testing may be delayed while the laboratory confirms the sample details. The requester is advised to check the identifiers and to obtain reassurance about the identifiers used for the linking between patient and sample.

The following information is mandatory* on samples:

• Surname and forename in full
• Date of birth
• NHS number (Mandatory where available)
• Date, and time if pertinent, of sample collection

*Certain exceptions apply, e.g. anonymised samples with a unique identifier such as a GUM clinic patient test request or for non-UK nationals where no NHS number exists.

The following additional information is also required on the request form:

• Requesting hospital name in full (including town or city)
• Known risk sample
• NHS or Non-NHS
• Type of investigations requested
• Diagnosis/treatment
• Other relevant clinical details
• Type of sample if not peripheral blood

NHSBT should be informed if samples are from non-NHS or private patients or whether from a patient or a donor. The terms and conditions of service provision for the NHS by NHSBT are agreed with the National Commissioning Group. Service provision for non-NHS patients may be charged differently.

Clinical information is essential for providing the most appropriate testing and advice. The quality of clinical advice will also depend on provision of adequate clinical information. Absence of clinical information may lead to a delay in the processing of the sample while the requester is contacted to clarify or ascertain the type of investigations required.

NHSBT stores patient and donor data on a national database and the use of hospital number without other points of identification may lead to errors as a hospital number is not a unique identifier. NHS number must be used in accordance with Department of Health requirements except in cases where the individual has no NHS number or where anonymity is mandated. If the address is provided, then it may be entered on the patient's NHSBT computer record and appear on some patient reports. If the address contributes to the quality of identification, then it may be used as a form of identification.

3.4 KEY FACTORS THAT MAY AFFECT TESTING

The following key factors may affect the tests that are performed by our laboratories:

Table 3: Summary of key factors that may affect testing

Factor	Advice to minimise impact.
Sample storage time	In general, samples should be sent to the laboratory with minimum delay ideally to arrive within 24 hours of sample collection
Sample storage and transportation temperature	In general, storage and transport of samples should be at ambient temperature
Anticoagulant	It is important to collect samples into the correct tubes. Please ensure the correct anticoagulant (usually EDTA) or no anticoagulant (clot) is used. It is also important to supply adequate volumes of blood to allow completion of testing (sample types and volumes are listed in Table 5).
Low white blood cell/ platelet count	Note low count on test request form
Condition and/or drug regime e.g. Immunosuppressants such as IVIg	Note medication/treatments on test request form

For further details please refer to NHSBT H&I test request forms (which can be accessed via http://tinyurl.com/h-i-forms) and information in Table 5: "Summary of volumes and type of blood samples required for each test" or contact your local H&I laboratory for help and advice.

3.5 ACCEPTANCE LIMITS FOR SAMPLE AGE

Acceptance limits for sample age is summarised in table 4:

Table 4: Summary of acceptance limits for sample age

Test	Sample age
DNA based tests	No restrictions if DNA of necessary quality can be extracted*
HLA specific antibody screening	In general, the sample age limit of 5 days of date bled*
HPA antibody screening	In general, the sample age limit of 5 days of date bled*
HNA antibody screening	In general, the sample age limit of 5 days of date bled*
HNA phenotype	Less than 24hrs of date bled
HIT testing	In general, the sample age limit of 5 days of date bled*
Chimerism analysis	Maximum is 72 hours of date bled to complete cell lineage separation
Cross-match	Samples where the cell viability is below approximately 80% may not be used
NAIN cross-match	Less than 24hrs of date bled
Platelet cross-match for NAIT	Less than 72hrs of date bled
Direct granulocyte tests	Less than 24hrs of date bled
Direct platelet tests	Less than 72hrs of date bled

* Testing can take place at a later date providing sample has been extracted (DNA) or separated (serum/plasma) and stored in accordance with assay manufacturer instructions.

Ideally samples should arrive at the laboratory within one day of bled date to ensure sample age is not a limitation factor for testing.

Please contact your local H&I laboratory for help and advice.

3.6 TIME LIMITS FOR REQUESTING ADDITIONAL EXAMINATIONS

We appreciate there are times when you need to request additional tests on a sample we have already received. Please be aware that not all specimens will be suitable for additional tests or retesting due to sample type, age, quantity or quality but please contact your local H&I laboratory for further help and advice.
Please note, the control of clinical material in the laboratories is in accordance with the requirements of the Human Tissue Act 2004, and the Royal College of Pathologists guidelines: The Retention and Storage of Pathological Records and Specimens. This lists the minimum storage requirements for the tests we provide therefore if requests for additional testing exceed these requirements, we may not still have the sample in our archive.

3.7 VOLUME AND TYPES OF SAMPLE

Refer to Table 5 for the volumes and type of blood samples required for each test. For further information, refer to the request forms via the NHSBT “Hospital & Science” website. Contact the relevant laboratory when referring samples of infants less than 6 months to discuss the minimum sample requirements.

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Table 5: Summary of volumes and type(s) of blood samples required for each test

Test	Sample requirements	Laboratory
Transfusion & Transfusion Reactions		
Initial investigation of Platelet refractoriness*	6ml EDTA and 6ml clot	Local H&I laboratory
* NB this investigation requires an HLA type and HLA antibody screen		
Follow up testing of platelet refractoriness	6ml clot	
HLA type	6ml EDTA	
HLA specific antibody screen	6ml clot	
Transfusion-Related Acute Lung Injury (TRALI)	Pre-transfusion serum sample 6ml clot & 6ml EDTA (patient) Donation numbers of all blood products transfused < 24hrs before event	Filton
Transfusion-Associated Graft Versus Host Disease (TA-GVHD)	Discuss sample requirements with H&I consultant	Local H&I laboratory
Granulocyte Immunology		
Neonatal AlloImmune Neutropenia (NAIN) for initial screen – Contact Filton lab to discuss crossmatch requirements	Maternal = 6ml EDTA & 6ml clot Paternal = 6ml EDTA Neonate = 1ml EDTA	Filton
Autoimmune neutropenia	6ml clot (smaller volumes permissible for infants)	
Drug related neutropenia*	6ml clot + sample of drug(s) Contact the laboratory before referring samples	
Platelet Immunology		
Foetal/Neonatal AlloImmune Thrombocytopenia (NAIT) for initial screen Contact Filton lab to discuss crossmatch requirements	Maternal = 6ml EDTA + 6ml clot Paternal = 6ml EDTA Neonate = 1ml EDTA	Filton
Heparin Induced Thrombocytopenia (HIT)	6ml clot	
Other drug related thrombocytopenias*	6ml clot + sample of drug(s) Contact the laboratory before referring samples	
HIT alert*		
Autoimmune thrombocytopenia	3 x 6ml EDTA and 6ml clot contact laboratory	
Post Transfusion Purpura (PTP)	6ml EDTA and 6ml clot	
Platelet glycoprotein estimation for thrombasthenia investigation	Contact the laboratory before referring samples	
Haematopoietic Stem Cell Transplantation		
Patients HLA type	6 – 80 ml EDTA * *Depending on WBC count.	Local H&I laboratory
Donor HLA type	6ml EDTA	
HLA specific antibodies	6ml clot	
Chimerism analysis	2 x 6ml EDTA	
Solid Organ Transplantation		
HLA type of patient, donors or family members	6ml EDTA	Local H&I laboratory
HLA specific antibodies	6ml clot	
Cross-match - live donor	60ml EDTA‡ (donor)	
	6ml clot (recipient)	
Cross-match - deceased donor	6ml clot (recipient)	
	60ml EDTA‡ (donor) OR spleen or lymph node as appropriate	
‡Newcastle require Li+ Heparin INSTEAD of EDTA for cross matching		
Auto cross match	20ml EDTA + 6ml clot (recipient)	Local H&I laboratory
Immunogenetics		
HLA typing for disease association and drug hypersensitivity	6ml EDTA	Local H&I laboratory

Please note:

- *Samples for ABO grouping MUST be sent directly to NHSBT RCI laboratories accompanied by an appropriate completed test request form – 1A. The NHSBT RCI User Guide can be accessed at: https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/16274/inf669-red-cell-immunohaematology-user-guide.pdf*
- **These assays are not included within our scope of accreditation for ISO15189 (UKAS). This will be highlighted on the report that you receive.*
- *Oragene DNA Genotek buccal swab kits may be used in cases where DNA concentrations in white blood cells are low. Please contact laboratory for advice.*

3.8 TYPE OF REQUEST

The type and reason for the request must be clearly identified using the appropriate test request form.

3.9 CONSENT

To comply with the Human Tissue Act legislation (Human Tissue Act, 2004), 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.

NHSBT may ask the requester to provide a copy of this information. 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.

Where patient or donor consent is required it is the responsibility of the requester to ensure the subjects of any tests have given informed consent. Unless written notice is received to the contrary, consent for investigations and the use of any surplus sample in scheduled purposes (quality control, staff development or ethics committee approved research) will be assumed.

All information provided to NHS Blood and Transplant is used in accordance with the General Data Protection Regulation (GDPR) and all other applicable privacy legislation. For more information on how we look after personal details or to find out more about privacy rights visit: www.nhsbt.nhs.uk/privacy or call 0300 123 23 23. NHSBT are committed to keeping data safe and confidential.

NHSBT H&I laboratories have developed a series of patient information leaflets to assist healthcare professionals to obtain informed consent for diagnostic testing. The leaflets explain what happens to their samples and why the tests are undertaken. In addition, there is a brief explanation of H&I investigations. The leaflets are available to download from the NHSBT “Hospital & Science” website at: https://hospital.blood.co.uk/diagnostic-services/histocompatibility-and-immunogenetics/patient-information-leaflets/

Table 6: Summary of H&I patient leaflets

Patient Information leaflet	Document No.
Histocompatibility testing for kidney transplant donors	INF253
Histocompatibility testing for kidney transplant patients	INF255
Histocompatibility testing for cardiothoracic transplant patients	INF254
Histocompatibility testing for platelet transfusion patients	INF256
Histocompatibility testing for stem cell transplant patients	INF257
Histocompatibility testing for possible donors or relatives of stem cell transplant patients	INF258
Immunogenetic markers and diagnosing diseases	INF259
Heparin-induced thrombocytopenia (HIT) : Your background guide to HIT and the associated laboratory testing	INF260
Information for mothers about neutrophil blood groups and Neonatal alloimmune Neutropenia (NAIN)	INF261
Platelet groups & antibodies in pregnancy	INF283

3.10 PACKAGING AND TRANSPORT

It is the responsibility of the requester to ensure that all samples are packaged in accordance with the current European agreement concerning Carriage of Dangerous Goods by Road Regulations, and IATA (packaging instructions PI650), to prevent breakage or spillage in transit.

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Mandatory regulations

For all transport purposes, pathogens are assigned according to categories A and B. Unless it is known or reasonably believed to contain infectious substance of category A (e.g. haemorrhagic fevers), all human or animal material is regarded as category B, UN 3373.

Category A

Category A includes higher risk infectious micro-organisms, defined as an infectious substance which is transported in a form that when exposure to it occurs is capable of causing permanent disability, life threatening or fatal disease in otherwise healthy humans or animals.
If sending category A substances, **please phone the testing laboratory** and discuss the arrangements before sending.

Category B

Category B includes infectious substances that do not meet the criteria for inclusion in category A, and include human and animal material such as, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluids, and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment or prevention. These are assigned to UN 3373 (diagnostic or clinical specimens) and must be packed to Packing Instructions PI650.

3.10.1 Sending specimens by post/ NHSBT Transport

All diagnostic samples sent are to be deemed and labelled Biological Substance Category B, UN 3373 and must meet PI650:

- Primary inner receptacle sealed and leak proof
- Secondary receptacle sealed and leak proof
- Rigid outer packaging (the outer package must also have one side with the minimum dimensions of 100mm x 100mm)
- Samples/packaging must be labelled with an emergency contact name and the fact that the package is a diagnostic specimen packed in compliance PI650
- When posting only first-class post or data post should be used
- All packages must use a visible diamond-on-point label UN3373 (min require dimension of this diamond are 50mm x 50mm)
- Adjacent to the diamond must be the label 'Biological Substance, Category B' and all text MUST be 6mm in height
- PI650 now permits up to 1 litre per primary receptacle with a total of 4 litres per package for liquids and 4kg for solids. Either primary or secondary receptacle must withstand pressure of 95kPa and a 1.2 meter drop test

The outside of the box or package containing the samples must be clearly addressed to the H&I Department at the appropriate Blood Centre where the testing laboratory is based including **“FAO: H&I Department - Diagnostic Samples”** as the first line of the address to prevent delivery to H&I sample reception being delayed.

Please note that the appropriate NHSBT laboratory may not be at your local blood centre. NHSBT reserves the right to refuse to handle any samples which are inappropriately packaged or labelled; customers sending unsatisfactorily packaged samples will be contacted.

Address label templates for H&I laboratories can be downloaded from:
https://hospital.blood.co.uk/diagnostic-services/histocompatibility-and-immunogenetics/labels-for-sample-boxes/

For advice from the Health and Safety Executive (HSE) on packaging for posting samples see:
http://www.hse.gov.uk/biosafety/blood-borne-viruses/transportation-of-infectious-substances.htm

3.11 WHERE TO SEND SAMPLES

Accurate completion of the request form and clear labelling is essential for an effective transfer of samples to the testing laboratory. Ensure external packaging is clearly addressed to the appropriate H&I laboratory where the testing is completed (This may not be your nearest NHSBT centre). Samples for non-urgent testing can be given to your NHSBT blood delivery driver via the hospital transfusion laboratory.

For investigations not available from your local blood centre it is advisable to send samples directly to the H&I laboratory conducting the testing (see table 5 for site specific testing).

Samples for platelet immunology and granulocyte immunology investigations should be sent **directly** by first class post to the H&I laboratory at Filton to avoid delays.

3.11.1 Urgent samples

For urgent testing of samples **please phone the testing laboratory** and discuss the arrangements for sending the samples. Urgent samples should be transported directly from the hospital transfusion laboratory, transplant unit or requesting clinician to the blood centre where tests are performed. Packages containing urgent samples must be clearly labelled (including “FAO: H&I Department - Diagnostic Samples - URGENT”) to ensure samples do not go astray.

3.12 REPORTING TIME

In 95% of cases NHSBT aim to issue reports for DNA investigations within seven working days **from receipt of the samples in the laboratory**. A longer turnaround time may apply to other investigations.

HLA specific antibody test reports for patients’ refractory to platelet transfusion will normally be issued within seven working days. Preliminary reports of HLA specific antibody positivity may be available sooner upon discussion with the local laboratory.

Drug dependent antibody screening (other than heparin induced thrombocytopenia) may take up to 20 working days, as these investigations often require additional studies.

Reports for complex cases e.g. samples for transplant requiring multi-stage testing, family or combined donor/recipient reports requiring collation of test results from multiple samples will take longer than seven days from receipt of the first test request/sample to the generation of the final report.

In 95% of cases NHSBT aim to issue reports for granulocyte immunology investigations within 14 working days **from receipt of the samples in the laboratory**. If further (specific) investigations are required, the turnaround time may extend to 21 working days.

Blood samples referred for foetal HPA typing will be tested and the results reported within 3 working days of receipt of the sample. Amniocytes referred for foetal HPA typing will be tested and the provisional results reported within 3 working days from the receipt of the sample in the laboratory. A final report is only issued after typing of cultured amniocytes. Depending on the number of viable cells, a further 21 days may be required before sufficient cells are available for confirmatory typing to be completed.

Details are summarised in Table 7.

Table 7: Summary of reporting time

Service	Report	95% within
Immunological refractoriness to platelets	HLA type	7 working days
	HLA specific antibody screen	7 working days
Platelet Immunology (PI)	Platelet antibody specificity (e.g. NAIT) **	5 working days
	HIT	5 working days
	HIT - urgent result *	1 working day
	Other drug induced thrombocytopenia	20 working days
	Foetal HPA typing	3 working days
Granulocyte Immunology (GI)	All tests	21 working days
Haematopoietic Stem Cell Transplantation (HSCT)	HLA type - class I and II	7 working days
Solid Organ Transplantation	HLA type - class I and II	7 working days
	HLA specific antibody screen	15 working days
	Urgent result	1 day
Immunogenetics	All tests	7 working days
Chimerism Analysis	All tests	7 working days

* This **must** be discussed with the laboratory ahead of sending the sample
** HPA -15 antibody testing may require 21 working days depending on donor availability

3.13 COMPUTER RECORDS AND REPORTS

3.13.1 Computer records

The H&I laboratories are supported by national computer systems (Hematos/PULSE) on which patient and donor data are stored. NHSBT computer systems are registered under the Data Protection Act. Access to the database is on a 'need to know' basis for 'clinical care purpose only' and confidentiality is respected at all times.

3.13.2 Reporting

NHSBT H&I has electronic reporting capabilities for those requesters with Health and Social Care Network (HSCN) and N3 NHS network access. This system is based on the Sunquest ICE electronic reporting system and is named SpICE (Specialist Services ICE).

SpICE will reduce significantly the time taken for reports to be available to requesters once they have been authorised for release: all reports should be visible to those with access to them on the system within one hour of the report being authorised as opposed to being delayed by the printing and postal delivery process. Hard copy reports will continue to be sent, in addition to the electronic reports, until NHSBT is informed by requesters that they are no longer required.

The basic principle for hard copy reports is for them to be sent to the requester. Please contact the laboratory if you require different hard copy reporting arrangements. When requested, urgent reports can be faxed to a requester, but the requester will be asked to fax this request on headed paper, as proof of identity is needed in order to protect patient confidentiality.

H&I also have the capability to allow the hospital to receive reports via secure email direct from the NHSBT Specialist Services IT System. The hospital must supply NHSBT with a nhs.net email address for their department. Hardcopy reports will not be available from NHSBT once the email

process is in place. Further information regarding how to set this up please contact your local laboratory.

More information about SpICE, including a user guide, presentation and FAQs can be found on the NHSBT "Hospital & Science" website at:
https://hospital.blood.co.uk/diagnostic-services/sp-ice/

3.13.3 Antibody cards

For patients with clinically relevant platelet (HPA) and / or neutrophil (HNA) alloantibodies or cell specific or drug dependent antibodies, an antibody card will be issued for the patient. SpICE will enable hospitals to access patient antibody cards for printing if required. Information leaflets will be sent for patients with a diagnosis of NAIT, NAIN or HIT. Antibody cards are not issued for patients with HLA specific antibodies.

CHAPTER 4: H&I SERVICES

Services are provided to support the diagnosis and/or treatment of a variety of conditions and are relevant in the following areas of clinical medicine shown in Table 8.

Table 8: Areas of clinical medicine involving H&I services

Areas of clinical medicine involving H&I services	Section
H&I service relating to transfusion and transfusion reactions	4.1
- Platelet refractoriness and provision of HLA/HPA selected platelets	
- Investigations of Serious Hazards of Transfusion (SHOT)	
Platelet Immunology (PI)	4.2
Granulocyte Immunology (GI)	4.3
Haematopoietic Stem Cell Transplantation (HSCT)	4.4
Solid Organ Transplantation	4.5
Immunogenetics	4.6

4.1 H&I SERVICES RELATING TO TRANSFUSION & TRANSFUSION REACTIONS

For Post-Transfusion Purpura (PTP) see section 4.2 Platelet Immunology

4.1.1 PLATELET REFRACTORINESS AND THE PROVISION OF HLA / HPA SELECTED PLATELETS

Platelet transfusion refractoriness may result from immune or non-immune platelet destruction. The identification of platelet refractoriness due to HLA / HPA specific antibodies is important to enable allocation of these specialised products to those patients who will benefit from them. Patients with non-immune platelet refractoriness will not gain any additional benefit from HLA / HPA selected platelets compared to non-HLA selected platelet units. In some patients with HLA specific antibodies, HPA specific antibodies may also be present requiring donor platelets compatible with both types of antibodies. If compatible platelets cannot be provided, either increasing the transfused dose or discontinuing prophylactic platelet support may be appropriate strategies.

4.1.1.1 CMV negative selected products

The Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) position statement on the provision of cytomegalovirus (CMV) tested blood components recommends that for patients other

than neonates (under 28 days and intra-uterine transfusions) leucodepleated products provide adequate risk reduction for the transmission of CMV.

Quote from SaBTO position statement:

"All blood components (other than granulocytes) in the UK now undergo leucodepletion, which provides a significant degree of CMV risk reduction. This measure is considered adequate risk reduction for all other patients requiring transfusion (haemopoietic stem cell transplant patients, organ transplant patients, and immune deficient patients, including those with HIV) without the requirement for CMV seronegative components in addition."

The full report of the SaBTO CMV Steering Group may be found at:
https://www.gov.uk/government/publications/sabto-report-of-the-cytomegalovirus-steering-group

Restricting selected products to CMV seronegative units severely reduces the number of units available and may result in a less beneficial unit being selected for your patient.

4.1.1.2 Alloimmunisation against platelets

Alloimmunisation is defined as the development of an immune response against alloantigens. In some transfusions this immune response may result in the production of HLA and / or HPA specific antibodies. Refractoriness is the failure to obtain satisfactory responses to transfusions of platelets from unselected but ABO compatible donors. A proportion of, but not all, alloimmunised patients will become refractory. It is generally accepted that as a consequence of universal leucocyte depletion of all blood components the rate of alloimmunisation has dropped to approximately 10-25%. However, the precise incidence is influenced by a number of factors including pregnancies and the number of transfusions. Non-immune mechanisms are an important cause of refractoriness and have been shown to cause transfusion failure in a significant group of patients on prophylactic platelet transfusion support.

4.1.1.3 Platelet refractoriness

Platelet refractoriness is defined as a failure of the platelet count to increase by greater than 10 x 10^9/L at between 1 and 24 hours after the transfusion of an adult dose of ABO compatible platelets (> 240 x 10^9 /L platelets). Refractoriness to random donor platelets can be of non-immune or immune cause, or a combination of both.

4.1.1.4 When to request HLA class I selected platelets

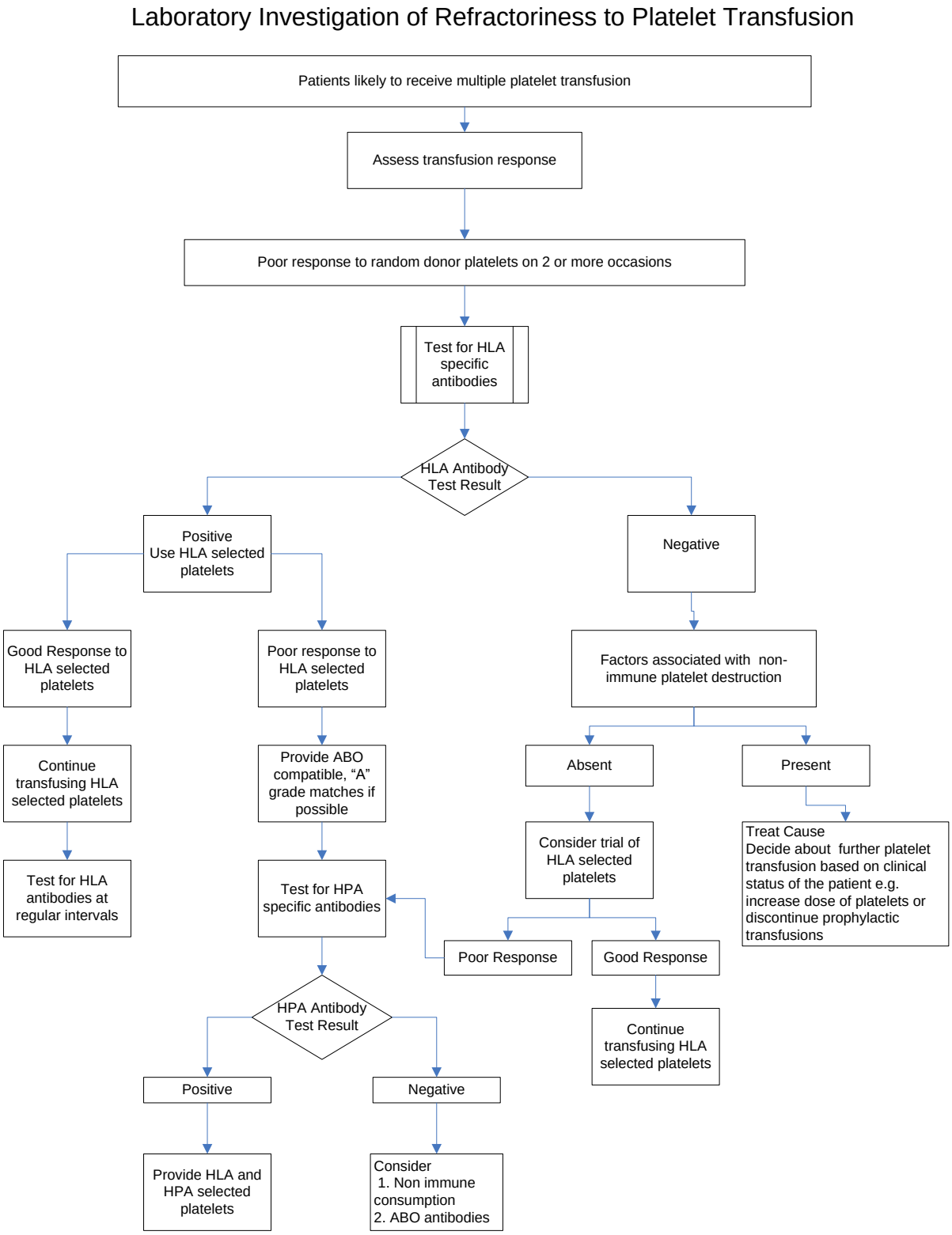
The majority of patients with immune refractoriness are best supported with platelet transfusions that are either HLA selected or HLA compatible between the donor and patient. HLA selected platelets are collected by apheresis and specific donors may have to be called to donate these platelets for a specific patient. The provision of this service is time consuming and expensive and should be reserved for those patients who really need them. See Figure 2 for guidance on the Laboratory Investigation of Refractoriness to Platelet Transfusion.

The following criteria should be met:

- Exclusion of non-immune causes of refractoriness
- Positive screen for HLA class I or HPA specific antibodies or both
- Refractoriness to an ABO compatible platelet concentrate on two occasions

User Guide for Histocompatibility and Immunogenetics Diagnostics Services

Figure 2: Laboratory Investigation of Refractoriness to Platelet Transfusion



4.1.1.5 Increments with HLA Class I selected platelets

Transfusion of selected platelets in patients with immune refractoriness results in a significantly improved post-transfusion increment in 60-70% of patients. The H&I laboratory needs to collect increment data to identify units that obtain satisfactory results from transfusions. The platelet count should be measured 1 hour after completion of the transfusion but can be obtained after 10 minutes (1). The laboratory can then identify those donors whose donation are most beneficial to patients and assign further units accordingly. Transfusion failure with HLA class I selected platelets may be due to co-existing non-immune causes of refractoriness, HPA alloantibodies, platelet autoantibodies, drug-dependent platelet antibodies and potent anti-A or anti-B antibodies. If increments with HLA class I selected platelets are poor, the case should be discussed with an H&I consultant clinical scientist. Assays for the detection and identification of HPA specific antibodies may then be recommended. In refractory patients with active bleeding, a dual investigation strategy of simultaneous investigations for HLA and HPA specific antibodies may be indicated.
The H&I laboratory will send FRM741 (HLA Selected platelet- follow up) with every order of HLA platelet. Please return the completed form back to your local H&I laboratory as soon as possible so the laboratory can make a decision which donation would be best for your patient.

4.1.1.6 HLA & HPA selected platelets

For patients with HPA as well as HLA class I specific antibodies, attempts will be made to provide dual selected platelets. This may not be possible if the platelet specific antibodies are against high frequency HPA alloantigens. In this case, HPA selected platelets alone may be provided to determine if these give a satisfactory increment.

4.1.1.7 Ordering HLA & HPA selected platelets

Orders for selected platelets should be made during normal working hours and with at least **24 hours' notice.** Planning in advance allows the H&I laboratory to source the best available HLA/HPA selected product from national stocks. For highly sensitised patients there may only be one or two suitable products available and the units most appropriate for the patient may not be in stock at your local NHSBT centre . Products which have to be supplied at short notice may need to be transported from any another NHSBT centre in the country which is why we require a minimum of 24 hours' notice. Outside normal working hours platelets can be supplied for existing patients for emergency care only.

4.1.1.8 New requests for HLA selected platelets

Hospital staff must first phone their local H&I laboratory to discuss patient information, testing and component requirements. An order form "First request for HLA selected Platelets" (FRM 558) will be e-mailed to the hospital by the H&I laboratory. The completed order form should be e-mailed back to the H&I laboratory. This form is not available on the website to ensure the correct process is followed. Please ensure that the first request and any special instructions e.g. RhD incompatible is acceptable and is authorised by an appropriate clinician treating the patient.
In line with the directive from the Department of Health and Social Care, we do not encourage faxing requests, but a fax number can be made available for business continuity purposes, if required.

4.1.1.9 Second and subsequent orders

For named patients who have previously received HLA selected platelets hospital staff should place orders using OBOS (Online Blood Ordering System) which is available through your Blood Transfusion department. Please see the NHSBT "Hospital & Science" website at https://hospital.blood.co.uk/diagnostic-services/histocompatibility-and-immunogenetics/ordering-hla/ .

[1] O'Connell B, Lee EJ, and Schiffer CA, The value of 10 minute post transfusion counts. Transfusion 1988;28:66-67

The selection and issue of HLA selected platelets is managed from two NHSBT sites. The contact details are dependent of the location of the hospital (see table 17).

4.1.2 SERIOUS HAZARDS OF TRANSFUSION (SHOT)

The overall incidence of serious side effects is small when compared with number of blood components used per annum by the NHS. In the majority of cases of serious reactions or hazards associated with transfusion, the diagnosis is a clinical one and in some, elaborate laboratory tests are required to confirm the diagnosis. A suspected adverse reaction should be discussed in the first instance with a NHSBT consultant Haematologist to agree on the best set of laboratory investigations. Samples can be referred to one NHSBT laboratory and will be distributed internally.

4.1.2.1 Reporting adverse reactions to transfusions

There is a regulatory requirement in the UK under the terms of the Blood Safety and Quality Regulations (BSQR) 2005 to report adverse reactions related to transfusion.
The MHRA has been appointed the Competent Authority on behalf of the Secretary of State to administer the regulations and has developed a web-based haemovigilance reporting system called SABRE (Serious Adverse Blood Reactions and Events) to facilitate reporting. SHOT (Serious Hazards of Transfusion) is the United Kingdom's independent, professionally led haemovigilance scheme.

All Trusts in the UK should be registered with the MHRA and must submit a 'notification' report to them as soon as possible following a reaction.

MHRA and SHOT have been working towards producing a joint haemovigilance reporting system that is fit for reporters' purposes as well as those of the MHRA and SHOT.

Current SHOT reporting categories and laboratory flowchart may be found at:
http://www.shotuk.org/sabre/.

The SHOT reactions that involve the H&I laboratories in the investigations are in Table 9.

Table 9: SHOT categories that involve the H&I laboratories in the investigations

Type of SHOT reaction	Abbreviation
Post Transfusion Purpura	PTP
Transfusion-Associated Graft Versus Host Disease	TA–GVHD
Transfusion Related Acute Lung Injury	TRALI
Severe Non-Haemolytic Febrile Transfusion Reactions (NHFTR) Classified as Acute Transfusion Reaction by SHOT	ATR

4.1.3 TRANSFUSION ASSOCIATED GRAFT VERSUS HOST DISEASE (TA-GVHD)

TA-GVHD is usually fatal but almost entirely preventable complication of transfusion. Patients at risk of this complication have been clearly defined, as have groups not considered to be at risk. Components implicated are red cells, platelet concentrates, fresh plasma and granulocytes. At risk patients should carry the card issued by the Department of Health, which can be obtained from NHSBT Hospital Services, and receive gamma-irradiated blood components. The dose of gamma irradiation should be a minimum of 2500 cGy to any part of the blood component.

4.1.3.1 Investigations

In supporting the clinical diagnosis, laboratory testing to demonstrate mixed chimerism is important. TA-GVHD is the result of engraftment and proliferation of alloreactive donor lymphocytes in the recipient. Inflammation and tissue damage follow. Tests for short tandem repeats (STR) on patient DNA and on DNA from pinch skin biopsy samples from affected and non-affected sites will be required to establish

the presence of infiltrating donor lymphocytes in the TA-GVHD skin lesions and to unequivocally identify cells of donor and patient origin.

4.1.4 TRANSFUSION RELATED ACUTE LUNG INJURY (TRALI)

TRALI is a serious complication of transfusion which usually occurs within 6 hours of a transfusion episode and is characterised by symptoms and signs of dyspnoea, cyanosis, hypoxaemia and pulmonary oedema and in the absence of other causes such as cardiac insufficiency and fluid overload. Chest X-ray shows characteristic pulmonary infiltrates. None of the clinical features are specific to TRALI and the diagnosis is essentially clinical. The clinical presentation is indistinguishable from the Acute Respiratory Distress Syndrome (ARDS) or the less severe form, Acute Lung Injury (ALI). The aetiology of TRALI is complex and is difficult to distinguish from ARDS/ALI on the basis of clinical symptoms and tests. TRALI is therefore a diagnosis made by exclusion where other causes of ARDS/ALI are not apparent and where there has been a recent transfusion of blood or other plasma containing blood products. Although rare, TRALI is a significant cause of transfusion associated morbidity and mortality. The risk of the latter can be reduced by early recognition of the cause and optimal treatment. Some cases were considered, after review, not to be TRALI, illustrating the difficulty of making a positive clinical diagnosis of the condition. However, in many cases, TRALI was thought either likely or possibly to have contributed to the patient's death. Leucocyte antibodies in the donor plasma generally cause this syndrome. Even a small volume of plasma containing leucocyte antibodies such as that found in SAG-M red cell concentrates is able to precipitate a reaction. On rare occasions TRALI can also be caused by leucocyte antibodies in the recipient or by immune complexes of leucocyte antigens and antibodies in platelet concentrates derived from pooled buffy coats.

4.1.4.1 Investigations

The logistics of TRALI investigations are complicated and time consuming. When referring a suspected case of TRALI full clinical details should be provided in order to assess the likelihood of the reaction having been due to TRALI. Clinical details should include, nature of transfusion reaction and time in relation to transfusion, components transfused including donation numbers, treatment given including ventilation and clinical response.

Leucocyte antibodies are generally against HLA class I antigens but HLA class II or HNA specific antibodies may also be implicated. Initial investigations will be performed with fresh donor samples. It is therefore important that donation numbers of all implicated units (blood, platelet concentrates, FFP) in the 24 hours preceding TRALI presentation are provided. Pre- and post-transfusion serum samples from the patient should be provided; together with the date and time the samples were taken.

The investigations for TRALI are done at the H&I laboratory at NHSBT Filton. The investigations aim to identify the presence of:

- HLA class I and class II specific cytotoxic antibodies
- HLA class I and class II specific non-cytotoxic antibodies
- Granulocyte-specific antibodies

If donor leucocyte alloantibodies are detected, then appropriate tests for the presence/absence of the antigen or allele in the patient/donor will be performed to determine whether the patient is positive for the cognate antigen. Even if this is the case, there is a good chance that the incompatibility is by chance and is not the cause of ARDS/ALI. TRALI does not always ensue even when a patient is positive for the cognate antigen.

4.1.4.2 Future transfusions

There is no clear evidence on the best transfusion support policy for patients who have experienced TRALI. However, it is generally accepted that in addition to donor leucocyte antibodies, patient factors may contribute to the risk of TRALI is generally accepted. Therefore, in a patient who has experienced

a TRALI, it is recommended not to use plasma containing blood products from female donors (FFP, cryoprecipitate, platelet concentrates) as the chance of leucocyte antibodies being present is greater in this group.

4.1.5 SEVERE NON-HAEMOLYTIC FEBRILE TRANSFUSION REACTIONS (NHFTR)

The incidence of NHFTR and of rigors have both reduced as a consequence of the introduction of universal leucocyte depletion. However, it remains a common consequence of transfusing blood or blood products. In the majority of cases pre-medication with paracetamol may alleviate symptoms. If severe and when combined with other features such as hypotension, then bacterial contamination of blood products (especially platelet concentrates) must be considered and an NHSBT medical consultant must be contacted urgently for advice and investigations.

4.1.5.1 Non-haemolytic febrile and allergic transfusion reactions with an immunological cause

Apart from bacterial contamination, severe febrile transfusion reactions may be caused by immunological reactions. Severe immunological reactions can be of the allergic / anaphylactic type with rashes, wheezing or dyspnoea. If febrile reactions recur and are refractory to paracetamol and corticosteroids other causes should be considered. It is advised that such cases be discussed with a NHSBT medical consultant. The straightforward method for prevention of both types of reactions is to alter the specification of the blood product; i.e. reactions to platelet transfusions may be simply resolved by replacement of the plasma by platelet suspension medium and to blood transfusions by removing the plasma proteins by washing.

If severe febrile reactions are not resolved by altering the component specification, then tests for leucocyte and platelet alloantibodies may be of use. If any of these antibodies are present in the patient, then reactions may be remedied by better matching. In such rare and complex cases, it is recommended to run investigations for HLA class I and class II, HNA and HPA specific antibodies in parallel. Samples should be referred to the local H&I laboratory. Tests for leucocyte and HPA alloantibodies have a low diagnostic specificity for NHFTR and the reactions may persist even if better selected blood or platelets are provided.

4.2 PLATELET IMMUNOLOGY (PI)

There are six clinical syndromes for which PI services are provided:

- Neonatal alloimmune thrombocytopenia
- Post transfusion purpura
- Refractoriness - HPA only tested for after HLA antibody investigation * (see section 4.1)
- Delayed engraftment of platelet lineage following bone marrow transplantation – investigated only after HLA antibody investigation
- Autoimmune thrombocytopenia – selected cases only
- Drug-induced antibody mediated thrombocytopenia e.g. heparin, antibiotics, quinine and gold
- Congenital and acquired thrombasthenias

4.2.1 Neonatal alloimmune thrombocytopenia (NAIT)

The frequency of NAIT is 1 in 1100 live births and is the most likely cause of severe thrombocytopenia in a term and otherwise healthy neonate. NAIT is caused by maternal IgG alloantibodies directed against a HPA antigen present in the foetus/neonate and absent in the mother. Many alloantigen systems have been described but the HPA-1a antigen is clinically most important and approximately 80% of severe cases are caused by anti-HPA-1a. Approximately a further 15% of NAIT cases are due to HPA-5b alloimmunisation and the strategy of providing HPA-1a (-), 5b(-) platelets in suspected NAIT cases will therefore be successful in 95% of cases involving Caucasians.

NAIT due to antibodies against HPA other than HPA –1a and – 5b and to isoantigens in the case of maternal platelet glycoprotein deficiencies account for approximately 5% of cases. In cases where antibodies to the major HPA are not detected and there is strong clinical evidence to support a diagnosis of NAIT, the maternal serum is also investigated for the presence of antibodies against 'private' or low frequency antigens by performing a crossmatch between maternal serum and paternal platelets.

4.2.1.1 Investigations for NAIT

The maternal serum will be screened for HPA specific antibodies using both an immunofluorescence test and a glycoprotein specific ELISA (MAIPA assay) with a panel of HPA and HLA typed platelets and, when appropriate, paternal platelets as soon as samples arrive at the laboratory. If the mother is found to have HPA specific antibodies, these results will be relayed to the requester as soon as possible. However, laboratory results should not delay transfusion of HPA-1a (-), 5b(-) platelets if NAIT is suspected and there is evidence of bleeding or if the platelet count is $<30 \times 10^9$/L. Maternal and paternal blood (and from infant if available) will be genotyped for the HPA -1, 2, 3, 4, 5, 6, 9 and 15 alleles.

4.2.1.2 Therapy

In a term neonate with normal clotting but severe thrombocytopenia ($< 30 \times 10^9$/L) or clinical signs of bleeding, the count should be corrected as soon as possible by transfusion of HPA-1a and HPA-5b negative donor platelets, without waiting for the results of the laboratory investigations. HPA-1a and HPA-5b negative platelets suitable for neonatal use are available 'from the shelf'. These platelets will be compatible with maternal HPA specific antibodies in over 90% of NAIT cases. If HPA-1a and HPA-5b negative platelets are not available from stock, then normal ABO and D compatible donor platelets should be administered together with high dose intravenous immunoglobulin.

4.2.1.3 Counselling and clinical questionnaires

If HPA alloantibodies are detected in the maternal serum, counselling should be provided to the parents about the risks to further pregnancies. Details of clinical outcome are sought by the laboratory in each confirmed case of NAIT.

4.2.1.4 Foetal HPA genotyping in future pregnancies if the partner is heterozygous

The HPA status of a foetus can be identified by analysis of genomic DNA derived from foetal blood or amniotic fluid. Please discuss with one of the consultant Haematologists before a decision for sampling is taken. In general, a 10ml sample of amniotic fluid (depending upon gestational age) or a chorionic villus biopsy is required. This should reach NHSBT H&I Filton within 48 hours of sampling. To avoid the possibility of contamination, it is preferable to dispatch the amniotic fluid without transferring it to a second container. If amniotic fluid is transferred from one container to another, precautions should be taken to avoid contamination with bacteria or with exogenous DNA.

4.2.2 POST-TRANSFUSION PURPURA (PTP)

PTP is a rare but serious transfusion reaction occurring 5 to 12 days after the transfusion of blood. A sharp decline in the number of confirmed PTP cases has been observed since the introduction of universal leucocyte depletion. PTP mainly occurs in women and HPA-1a specific antibodies are generally detected.

However, other HPA specific antibodies can also cause PTP. Severe thrombocytopenia occurring immediately after the transfusion of whole blood, a platelet concentrate, or fresh frozen plasma can be caused by potent HPA antibody in the transfused plasma. All cases in which there is a precipitous fall in the platelet count either immediately or some days after transfusion (except in case of massive transfusion) should be referred for investigations and reported. Patients who require blood peri-operatively and in whom a severe thrombocytopenia develops will often also receive heparin. However, the development of thrombocytopenia in PTP is more precipitous than in HIT (Heparin Induced

Thrombocytopenia) and purpura and bleeding are characteristic of PTP. If PTP investigations are requested then it is important to inform the laboratory whether the patient was receiving heparin, even if this was only to flush an in-dwelling line.

4.2.2.1 Investigations

Tests for HPA specific antibodies and where appropriate heparin-platelet factor 4 specific antibodies will be performed.

4.2.2.2 Therapy

High dose intravenous immunoglobulin (1.0 g/kg body weight on two to three consecutive days) is the treatment of choice. Platelet transfusion is usually contra-indicated in the acute phase. Plasmapheresis needs to be considered as an additional therapy if intravenous IgG does not result in a satisfactory rise of the platelet count. High dose corticosteroids are not recommended.

4.2.2.3 Transfusion support

In the acute phase of PTP, random ABO/D compatible blood components are advised. HPA compatible blood and platelets must be used if a patient requires transfusion after recovery.

4.2.2.4 Delayed engraftment of platelet lineage following stem cell transplantation

Isolated failure of platelet engraftment following stem cell transplantation can be due to the presence of pre-existing HPA specific antibodies in the recipient. These patients should be investigated in the same way as for platelet transfusion refractoriness.

4.2.3 Autoimmune Thrombocytopenia (AITP)

A raised level of Platelet Associated Immunoglobulin (PAIg) is detected in the majority of patients with AITP. However, the diagnostic specificity and therefore the clinical usefulness of the PAIg test by immunofluorescence is poor. Normally, these investigations are only indicated if the patient's platelet count is < 100 x 10^9/L. The diagnostic specificity is increased if platelet glycoprotein specificity of the PAIgG can be determined by direct MAIPA assay, but this assay requires a significant number of platelets which may be difficult to obtain from severely thrombocytopenic patients. The detection of serum platelet autoantibodies may be indicated, if the patient's platelets cannot be tested, but the results may be difficult to interpret because both alloantibodies and autoantibodies may be present in the serum.

These investigations are recommended only in the following categories of thrombocytopenic patients:

- Bone marrow failure possibly combined with immune-mediated thrombocytopenia
- AITP patient's refractory to first and second line treatment
- Monoclonal gammaglobulinopathies
- Acquired autoantibody mediated thrombasthenia

4.2.3.1 Bone marrow failure and immune-mediated thrombocytopenia

In some patients with thrombocytopenia due to inadequate thrombocytopoiesis, antibody - mediated platelet destruction may compound the thrombocytopenia, e.g. patients with proliferative disorders such as Chronic Lymphocytic Leukaemia (CLL) or stem cell transplant recipients. Reactive megakaryocytopoiesis is a diagnostic cornerstone of AITP but is not diagnostic if platelet autoimmunity is present in addition to bone marrow infiltration/failure. A PAIg test and determination of autoantibody specificity may be of use.

4.2.3.2 AITP patients refractory and first or second line treatment

For AITP patients for whom third line treatment is considered, a PAIg test, direct MAIPA assay and/or determination of antibody specificity may be indicated.

4.2.3.3 Monoclonal Gammaglobulinopathies

Patients with a paraprotein in their serum (Monoclonal Gammaglobulinopathies of Unknown Significance (MGUS), myeloma, secretory lymphoma) and a profound and unexplained thrombocytopenia should be investigated to determine whether the paraprotein is platelet reactive. Although rare, reactivity of paraproteins with platelets and thrombocytopenia has been reported and is the platelet equivalent of cold haemagglutinin disease.

4.2.4 Drug-Dependent Immune Thrombocytopenia (DDITP)

Many drugs are associated with thrombocytopenia. For some drugs there is firm evidence that the thrombocytopenia is antibody mediated. We recommend testing for DDITP for the following drugs:

- Heparin
- Antibiotics (penicillin type, beta-lactams and glycopeptide)
- Quinine and quinidine
- Gold salts

4.2.4.1 Heparin Induced Thrombocytopenia (HIT)

An ELISA test for heparin/platelet factor 4 specific antibodies can be of use in patients with a clinical diagnosis of HIT and in whom continued anticoagulation is required. In such patients, prompt withdrawal of heparin and alternative anticoagulation with recombinant hirudin or an alternative heparinoid should be considered without waiting for laboratory results. The BCSH guidelines for the management of heparin induced thrombocytopenia[2] describe a scoring system (based on the **4T**s) that can be used to assess the probability of a patient developing HIT:

- **T**hrombocytopenia
- **T**iming of platelet count fall
- **T**hrombosis
- O**T**her causes for thrombocytopenia are not evident

Table 10 should be used to assess the probability that a patient has HIT.

A score of: **6-8** means there is a high probability of HIT
4-5 means the probability is intermediate
0-3 means there is a low probability

If you think your patient has HIT, stop heparin and switch to an alternative antithrombotic agent.

[2] BCSH, Guidelines on the diagnosis and management of heparin induced thrombocytopenia: second edition – issue 2012, which can be found at: https://b-s-h.org.uk/guidelines/guidelines/diagnosis-and-management-of-heparin-induced-thrombocytopenia-second-edition/

Table 10: Estimating the probability of HIT: 'The 4 Ts'

Probability of HIT score:	Points (0, 1 or 2 for each of 4 categories: maximum possible score = 8)		
	2	**1**	**0**
Thrombocytopenia	>50% fall and/or platelet nadir 20-100 x 10^9/l	30-50% fall and/or platelet nadir 10-19 x 10^9/l	fall <30% and/or platelet nadir <10 x 10^9/l
Timing* of platelet count fall or other sequelae	Clear onset between days 5-10; or less than 1 day (if heparin exposure within past 100 days)	Consistent with immunisation but not clear (e.g. missing platelet counts) or onset of thrombocytopenia after day 10	Platelet count fall too early (without recent heparin exposure)
Thrombosis or other sequelae (e.g. skin lesions)	New thrombosis; skin necrosis; post heparin bolus acute systemic reaction	Progressive or recurrent thrombosis; erythematous skin lesions; suspected thrombosis not yet proven	None
O**T**her causes for thrombocytopenia are not evident	No other cause for platelet count fall is evident	Possible other cause is evident	Definite other cause is present

*First day of immunising heparin exposure considered day 0; the day the platelet count begins to fall is considered the day of onset of thrombocytopenia (it generally takes 1-3 days more until an arbitrary threshold that defines thrombocytopenia is passed).

It is requested that the 4Ts test be applied to all patients for whom samples are referred for heparin dependent antibody investigation and the score entered on the request form. In selected circumstances additional information may be requested regarding some referrals.

In cases where patients have received a transfusion in the previous 12 days followed by a precipitous drop in platelet count, a diagnosis of PTP should also be considered.

4.2.4.2 Other drugs

Investigation of the presence of platelet specific antibodies against other drugs is time consuming and positive control drug dependent antibody samples are typically not available. Reporting time will be extended since these investigations are not 'routine'. It is the referring centre's responsibility to provide samples of the implicated drug(s) (preferably in an aqueous form). Without such samples, the drug dependent antibody test will not proceed.

4.2.5 THROMBASTHENIA

4.2.5.1 Acquired thrombasthenia

Platelet autoantibodies generally target epitopes on GPIIb/IIIa (CD41), GPIb/IX/V (CD42), GPIa/IIa (CD49) or GPVI and, in some patients, the autoantibody may target the ligand binding site of these glycoproteins in cases with severe thrombocytopenia, a diagnosis of AITP is likely to be made. However, when the platelet count recovers during therapy a discrepancy between bleeding tendency and platelet count may be apparent. In such cases, platelet aggregation studies may be consistent with Glanzmann's Thrombasthenia (GT), Bernard Soulier Syndrome (BSS) or a collagen receptor deficiency of the acquired type. PAIg and autoantibody specificity investigations are important in these rare cases to confirm the true pathophysiology.

4.2.5.2 Glanzmann's thrombasthenia (GT), Bernard Soulier Syndrome (BSS), collagen receptor deficiencies

Homozygous or compound heterozygous mis-sense, non-sense mutations or deletions/insertions in genes encoding platelet membrane receptors can cause congenital bleeding disorders of the platelet type. Classic examples are GT and BSS, which are both rare autosomal recessive disorders with an absence or reduced expression of the platelet αIIBß3 integrin (GPIIb/IIIa, CD61/41) and the Von Willebrand Factor receptor complex (GPIb/IX/V, CD42), respectively. Reduced expression of the platelet collagen receptors (GPIa/IIa or α2ß1 integrin and GPVI) have also been reported as a cause of congenital thrombasthenia.

4.2.5.3 Investigations

The diagnosis of GT, BSS and collagen receptor deficiencies is made on bleeding phenotype, by the results of platelet aggregation studies and, in classic BSS, on platelet count and morphology. However, mis-sense mutations associated with a mild phenotype might be missed in aggregation studies depending on the dose of agonist used and BSS without the striking morphology of giant platelets has been reported. Monoclonal platelet glycoprotein antibodies against CD41/61, CD42 and CD49 and flow cytometry provide a sensitive method to confirm the diagnosis. However, many laboratories only use a single monoclonal antibody for each CD marker, which limits the diagnostic sensitivity; null mutants will be identified but subtler mis-sense mutations may remain undetected. Consequently, the patient's platelets are tested with a large panel of the relevant monoclonal antibodies to improve diagnostic sensitivity. These tests can only be performed after discussion with the H&I laboratory at Filton.

If the diagnosis of GT, BSS or inherited collagen receptor deficiency is confirmed, advice regarding transfusion support will be provided.

In addition, direct sequencing of the coding regions of the relevant genes, BSS – *GPIbα, GPIbβ GP/IX* GT – *ITGA2B* and *ITGB3* is now available; please contact H&I laboratory at NHSBT Filton.

4.3 GRANULOCYTE IMMUNOLOGY (GI)

There are seven clinical syndromes for which services are provided:

- Autoimmune neutropenia
- Neonatal alloimmune neutropenia
- Severe and persistent non-haemolytic febrile transfusion reactions (see section 4.1)
- Transfusion-related acute lung injury (see section 4.1)
- Persistent isolated neutropenia after allogeneic bone marrow transplant
- Drug-induced antibody mediated neutropenia
- Severe reactions to granulocyte transfusions (see section 4.1)

4.3.1 Autoimmune Neutropenia (AIN)

AIN is a rare clinical condition caused by granulocyte autoantibodies, which may occur either in children or adults, but which often remains undiagnosed. Autoimmune neutropenia commonly occurs in children between the ages of 6 months and 5 years (where it is referred to as Autoimmune Neutropenia of Infancy (ANI), although applied strictly the term 'infancy' describes children under one year of age). ANI tends to be a self-limiting autoimmune condition but can last several years. In adult patients, AIN presents as a chronic disorder either as an isolated (primary) neutropenia or as a neutropenia secondary to other disorders, such as rheumatoid arthritis, systemic lupus erythematosus, Felty's syndrome and chronic lymphocytic and large granulocytic leukaemias.

Granulocyte autoantibodies may target the low affinity Fc receptor for IgG (FcγRIIIb or CD16); GP 56-64 kDa related antigens (CD177) or CD11/18. Autoantibodies can demonstrate human neutrophil

antigens (HNA) related specificity and therefore the sera are screened against a panel of granulocytes typed for HNA. On occasion, it is important to determine whether antibodies with HNA specificity are autoimmune or alloimmune in origin. This can be achieved by typing the patient for the relevant HNA and/or performing a direct granulocyte immunofluorescence test. Immune complexes may also bind to granulocytes. There is no simple procedure to distinguish between immune complexes and pan-reactive autoantibodies.

4.3.1.1 Investigations

The serum will be investigated by the indirect granulocyte immunofluorescence and chemiluminescence tests using granulocytes from donors typed for HNA-1, -2, –3, 4 and 5. These investigations are only indicated if the patient has a neutrophil count < 2.0 x 10^9/L and the results will affect clinical management. Referrals without a stated neutrophil count or if the neutrophil count is >2.0x10^9/L or if inadequate clinical information is provided may not be investigated. If the serum test is negative, a direct granulocyte immunofluorescence test for IgG and IgM can be arranged with the laboratory but only in cases where there is strong evidence to support the diagnosis and where the result will influence clinical management. Elevated granulocyte bound immunoglobulins have been found in patients who lack demonstrable serum autoantibodies. Direct tests cannot be performed on patients with a neutrophil count < 0.4 x 10^9/L or if the patient has received G-CSF or IVIGG within the previous 3 weeks. Granulocytes are labile cells that deteriorate rapidly in vitro. Consequently, blood samples for direct tests must reach the GI section of the H&I laboratory at NHSBT Filton within 24 hours of venesection. The laboratory must be contacted prior to sending samples for direct tests so appropriate control samples can be arranged.

4.3.2 Neonatal Alloimmune Neutropenia (NAIN)

NAIN is caused by maternal alloantibodies against a granulocyte-specific antigen, which is present on the neutrophils of the neonate and absent from the maternal neutrophils. The condition is rare (< 1 in 1000 births) but may be under-diagnosed. Profound neonatal neutropenia places the child at risk of infectious complications. The neutropenia may persist for up to six months. In the majority of cases, the maternal alloantibodies are directed against HNA. Occasionally, NAIN can arise due to the formation of isoantibodies against granulocyte membrane glycoproteins, e.g. FcγRIIIb (CD16) which is absent in approximately 1 in 2000 of the population. Clinical management consists of the use of antibiotics either prophylactically or in response to infections. G-CSF may be required where there is severe persistent neutropenia and infection.

4.3.2.1 Investigations

Serum investigations are similar to those for autoimmunity, but a crossmatch of maternal serum versus paternal granulocytes may be performed to determine the presence of low frequency granulocyte-specific antibodies if initial investigations are negative. In the event of HLA specific antibodies being present, the serum sample will be further investigated by a glycoprotein capture ELISA (MAIGA assay). The maternal and paternal HNA type will be determined. In serologically confirmed cases of NAIN involving HNA specific antibodies, the zygosity of the father of the child should be determined so that the risk to future pregnancies can be assessed.

4.3.3 Persistent isolated neutropenia after bone marrow transplant

Both HNA alloantibodies and autoantibodies can cause persistent isolated neutropenia after bone marrow transplantation. Granulocyte immunology investigations can be informative in such cases. Investigations are similar to those described above.

4.3.4 Drug-induced antibody mediated neutropenia

A wide range of drugs can cause immune mediated neutropenia. However, these idiosyncratic reactions only occur in a small number of patients. There are several mechanisms for drug induced antibody

mediated neutropenia. One established mechanism occurs when membrane glycoproteins bind to the drug to form a hapten. This causes the formation of antibodies which only bind to granulocytes in the presence of the drug. Quinine, and its stereoisomer quinidine, is known to cause drug dependent antibody formation via this hapten mechanism.

Other drugs (e.g. ß-lactams) have been reported to elicit the formation of antibodies. Alternatively, some drugs induce the formation of 'true' autoantibodies, which are able to bind granulocytes in the absence of any drug. These drugs (e.g. levamisole) appear to alter the homeostasis of the immune system resulting in autoimmunity against granulocytes in a small number of patients.

The investigation of cases with drug dependent antibodies can be complicated. Furthermore, some antibodies have been reported to only be detected at specific concentrations of the drug, by specific techniques or in the presence of drug metabolites. Please phone the H&I laboratory at NHSBT Filton before referring such cases.

4.3.4.1 Investigations

The patient serum sample is investigated for granulocyte-specific antibodies by granulocyte immunofluorescence tests using a panel of granulocytes typed for HNA-1 to -5 in the presence and absence of the implicated drug. The referring centre must provide a sample of the implicated drug(s). Further investigations may require the provision of anti-coagulated blood from the patient.

4.3.5 Granulocyte transfusion reactions

An increment in granulocyte count greater than 0.5 x 10^9/L is not always achieved in profoundly granulocytopenic recipients by granulocyte transfusions. An incremental count would be expected to be seen with granulocyte doses of at least 1x10^{10} granulocytes/m^2 of recipient surface area. Severe reactions to granulocyte transfusions and failure to increment despite adequate granulocyte dosage may suggest HLA or granulocyte specific antibody formation in the recipient and in these cases referral for antibody screening is advised. The investigations are similar to those described previously and where necessary HNA typing of the patient and implicated and/or prospective donors will be undertaken.

4.4 HAEMATOPOIETIC STEM CELL TRANSPLANTATION

4.4.1 HLA typing of recipients and related or unrelated donors

Incompatibility in the HLA expressed by the recipient and the stem cell donor is one of the most important factors influencing the outcome of transplantation. It is therefore crucial that the most up-to-date techniques are used to identify these incompatibilities at the DNA level. The implementation of NGS for allelic level HLA typing is part of NHSBT's strategy to support improved patient outcomes with the latest technological advances in HLA typing. NHSBT H&I laboratories are perfectly placed to carry out these tests since a significant number of patients prepared for haematopoietic stem cell transplant are also investigated by NHSBT for their platelet transfusion support. All aspects of the service are compliant with the relevant standards for haematopoietic stem cell transplantation, specifically:

- Standards for Histocompatibility Testing Version 8.0 European Federation for Immunogenetics (EFI) January 2020 https://www.efi-web.org/efi-committees/standards-committee.html

4.4.2 HLA antibody screening for haematopoietic stem cell transplant patients

For certain patients undergoing allogeneic stem cell transplantation it is advisable to perform HLA class I (and class II) specific antibody screening well in advance. As the use of alternative donors (e.g. HLA mismatched adult donors, haploidentical donors and cord blood) for HSCT is increasing the relevance

of HLA specific antibodies on donor compatibility becomes critical. Knowledge of the patient's antibody status is of value when selecting the final donor for transplant and assessing overall risk. Platelet transfusion support may also be complicated in HLA antibody positive patients.

4.4.3 Unrelated donor searches of the stem cell and cord blood registries

NHSBT provides a facility for searching national and international unrelated stem cell and cord blood registries for patients requiring haematopoietic stem cell transplantation where no HLA compatible family member has been identified. Requests from transplant centres for searches of registries should be made via the local NHSBT H&I laboratory. A search via NHSBT H&I laboratories will automatically be referred to the Anthony Nolan Trust (ANT) who, on behalf of the aligned registries, will undertake a search of volunteer unrelated donors held on the British Bone Marrow Registry (BBMR), Welsh Bone Marrow Donor Registry (WBMDR) and Anthony Nolan Registry. When required, searching of international registries can also be initiated. For cord blood stem cells, the UK cord blood registries and other international cord blood registries are searched. The H&I laboratory will co-ordinate the donor searches and the request for confirmatory typing of potential donors. They will also advise on the final selection of the most suitable donor and will liaise on behalf of the requester with relevant donor registry.

4.4.4 Graft Information Advisory Service (GIAS) and compatibility assessment

Graft Information Advisory Service (GIAS) and compatibility assessment is an integral part of haematopoietic stem cell transplantation support from our laboratories. Our services are always supported by the highest standards of advice from Consultant Clinical Scientists and their staff. Clinical Scientist staff will support the identification and selection of donors most advantageous to your patients. GIAS encompasses the whole process from diagnosis through to post transplant and beyond including transplant and antibody monitoring and supply of specialised selected blood products when needed.

4.4.5 Chimerism investigation for post-transplant monitoring

Assessment of the chimeric status of a patient following transplantation can be undertaken using Short Tandem Repeat (STR) analysis. Fluorescently labelled PCR primers are used to amplify STR loci resulting in an 'STR profile' for the patient pre-transplant, the donor and the patient post-transplant.

A pre-transplant sample from the patient and an EDTA peripheral blood sample or bone marrow aspirate post-transplant is required for STR analysis. A donor sample is also required. Where possible, if sample size allows, both patient pre-transplant and donor DNA will be stored at laboratories where HLA typing has been performed. If a stored sample is not available it is possible to isolate DNA from the buccal cells of the patient, with the resulting DNA being the equivalent of a pre-transplant sample. Your local H&I laboratory will be able to advise.
Data has demonstrated that increased sensitivity can be achieved in the investigation of chimerism when isolating specific cell lineages e.g. T cells. This may be particularly relevant for patients with certain malignancies, where cell lineage isolation prior to STR analysis can detect changes in chimeric status otherwise undetectable by whole blood analysis. NHSBT H&I laboratories are able to perform STR analysis on specific cell lineages, e.g. T cells, the myeloid compartment and B cells. Again, an EDTA blood sample from the patient post-transplant is required for this analysis. Testing follows recently published consensus best practice guidelines and recommendations for STR chimerism testing[3].

STR analysis is also a critical diagnostic tool in the investigation of TAGVHD. STR profiles can be established for the patient pre-transfusion, the implicated donor and the patient post-transfusion. This allows assessment of the chimeric status of the patient post-transfusion. Samples required for this analysis would be a patient pre-transfusion sample (if no DNA has previously been isolated from this patient, then a buccal scrape would provide cells for DNA isolation), an EDTA blood sample from the donor and an EDTA sample from the patient post-transfusion.

[3] British Journal of Haematology, 2015, 168, 26-37

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4.5 SOLID ORGAN TRANSPLANTATION

The NHSBT H&I laboratories support Solid Organ Transplantation (SOTx) by identifying and characterising immunological risk factors that determine outcome and provide advice accordingly. These risk factors are the degree of HLA mismatch between donor and recipient and specific sensitisation to non-self HLA. A 24 hour on-call service operates every day of the year for deceased donor HLA typing and crossmatching of local patients for renal and, where appropriate, cardiothoracic transplantation. Our aim is to work in partnership with the transplant and clinical units as part of the overall transplant team. Accountability for service provision, development and governance lies with the local H&I Consultant. Development of local transplant policies, particularly allocation rules should include liaison with the Head of Laboratory. Clinical and scientific advice from an H&I consultant clinical scientist relating to solid organ transplantation is also always available.

4.5.1 HLA typing for SOTx

In the UK, deceased donor kidneys are currently allocated though NHSBT Organ Donation & Transplantation (ODT) using matching algorithms in which HLA match is a key factor. Thus, all patients are required to be HLA typed before being placed on the transplant waiting list. Donors will be HLA typed and then allocated to recipients on the list based on factors including HLA match. The matching schemes for organ allocation can be found on the ODT web site at: http://www.odt.nhs.uk/transplantation/guidance-policies/.

Because of the extreme variability of HLA in the population, most patients will receive a graft from a donor mismatched to some degree for HLA. The greater the degree of mismatch the greater risk of immunological rejection, however, by modifying the immunosuppression this may be compensated for. HLA matching is normally not a primary consideration in other forms of transplantation (cardiothoracic, liver, etc.). Graft failure is often associated with immunological sensitisation to mismatched donor HLA and this can severely limit the possibility of re-transplantation if this were to be an option. It is the responsibility of the clinical teams to inform the laboratory if a patient has been exposed to a specific sensitisation event and provide a test sample.

4.5.2 Routine testing for HLA-specific antibodies

HLA specific sensitisation is best investigated by serological analysis for antibodies. Any exposure to non-self HLA, such as from transplantation, transfusion or pregnancy can stimulate the production of HLA specific antibodies. These can vary in their potency and persistence depending on the nature and number of stimulating events but represent a significant risk of graft failure. All patients on a transplant waiting list should therefore be monitored regularly for the presence of HLA specific antibodies. For prospective kidney and cardiothoracic transplant patients the recommendation is that each patient should be tested at least at three monthly intervals and after each potential sensitising event[4].
All antibody positive sera will be characterised for specificity for all known HLA A, B, C, DR, DP and DQ antigens. For some sera (i.e. those from highly sensitised patients, reacting with over 80% of the donor population) this may require successive testing by increasingly sensitive and specific techniques. In such cases the completion of testing may take significantly longer than for less complex cases.

For certain highly sensitised patients pre-transplant antibody removal (desensitisation) may offer the only possibility of being transplanted. NHSBT H&I laboratories can support such procedures, but because it is excessively labour-intensive for the laboratory this must be discussed and planned with the Head of the Laboratory before proceeding.

Post-transplant antibody monitoring is recommended for most types of solid organ transplantation[4]. For immunologically high-risk transplants antibody monitoring should be intensified. For any transplant, if

[4] BTS/BSHI Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation, Version 4.0 January 2016
https://bts.org.uk/wp-content/uploads/2016/09/06_BTS_BSHI_Antibodies-1.pdf

rejection is suspected, a test for donor specific antibodies can confirm a diagnosis of rejection and indicate a course of management. Such testing can be performed on demand but usually only during normal working hours.

4.5.3 Crossmatching

If present at a high concentration, patient antibodies corresponding to mismatched donor HLA can cause immediate and irreversible rejection (hyperacute rejection) of the transplanted organ. The presence of donor HLA specific antibodies in the serum of the patient at any time prior to transplant is an indication of prior sensitisation and even in cases where these antibodies are not present at a high concentration at the time of transplant they indicate there may be an increased risk of accelerated acute or acute rejection. Performing a prospective crossmatch between donor and recipient can prevent hyperacute rejection and identify some patients at risk of acute rejection. A pre-transplant crossmatch can therefore avoid an unintentional antibody incompatible transplant and is performed in one of two ways. Firstly, using donor cells (peripheral blood leucocytes or leucocytes obtained from spleen or lymph nodes) donor reactive antibodies can be assessed directly by either Complement Dependant Cytotoxic (CDC) or flow cytometry; the latter being a more sensitive assay. Secondly, using the results of HLA antibody specificity tests on the recipient together with the HLA type of the donor a virtual crossmatch (VXM) can be performed. Essentially the VXM predicts the result of a donor cell-based crossmatch and is dependent on a comprehensive knowledge of the specificity of any detected antibody and its potential reactivity with a donor of given HLA type. Virtual crossmatching is routinely used in cardiothoracic transplantation where time does not allow for a cell-based crossmatch to be completed. In renal transplantation virtual crossmatching may be used for a well-defined population of potential recipients but is not currently recommended for highly sensitised patients.

A prospective crossmatch is required or recommended in renal, pancreatic, cardiothoracic and small bowel transplantation. The choice of pre-transplant crossmatch can vary with transplant type and should be controlled by local policies guided by national policies, guidelines and accreditation standards. Where the prospective crossmatch was a virtual crossmatch, a retrospective donor leucocyte confirmatory crossmatch should always be done.

The time taken to perform the leucocyte crossmatch is usually between 3 and 6 hours of laboratory time. A VXM can usually be completed in 30 minutes but this does require the on-call H&I scientist to go to the laboratory in order to review the patient's serological history.

The results of crossmatch tests can be highly 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 will be provided by the H&I consultant clinical scientist, as required.

4.5.3.1 Pre-transplant antibody removal (Desensitisation)

Pre-transplant antibody removal, undertaken to allow transplantation in crossmatch positive cases (Antibody incompatible Transplantation, AiT) is termed desensitisation. Incompatibility is either due to ABO mismatch or preformed HLA Donor Specific Antibodies (DSA). Desensitisation is achieved by extracorporeal antibody removal using various techniques.

During the desensitisation process antibody removal should be monitored so that the effectiveness of the process can be assessed, and a safe level of residual antibody can be determined before the transplant can proceed. During the early post-transplant phase DSA can be re-synthesised and cause rejection. Early detection of an emerging response allows effective treatment and management of rejection. Frequent DSA monitoring with fast turnaround times are therefore essential for a safe desensitisation programme.

Rapid DSA testing requires significant resources and scientific staff need to be available on demand. Therefore, if laboratory support for AiT is required there must be a formal agreement with the laboratory

to allocate the necessary resources. Effective communication between the laboratory and the transplant unit is essential for these high-risk procedures to be undertaken with safety. A reliable and unimpeded sample transport system must be established.

4.5.3.2 Tests

4.5.3.2.1 ABO AiT

Antibody levels will be monitored in terms of titre of IgG and IgM using reagent red cells of the donor group. Transplantation would not normally proceed if the titre of the corresponding antibody exceeds 1:8. It is therefore very important to test immediately before the transplant is to proceed. In NHSBT ABO testing is carried out by Red Cell Immunohematology (RCI) who will make a separate charge for this service.

4.5.3.2.2 HLA AiT

The amount of desensitisation required will depend on the pre-treatment levels of DSA. The highest levels of DSA will be cytotoxic and a cytotoxic titre assay should be performed to measure these against donor lymphocytes (CDC crossmatch). The strength of non-cytotoxic DSA should be assessed by a flow cytometric crossmatch (FCXM).

DSA levels and specificity are most effectively monitored using antigen coated beads in an immunofluorescence assay (e.g. Luminex™). These assays are significantly more sensitive than previous methods and our experience shows that reducing DSA to undetectable by desensitisation is rarely achieved. An assessment of a safe level for transplantation needs to be determined for each case by discussing with the H&I consultant. A pre-transplant crossmatch should always be performed, normally a FCXM is sufficient.

4.5.3.2.3 Frequency of testing

During antibody removal, pre- and post-treatment serum samples should be sent directly to the laboratory. Throughout the early post-transplant phase (up to three weeks) daily serum samples should be taken and sent to the laboratory. Early post-transplant antibody re-synthesis can be treated with antibody removal if accompanied by rejection and this should be monitored as above. From week three approximately weekly serum samples should be taken for antibody testing until a stable antibody profile is established (usually 3-5 months). Thereafter monthly samples should be taken to year one followed by six-monthly samples.

4.5.4 Additional Services

In addition to performing and reporting tests, there are certain supporting and administrative elements provided by the laboratory, which may constitute part of the H&I service required for a transplant programme. It is important that close liaison is maintained between the laboratory and the clinical transplant units in order to establish good working relationships with the medical and nursing staff. Senior laboratory staff should attend relevant clinical and audit meetings. The H&I laboratory should play a major role within the multidisciplinary team involved in the provision, planning and development of clinical transplantation services.

The laboratory maintains a database of successive test results for all patients and their donors. From this we can establish and review each patient's immunological history and where necessary provide advice on general transplant suitability and specific advice regarding risk of individual transplants.

For patients on the national renal transplant waiting list (at Organ Donation and Transplantation (ODT)), the H&I laboratory will be responsible, if required, for updating the ODT database with HLA typing and antibody data and collating other information as requested. In addition, registration of new patients with

ODT can be performed by the H&I laboratory. To do this, additional information, such as demographics, virology status, and blood group must be given to the laboratory.

Blood grouping of donors and recipients can be undertaken by NHSBT RCI. Samples should be sent direct to your local NHSBT RCI department with an appropriate test request form (1A). RCI will charge separately for grouping tests.

Further information about NHSBT RCI services can be found in their user guide at: https://hospital.blood.co.uk/diagnostic-services/user-guides/

Where a local transplant waiting list is required, this can also be maintained and distributed by the laboratory. The laboratory database has the functionality to identify patients from whom we have not received sufficiently recent samples. We can send written reminders to the clinical units, as part of our service. Failure to provide up-to-date serum samples can compromise the chance of a patient receiving a transplant.

4.5.5 Out of hours

Out of hours on-call is provided by H&I laboratories supporting solid organ transplant programmes, 24 hours a day, 365 days a year. A consultant clinical scientist can be contacted on your request in case of clinical emergency via your local Hospital Services department.

4.6 IMMUNOGENETICS - HLA TYPING FOR DISEASE ASSOCIATION AND DRUG HYPERSENSITIVITY

Genetic variations (mutations or polymorphism) within genes are now known to occur frequently throughout the human genome. Amongst these are mutations in genes located on or in proximity to the Major Histocompatibility (MHC) locus on the short arm of chromosome 6. Genetic markers determining the risk for the development of certain diseases can be identified by testing performed by the NHSBT H&I laboratories.

Examples of HLA genes associated with disease include HLA-B27 with ankylosing spondylitis and specific HLA-DQ genes with coeliac disease (CD). In CD only certain HLA-DQ heterodimers are able to present the gluten peptides to immune cells and initiate the response which leads to CD. Complete testing of both DQ alpha and DQ beta genes is required in order to identify the implicated alleles.

The HFE gene associated with hereditary haemochromatosis is another gene found in the MHC region. Two mis-sense mutations in the HFE gene, a cysteine282tyrosine and a histidine63aspartic acid, have both been shown to be associated with the development of disease. Between 80-90% of haemochromatosis cases are homozygous for the tyrosine282 codon. In addition, up to 78% of individuals heterozygous for both mutations may exhibit evidence of iron overload. A DNA based technique that allows the simultaneous identification of both HFE mutations has been developed and validated by participation in an appropriate EQA scheme.

HLA genes have also been found to be markers of some drug hypersensitivity responses, most notably HLA-B*57:01 is associated with hypersensitivity to the anti-retroviral drug abacavir. The H&I laboratories routinely provide HLA-B*57:01 testing. Testing for other HLA alleles implicated in drug hypersensitivity reactions can also be provided on request. Such associations include HLA-B*15:02 hypersensitivity to the anti-epileptic drug carbamazepine in individuals of Chinese descent and with A*31:01 in Caucasians. Numerous other associations between HLA alleles and drug hypersensitivities have been reported, including associations between a diverse range of drugs and Drug-Induced Liver Injury (DILI) and other hepatic adverse events. DILI is an important cause of serious liver disease and is responsible for 11-17% of cases of acute liver failure in Europe and the US. The most commonly reported HLA and DILI associations are listed in Table 13, along with examples of other reported HLA associated drug hypersensitivities.

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Tests for the detection of polymorphisms in genes involved in the metabolism and absorption of immunosuppressive drugs used in organ transplant patients are being developed and may be offered by the H&I laboratories supporting solid organ transplant programmes. In particular, current Clinical Pharmacogenetics Implementation Guidelines indicate if *CYP3A5* genotype is known, this can be used to guide tacrolimus dosing, with extensive (*CYP3A51/*1*) and intermediate (*CYP3A5*1/X*) metabolisers given a tacrolimus starting dose x1.5-x2.0 that given to patients with reduced CYP3A5 expression (CYP3A5*3/*3.

H&I laboratories also provide molecular typing for detecting polymorphisms in other immune related genes such as minor Histocompatibility (mH) genes, natural killer (NK) cell receptor genes and cytokine genes. All polymorphisms are detected using DNA based methods.

Examples of HLA associated, and HLA linked diseases are shown below (see tables 11, 12, 13 and 14). There are additional HLA associated diseases for which a typing service can be provided. Please contact the Head of Laboratory of your local NHSBT H&I laboratory to discuss the appropriate test.

Table 11: HLA associated diseases

Birdshot chorioretinopathy	HLA-A*29
Behcet's disease	HLA- B*51
Ankylosing spondylitis	HLA-B*27
Rheumatoid arthritis	Amino acids 70-74 on the DRB1 gene (QKRAA or QRRAA)
Narcolepsy	HLA-DQB1*06:02/DQA1*01:02
Coeliac disease	HLA-DQA1/DQB1
Selective IgA deficiency	HLA-DRB1*0301/DQB1*02
Development of anti-HPA-1a in NAIT*	HLA-DRB3*01:01

* Neonatal Alloimmune Thrombocytopenia

Table 12: HLA linked diseases

Haemochromatosis	HFE gene C282Y and H63D
21 OH deficiency	(HLA-B*47) 21 OH gene

Table 13: HLA genes associated with drug hypersensitivity / Adverse Drug Reactions (ADR)

Drug/reaction	Use of drug	HLA association
Abacavir hypersensitivity	Anti-retroviral	HLA-B*57:01
Allopurinol induced severe cutaneous adverse reactions (SCARs)	Treatment of Gout	HLA-B*58:01
Amoxicillin-clavulanate induced DILI	Anti-microbial	HLA-DRB1*15:01/DQB1*06:02
Carbamazepine induced Stevens-Johnson Syndrome / Toxic Epidermal Necrosis (SJS/TEN)	Anti-epileptic	HLA-B*15:02 (Han Chinese / Thais) HLA-A*31:01 (Japanese / Caucasian)
Clopazine and agranulocytosis, neutropenia	Treatment-resistant schizophrenia	HLA-DQB1 126Q (DQB1*05:02), HLA-B 158T (B*38, B*39, B*67)
Co-trimoxazole induced SJS/TEN	Anti-microbial	HLA-B*15:02, C*06:02, C*08:01 (Thais, but may apply in other ethnic groups)
Dapsone hypersensitivity syndrome	Anti-microbial, used in combination for treatment of leprosy	HLA-B*13:01
Fenofibrate induced DILI	Cholesterol reduction in patients at risk of cardiovascular disease	HLA-A*33:01
Flucloxacillin induced DILI	Anti-microbial	HLA-B*57:01 (on A*01:01-B*57:01-C*06:02-DRB1*07:01-DQB1*03:03 haplotype)
Flupirtine induced DILI	Non-opioid analgesic	HLA-DRB1*16:01-DQB1*05:02 haplotype
Lamotrigene induced SCARs	Anti-epileptic, anti-bi-polar	HLA-B*15:02, A*02:07 (Thai and other Asian) HLA-B*38:01, A*24:02 (Spanish)
Lapatinib induced DILI	Breast cancer, other solid tumours	HLA-DRB1*07:01/DQA1*02:01
Nevirapine hypersensitivity, including hepatotoxicity, maculopapular exanthema, SJS and TEN.	Anti-retroviral	HLA-C*04:01 (Sub-Saharans, Thais, Chinese HLA-DRB1*01:01 (Caucasians) HLA-C*08 (Thais); HLA-B*35:*05* Thais)
Oxcarbazepine induced SJS/TEN	Anti-epileptic	HLA-B*15:02 (Han Chinese)
Phenytoin induced SCARs	Anti-epileptic	HLA-B*15:02 (Chinese)
Ticlopidine induced DILI	Anti-platelet/anti-coagulant	HLA-A*33:01 HLA-A*33:03 (Japanese)

Terbinafine induced DILI	Anti-fungal	HLA-A*33:01
Trichloroethylene and neurotoxicity, hepatotoxicity, nephrotoxicity, immunotoxicity	Industrial chemical – degreasing, extraction, dry-cleaning	HLA-B*13:01 (Chinese)
Ximelagatran and elevated serum alanine aminotransferase	Thromboembolism	HLA-DRB1*07:01/DQA1*02

Please enquire for other testing you may require.

Table 14: Polymorphism in other immune-related genes

Cytokines and cytokine receptor genes	e.g. *TNFA*, *IL-10* and *IL-6*
NK cell receptors	
Minor Histocompatibility antigens	(HA-1)

CHAPTER 5: TECHNIQUES USED IN NHSBT H&I LABORATORIES

The following techniques are routinely used in the H&I laboratories, although further techniques are also used for the investigation of suspected thrombasthenias and further studies. Please refer to the appropriate section in this guide for details regarding techniques used in specific clinical conditions.

5.1 Screening for cytotoxic and non-cytotoxic HLA specific antibodies

Screening for HLA class I and class II specific antibodies is performed using one or more of a number of different techniques including the Complement Dependant Cytotoxic test, Luminex™ and flow cytometric based methods. If the screening is positive, further tests are carried out to identify the specificity of the antibodies.

5.2 Molecular HLA class I and class II typing

HLA class I (A, B, C) and class II (DR, DQ, DP) DNA typing is carried out using a variety of DNA based techniques including Sequence Specific Priming (SSP), Sequence Based Typing (SBT) and Next Generation Sequencing (NGS). All molecular techniques used for HLA class I and class II molecular typing have been fully validated as part of the participation in national and international histocompatibility workshops and external quality assurance schemes.

5.3 High through put HLA class I and class II typing by (NGS)

NHSBT has developed and implemented NGS for allelic level HLA class I (A, B, C) and class II (DR, DQ and DP) typing. NHSBT has employed a whole gene sequencing approach which allows unambiguous assignment of allelic level HLA types. NGS technology is currently applied to typing the following loci; HLA-A, B, C, DRB1 and DQB1 for BBMR donors and donations banked by the NHS Cord Blood Bank and is available for other categories of patients, donors and research studies.

For more information about HLA typing by NGS please visit our 'Hospitals and Science' site: https://hospital.blood.co.uk/diagnostic-services/histocompatibility-and-immunogenetics/next-generation-sequencing/.

5.4 Crossmatching

Different techniques are used by the laboratories which includes Complement Dependant Cytotoxic test and flow cytometric based methods.

5.5 Short Tandem Repeat (STR) analysis for the detection of chimerism

Fluorescently labelled PCR primers are used to amplify STR loci resulting in an 'STR profile' for the patient pre-transplant, the donor and the patient post-transplant. This allows assessment of the chimeric status of the patient following transplantation or during investigation of suspected TAGVHD. NHSBT H&I laboratories are able to perform STR analysis on specific cell lineages, e.g. T cells, the myeloid compartment and B cells.

5.6 Screening for platelet specific antibodies

The Platelet Immunofluorescence Test (PIFT) with a flow cytometric endpoint and the Monoclonal Antibody Immobilisation of Platelet Antigen (MAIPA) assay together with a panel of HPA typed platelets are used to facilitate the detection and identification of antibodies directed against platelet membrane glycoproteins. Both techniques are usually performed as an indirect test using patient serum but both tests can also be used as a direct test to detect immunoglobulins bound to patient's platelets and identify platelet glycoprotein specificities. Typically, the direct MAIPA assay is only performed for specific patients after discussion with the H&I laboratory at NHSBT Filton.
In addition, a Luminex™ based assay is now in use.

5.7 Molecular typing for HPA alleles (1, 2, 3, 4, 5, 6, 9 and 15)

Determination of HPA alleles (1, 2, 3, 4, 5, 6, 9 and 15) is performed using Polymerase Chain Reaction Sequence Based Typing (PCRSBT).

5.8 Heparin Induced Thrombocytopenia

ELISA tests for heparin dependent platelet factor 4 specific antibodies are performed in suspected cases of Heparin Induced Thrombocytopenia (HIT). Excess heparin is added to the test system to confirm that positive reactions are heparin dependent rather than autoantibodies. Additional testing using different ELISA assays is available for use in specific cases where initial test results are ambiguous.

5.9 Screening for granulocyte specific antibodies

The Granulocyte Immunofluorescence Test (GIFT) with a flow cytometric endpoint, the Granulocyte Chemiluminescence Test (GCLT), and the Monoclonal Antibody Immobilisation of Granulocyte Antigen (MAIGA) assay are used together with an HNA typed granulocyte panel to facilitate the detection and identification of antibodies directed against granulocyte membrane glycoproteins. These techniques are usually performed as indirect tests using patient serum. Direct immunofluorescence tests using the patient's granulocytes can be performed in certain cases, but these investigations are restricted by the patient's neutrophil count and the necessity to test the samples within 24 hours of venesection. A direct test will only be performed after tests for granulocyte serum antibodies have been performed and must be arranged in advance with the H&I laboratory at NHSBT Filton. Granulocyte immunology investigations can be prolonged compared to other investigations because granulocytes are labile cells and cannot be stored for testing.
In addition, a Luminex™ based assay is now in use.

5.10 Typing for HNA

HNA (-1, -2, -3, -4 and -5) are typically performed either by serology (HNA-2) or polymerase chain reaction by PCR SBT(HNA-1, -3, -4, -5).

CHAPTER 6: ORDERING SPECIALIST PRODUCTS

6.1 Ordering blood products

All standard blood products are ordered from NHSBT Hospital Services, which can be contacted 24 hours per day, each day of the year. Use direct dial numbers during normal working hours and for out of hours. Medical and scientific advice is available 24 hours a day. Refer to Chapter 7 for contact details.

6.1.1 Specialist products issued by the H&I Function

HLA and or HPA selected platelets are ordered directly from NHSBT H&I during laboratory operating hours (09:00 to 17:00). The laboratory staff can be contacted directly and will liaise on your behalf with the respective NHSBT Hospital Services and Transport departments to organise delivery either directly to your hospital or via your local blood centre.

6.1.2 Ordering of HLA selected platelets

Refer to sections 4.1.1.8, 4.1.1.8 and 4.1.1.9.

6.1.3 Order notice time

Refer to section 2.1.

When an order is placed for the first time for a patient the following information is required:

- Patient surname, first name, date of birth and hospital name in full
- NHS number
- ABO and D groups
- HLA class I type (if known)
- Clinical diagnosis (Bleeding grade if known)
- CMV antibody status of patient
- Period of expected thrombocytopenia
- Contact person at hospital transfusion laboratory
- Consultant or Specialist Registrar responsible
- Current platelet support
- Patient weight

In case of an allogeneic stem cell transplant, the following information is required for the donor:

- CMV antibody status
- HLA class I type (if known)
- ABO and D groups

6.1.4 Ordering of HPA selected platelets (refractory) and red cells (non-refractory)

Refer the section 4.1.1.7, 4.1.1.8 and 4.1.1.9. When an order is placed for the first time for a patient please contact the H&I laboratory at NHSBT Filton (Monday - Friday 09:00 to 17:00) to discuss the case.

The following information is required:

- Patient surname, first name, date of birth and hospital number or patient address
- NHS number
- Hospital name
- ABO and D groups
- Contact person at hospital transfusion laboratory
- Consultant or specialist registrar responsible
- Clinical diagnosis
- Red cell specific antibodies present
- Type, quantity of product required, date and time needed

6.1.5 HPA typed products

Please note that the HLA investigation **must** be completed prior to the HPA investigation.
The following HPA typed products are available:

- HPA-1a negative red cells
- HPA-1a and HPA-5b negative apheresis platelet concentrates for neonates (neonatal dose)
- HPA-1a or HPA-5b negative platelet hyper-concentrates for foetal use from accredited donors#
- Red cells or platelet concentrates typed for other HPA antigens *

These must be ordered at least 7 days in advance
* These products may not be available 'off the shelf'

HPA-1a and 5b negative typed platelet concentrates and red cells are banked at a limited number of blood centres. Orders for these products are processed during working hours from the H&I laboratory at NHSBT Filton. During 'out of hours', contact the Hospital Services department at your local blood centre.

HPA-1a negative red cell SAG-M concentrates and HPA-1a and HPA-5b negative apheresis platelet concentrates (neonatal dose, 1/4 of a standard adult dose) are normally available 'off the shelf' at selected centres. Apheresis platelet concentrates, or red cells negative for other HPA antigens need to be ordered well in advance (ideally at least 4-7 working days). Please discuss with the H&I laboratory at NHSBT Filton if additional HPA typing is required.

HPA-1a negative platelet hyper-concentrates for use are provided from specially accredited apheresis donors who lack antibodies against red cells, HLA or HPA and are CMV negative. The first hyper-concentrate needs to be obtained from an RhD negative donor before the type of foetus is determined. When ordering hyper-concentrates, please contact the laboratory ideally with at least 7 working days' notice as this product has a shelf life of 24 hours and is not a stock item. A request form for the ordering of platelet hyper-concentrates is available from the H&I laboratory at NHSBT Filton. The laboratory must be informed on pre- and post- transfusion platelet counts to ensure the effectiveness of the treatment.

6.1.6 Out of hours

Refer to section 2.1. H&I platelet immunology services do not provide a laboratory 'out of hours' service. However, HPA -1a(-), 5b(-) typed platelets can be issued from stock on request. Requests for HPA selected neonatal platelets and HPA selected red cells, should be discussed with a NHSBT medical consultant. Phone NHSBT Hospital Services and they can put you in contact with the 'on call' medical consultant.
For further details please refer to:
https://hospital.blood.co.uk/diagnostic-services/histocompatibility-and-immunogenetics/ordering-hpa/

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CHAPTER 7: COMMUNICATING WITH NHSBT CENTRES

NHSBT staff can be contacted by telephone, fax or e-mail either directly using their personal details or through the centre switchboards (provided in Table 18). Contact details can also be found on test request forms and patient reports.

By direct dialling
All departments and senior staff can be contacted using direct dial numbers in this guide. Our internal telephone system allows external calls to be transferred between departments, centres and to mobile phones.

Via switchboard
Alternatively, all centres can be contacted 24 hours per day, 7 days a week via the switchboard number during office hours and via NHSBT Hospital services outside office hours.

Via mobile phone
Mobile phones are used by the majority of medical & clinical scientist consultants, senior scientific and managerial staff. External calls to any of the blood centres in the country can be directly transferred to a mobile phone. The secretariats can advise on how to contact a member of staff when he/she is not at their base centre.

Sending a fax
In line with the directive from the Department of Health and Social Care, we do not encourage the use of faxes, but a fax number can be made available for business continuity purposes, if required. All centres have central fax facilities. It is therefore important that your fax is labelled clearly with the name of the person to who you wish to send it and if urgent, please indicate accordingly. Nearly all H&I laboratories and all Hospital Services departments have their own fax.

Via e-mail
All H&I staff can be contacted by e-mail using the following address format:
firstname.surname@nhsbt.nhs.uk. Alternatively, please use hinational@nhsbt.nhs.uk for generic enquiries.

For safety reasons attachments with incoming e-mails will be scanned and can be placed in quarantine. The sender and the addressee will be informed automatically when this safety mechanism is triggered.

NHSBT maintains several websites including:
http://www.nhsbt.nhs.uk/ for organisation wide information,
www.blood.co.uk for blood donation information and
https://hospital.blood.co.uk/ for healthcare professionals where information regarding all aspects blood donation, blood stock levels and all our services can be found.

7.1 Customer Services

If you have a query regarding the services provided by NHSBT you can also contact one of our Customer Services Managers. Each centre has a Customer Services Manager who works closely with local consultants and scientists. The Customer Services Managers are responsible for understanding the requirements of service users and act as the central point for contacts for technical, operational and financial issues. For contact details refer to: https://hospital.blood.co.uk/contact-us/.

7.2 How to enrol as a donor

Should you wish to enrol as a donor or want information on blood or platelet donation and donation session times please contact NHSBT national donor call-centre on 0300 123 2323, which is open 24 hours per day, 7 days per week or visit our website www.blood.co.uk.

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Table 15: H&I Heads of Laboratories contact details

Title	Name	Surname	Role	Location	Regional code	Local code	Fnet
Dr	Andrea	Harmer	National Head of H&I	Sheffield	0114	358	4914
	Debra	Marples	PA	Sheffield	0114	358	4935
Prof.	David	Briggs	Head of Laboratory	Birmingham	0121	278	4099
	Anita	Chambers	PA	Birmingham	0121	278	4109
Dr	Colin	Brown	Head of Laboratory	Colindale	0208	957	2811
	Usha	Mistry	PA	Colindale	0208	957	2824
Dr	Anthony	Poles	Head of Laboratory	Filton	0117	921	7533
	Tuarita	Lawson	PA	Filton	0117	921	7478
Mr.	Tim	Key	Head of Laboratory	Sheffield	0114	358	4876
	Claire	McFarlane	PA	Sheffield	0114	358	4935
Dr	Arash	Akbarzad-Yousefi	Head of Laboratory	Newcastle	0191	202	4475
	Alison	Campbell	PA	Newcastle	0191	202	4558
Dr	Deborah	Sage	Head of Laboratory	Tooting	0203	123	8567
	Jackie	Davis	PA	Tooting	0203	123	8387

Table 16: Laboratory contact details

H&I service	Centre	Regional	Local code	Lab	Fax
H&I	Birmingham	0121	278	4105/ 4108	4110
H&I/PI/GI	Filton	0117	921	5733	5731
H&I	Colindale	0208	957	2812	2973
H&I	Newcastle	0191	202	4410	4564
H&I	Sheffield	0114	358	4839 / 4830	4850
H&I	Tooting	0203	123	8347	8486

Table 17: HLA Selected Platelet Desk contact details

H&I service	Centre	Telephone	Fax	Email Address
HLA Selected Platelet Desk	Colindale	0208 957 2814	0208 957 2973	HLAMatchedPlatelets@nhsbt.nhs.uk

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Table 18: Blood Centre details

Centre	Address	Postcode	Switchboard		Hospital Services	
			Telephone	FAX	Telephone	FAX
Birmingham	**Vincent Drive, Edgbaston, Birmingham**	**B15 2SG**	**0121 278 4000**	**4005**	**4037**	**4039**
Basildon SHU	Wilber House Swinborne road Burnt Mills Industrial Estate Basildon	SS13 1EH			01268504421	
Filton	**500 North Bristol Park, Northway, Filton, Bristol**	**BS34 7QH**	**0117 912 0117 921 7200**	**7201**	**5724**	**5783**
Cambridge	Long road, Cambridge	CB2 0PT	01223 58 8000	8114	8021	8121
Colindale	**Charcot Road, Colindale, London**	**NW9 5BG**	**020 8957 2700**	**2970**	**2800**	**2971**
Lancaster	Ashton Road, Lancaster	LA1 4GT	01524 89 6220	6222		
Leeds	Bridle Path, Leeds	LS15 7TW	0113 820 8600	8737	8607	8738
Liverpool	14 Estuary Banks, Speke, Liverpool	L24 8RB	0151 268 7000	7001	7170	7173
Manchester	Plymouth Grove, Manchester	M13 9LL	0161 423 4200	4245	4201	4358
Newcastle	**Holland Drive, Barrack Road, Newcastle upon Tyne**	**NE2 4NQ**	**0191 202 4400**	**4505**	**4500**	**4514**
Oxford	John Radcliffe Hospital, Headington, Oxford	OX3 9BQ	01865 38 7900	7915	7963	7997
Plymouth	Derriford Hospital, Derriford Road, Plymouth	PL6 8DH	01752 63 7815	7816	7802	7810
Sheffield	**Longley Lane, Sheffield**	**S5 7JN**	**0114 358 4800**	**4911**	**4817**	**4952**
Southampton	Coxford Road, Southampton	SO16 5AF	023 8035 6700	6760	6712	2060
Tooting	**75 Cranmer Terrace, Tooting, London**	**SW17 ORB**	**020 3123 8300**	**8453**	**8352**	**8449**

Bold type indicates centres with H&I laboratories

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CHAPTER 8: STANDARDS, GUIDELINES & ACRONYMS

British Committee for Standards in Haematology guidelines
https://b-s-h.org.uk/guidelines

SaBTO Microbiological Safety Guidelines (2017)
http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_121497

Guidelines for the Blood Transfusion Services in the United Kingdom 8th edition (2013)
The Stationery Office, London, UK
http://www.transfusionguidelines.org.uk/red-book

Guidance from The Royal College of Pathologists and the Institute of Biomedical Science 'The retention and storage of pathological records and archives' 5th edition (2015)
https://www.rcpath.org/uploads/assets/049ea966-df5c-4a9f-9353ba24a69bb808/The-retention-and-storage-of-pathological-records-and-specimens-5th-edition.pdf

Human Tissue Authority Codes of Practice
https://www.hta.gov.uk/hta-codes-practice-and-standards-0

FACT-JACIE International Standards for Hematopoietic Cellular Therapy Product Collection, Processing and Administration Seventh Edition 7.0 (2018)
https://www.ebmt.org/sites/default/files/2018-06/FACT-JACIE%207th%20Edition%20Standards.pdf

Renal Association Guidelines: Assessment of the Potential Kidney Transplant Recipient 5th edition (2011)
https://bts.org.uk/wp-content/uploads/2016/09/10_RA_KidneyRecipient-1.pdf

Sixth Edition NetCord- FACT International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection and Release for Administration (2016)
http://www.factweb.org/forms/store/ProductFormPublic/sixth-edition-netcord-fact-international-standards-for-cord-blood-collection-banking-and-release-for-administration-free-download

EFI Standards for Histocompatibility Testing, Version 8.0 January (2020) https://www.efi-web.org/efi-committees/standards-committee.html

BTS/BSHI Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation, Version 4.0 January 2016
https://bts.org.uk/wp-content/uploads/2016/09/06_BTS_BSHI_Antibodies-1.pdf

All links verified February 2020

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Table 19: Acronyms

AiT	Antibody incompatible Transplantation
AIN	Autoimmune Neutropenia
AITP	Autoimmune Thrombocytopenia
ALI	Acute Lung Injury
ANI	Autoimmune Neutropenia of Infancy
ARDS	Acute Respiratory Distress Syndrome
ATR	Acute Transfusion Reaction
BBMR	British Bone Marrow Registry
BSS	Bernard Soulier Syndrome
CIT	Cold Ischemia Time
CMV	Cytomegalovirus
CPA	Clinical Pathology Accreditation
DDITP	Drug Dependent Immune Thrombocytopenia
DH	Department of Health
DSA	Donor Specific Antibodies
EFI	European Federation of Immunogenetics
FCXM	Flow Cytometric Crossmatch
FFP	Fresh Frozen Plasma
GCLT	Granulocyte Chemiluminescence Test
GI	Granulocyte Immunology
GIFT	Granulocyte Immunofluorescence Test
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GT	Glanzmann's Thrombasthenia
H&I	Histocompatibility & Immunogenetics
HIT	Heparin Induced Thrombocytopenia
HLA	Human Leukocyte Antigen
HNA	Human Neutrophil Antigen
HPA	Human Platelet Antigen
HTA	Human Tissue Authority
MAIGA	Monoclonal Antibody Immobilisation of Granulocyte Antigen
MAIPA	Monoclonal Antibody Immobilisation of Platelet Antigen
MHRA	Medicines and Healthcare products Regulatory Agency
NAIN	Neonatal Alloimmune Neutropenia
NAIT	Neonatal Alloimmune Thrombocytopenia
NGS	Next Generation Sequencing
NHFTR	Non-Haemolytic Febrile Transfusion Reaction
NHSBT	National Health Service Blood and Transplant
OBOS	Online Blood Ordering System
ODT	Organ Donation and Transplantation
PAIg	Platelet Associated Immunoglobulin
PI	Platelet Immunology
PIFT	Platelet Immunofluorescence Test
PTP	Post Transfusion Purpura
SaBTO	Advisory Committee on the Safety of Blood, Tissues and Organs
SHOT	Serious Hazards of Transfusion
SOP	Standard Operating Procedure
SSOP	Sequence Specific Oligonucleotide Probing
SSP	Sequence Specific Priming
STR	Short Tandem Repeat
TAGVHD	Transfusion Associated Graft Versus Host Disease
TRALI	Transfusion Related Acute Lung Injury
UKAS	United Kingdom Accreditation Service
UKNEQAS	United Kingdom National External Quality Assurance Scheme