Welsh Histocompatibility & Immunogenetics Service (WHAIS)

USER GUIDE

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Consultant Clinical Scientist

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Introduction

The Welsh Histocompatibility and Immunogenetics Service (WHAIS), together with the Welsh Bone Marrow Donor Registry (WBMDR), form the Welsh Transplantation and Immunogenetics Laboratory (WTAIL) and hosts the UK National External Quality Assessment Services for Histocompatibility and Immunogenetics (UK NEQAS for H&I).

WHAIS provides Histocompatibility and Immunogenetics (H & I) support to a variety of health care providers ranging from specialist transplant units to General Practitioners. This service is critical to both solid organ and blood stem cell transplant programmes. The services provided include HLA typing, patient/donor crossmatching, HLA antibody testing, solid organ and stem cell donor/recipient matching, platelet typing and platelet antibody testing. Our staff work closely with clinicians in the management of solid organ transplant, blood stem cell transplant and thrombocytopenic patients.

Service Provision

Histocompatibility Testing for Solid Organ Transplantation

WHAIS provides laboratory testing and clinical advice for patients requiring a pancreas or kidney who are transplanted in Wales. The service supports transplant of various donor types including:

- Local deceased donors
- Deceased donors allocated via NHSBT Organ Donation and Transplant (ODT)
- Potential living renal donors
- ABO incompatible donors
- HLA incompatible donors
- Altruistic donors
- Paired-pool exchange donors

Laboratory testing encompasses HLA typing, crossmatching and HLA antibody detection/identification. HLA typing is performed by polymerase chain reaction (PCR) - based methods at the resolution required for patient registration and donor allocation by NHSBT ODT. Additional typing is performed to assist in antibody definition.

HLA antibody screening is routinely performed on potential transplant patients every 3 months, and following any sensitisation events, by complement dependent cytotoxicity (CDC) and Luminex based methods. Testing schedules for patients receiving antibody incompatible transplants are discussed and agreed with the transplant team on an individual basis. Donor specific antibody testing is performed for transplant patients where antibody mediated rejection is suspected and to monitor effectiveness of treatment.

Allogeneic and autologous crossmatching is performed by CDC and Flow Cytometry.

The Laboratory manages patient registration and updates with ODT.
Clinical advice in relation to risk and donor selection is provided.

Histocompatibilty Testing for Haematopoietic Stem Cell Transplants

WHAIS supports services to the local transplant centre for allogeneic stem cell transplants from related and unrelated donors. HLA-typing of patients and potential donors is performed by PCR-based methods, Sequence-based typing and serology. If required the patient is tested for HLA antibodies by Luminex based methods.
A graft identification advisory service is provided and WHAIS liaise with WBMDR staff to facilitate local, national and international unrelated donor searches, verification typing sample provision and stem cell products. WBMDR is licensed by the Human Tissue Authority to import haemopoietic stem cells.

Platelet Immunology Service

The laboratory provides investigations for a variety of immune thrombocytopenias which include:

- Platelet refractoriness
- Neonatal alloimmune thrombocytopenia
- Drug induced thrombocytopenia (excluding Heparin)
- Idiopathic thrombocytopenia (ITP)
- Gestational ITP
- Post transfusion purpura

HLA and HPA antibody screening is performed by Luminex based methods and the Monoclonal Antibody Immobilization of Platelet Antigen (MAIPA) test, respectively. HLA and HPA typing is performed by PCR-based methods.
The Laboratory works in close collaboration with the Welsh Blood Service, selecting suitable HLA/HPA matched platelets when clinically required.

The 'All Wales Guidelines for HLA and HPA Selected Platelets: Requesting and Feedback' can be accessed from the Better Health Team Transfusion Resources pages (https://wbs-intranet.cymru.nhs.uk/bht/wp-content/bht-uploads/sites/4/2019/01/All-Wales-Guideline-for-HLA-and-HPA-Selected-Platelets-2019.pdf). Forms for requesting selected platelets and providing increment data can also be obtained from this website (https://wbs-intranet.cymru.nhs.uk/bht/policies-guidance-forms/forms/).

Disease Diagnosis and Drug Hypersensitivity

HLA typing is provided to support disease diagnosis for a number of diseases e.g. HLA B27 and ankylosing spondylitis, DQA/DQB for coeliac disease and HLA*B57:01 for Abacavir sensitivity.

Transfusion Related Acute Lung Injury
Investigations for this include HLA antibody screening of the implicated donors and the patient using Luminex technology and HLA typing where appropriate. Investigations for HNA antibodies are referred to the NHSBT.

Miscellaneous

The laboratory is happy to discuss requirement for ad-hoc HLA typing or antibody definition e.g. for appropriate research projects. Please contact Dr T Rees for further information.

Service Provision

Laboratory Tests

The current range that can be undertaken are given below

1. Serological Phenotyping Tests

- **HLA-A, -B, -C, phenotyping**
  Serological typing of HLA Class I (A, B & C) specificities using the CDC assay for the purpose of assisting in the definition of expression variants.

2. Serological Crossmatching and Antibody Screening Tests

- **Complement dependent cytotoxic crossmatch**
  CDC crossmatch (XM) to detect donor or auto-reactive specific cytotoxic antibodies in recipient sera using donor/recipient lymphocytes. This technique uses purified B cells, T cells or peripheral blood lymphocytes to detect complement fixing antibody. This can also be performed using dithiothreitol treated sera to determine the IgG or IgM class of the antibody. Donor specific HLA antibodies detected using this technique are normally considered a contraindication to solid organ transplantation.

- **Flow cytometry crossmatch (FCXM)**
  This is a more sensitive crossmatch than CDC and uses donor or recipient lymphocytes and recipient sera using a flurochrome labelled secondary antibody in order to detect IgG donor-specific antibodies against T and/or B cells. Donor specific antibodies detected using these techniques are considered an intermediate/high risk to transplant.

- **Serum screening for HLA Class I and II antibodies**
  CDC assay to determine the presence of specific HLA-A, -B and -Cw antibodies using an HLA-typed reference panel representing known specificities and incorporating uncommon antigen associations. HLA -DR and -DQ antibodies are specified using a random panel of HLA typed purified B cells. Donor specific antibodies detected using this technique are normally considered a contraindication to solid organ transplantation.

- **Serum screening for HLA Class I & Class II antibodies by Luminex based methods**
  This is specific for IgG HLA class I and/or II antibodies, including both complement and non-complement fixing sub classes, utilising Luminex technologies. This technique is more sensitive that CDC, is highly specific and can resolve complex antibody profiles in highly sensitised patients using beads coated with a single HLA antigen. This technique is used to detect and specify antibodies in solid organ recipients, potential HLA mismatched HSCT recipients, platelet refractoriness and for monitoring HLA antibodies in HLA antibody incompatible transplants.

- **MAIPA (Monoclonal Antibody Immobilisation of Platelet Antigen)**
  This sensitive technique detects antibodies directed to different platelet glycoproteins. This allows the identification of mixtures of platelet specific antibodies in the presence of HLA antibodies. The patient's serum is incubated with a panel of HPA typed platelets and defined antibodies reported.

3. DNA-based HLA and HPA typing

- **HLA Class I & II DNA typing: low resolution**
  Determination of HLA-A, -B, -C,-DR, -DQ and DP alleles or groups of alleles by DNA analysis giving definition comparable to serological typing, for the purpose of HLA typing in solid organ transplantation, as an aid to disease diagnosis and for selection of HLA compatible platelet transfusions.

- **HLA Class I & II DNA typing: high resolution (2nd field)**
  Determination of HLA-A, -B, -C,-DR, -DQ and -DP alleles by DNA analysis giving high resolution definition. This is used for matching in haemopoietic stem cell transplantation and assists in HLA antibody specification.

- **Human Platelet Antigen (HPA) Typing**
  Determination of HPA 'antigens' by typing for HPA alleles by DNA analysis.

- **HLA B*57:01 typing by molecular techniques**
  Determination of HLA-B57:01 (positive or negative) and differentiation of the 'common' HLA B57 subtypes (subtypes determined by sequence based typing). This is used to determine patients at risk of hypersensitivity type reactions to Abacavir.

4. Additional tests

- **HLA-B27 typing by flow cytometry**
  Determination of HLA-B27 (positive or negative) and differentiation of the common HLA-B27 subtypes 'B*27:02', 'B*27:05' and B*27:08 (subtypes 'B*27:02' and B*27:08 confirmed by PCR based methodology).
  Associations of HLA antigens with rheumatic diseases have been well established over the last two decades. Notably HLA-B27 typing has been particularly useful in the diagnosis of the atypical spondyloarthropathies.
  Determination of patient HLA-B27 status by flow cytometry (Coates & Darke, *European Journal of Immunogenetics* 1998,**25**, 29).

- **Miscellaneous disease association**
  WTAIL performs routine HLA typing in relation to disease association investigations for specific patients, e.g. patients being investigated for suspected coeliac disease.
  In consultation with our customers we can undertake any specific HLA typing request, or family studies. We also undertake disease susceptibility/association projects via prior agreement with the Head of WTAIL.
  Determination of HLA-A, B, C, DRB1/3/4/5, DQA1/DQB1, DPB1 HLA types in combinations and resolution appropriate to user requirements.

- **ABO titres**
  This is performed using donor cells and recipient plasma for assessing and monitoring ABO antibody removal for ABO incompatible transplants. A diamed gel card method is used to determine titres by saline and AHG.

  In addition to these tests, expert H&I advice is available from our staff.

Laboratory Hours

Routine

Monday to Friday 09.00-17.00 (excluding Bank Holidays)

Out of Hours Requests

The H&I service for deceased kidney and pancreas donor HLA typing, crossmatching and matching is available 24 hours a day, 365 days a year.

The out of hours service is staffed by HCPC registered Clinical Scientists and Biomedical Scientists, all of whom are employed within the WTAIL.

For each on call period two laboratory staff are available. This team consists of experienced Clinical Scientists who perform HLA typing and patient/donor crossmatching.

Contact Arrangements out of hours

On-call WTAIL Scientist

Contact WBS main switchboard (01443 622000) and ask for either the 'HLA Typer' or 'Crossmatcher' on-call for WTAIL.

Consultant advice may be obtained from the Head of WTAIL or Duty Consultant for WBS, both available via WBS switchboard as above.

Sample Requirements and Reporting

Request Forms

Please use the standard WTAIL request forms for renal/pancreas transplantation, stem cell transplantation and disease association testing available from the WTAIL website: (https://portal.welsh-blood.org.uk/wtail/request-forms/). Complete ALL sections as appropriate.
Request forms for platelet immunology can also be accessed from the Better Health Team Transfusion Resources website (https://wbs-intranet.cymru.nhs.uk/bht/wp-content/bht-uploads/sites/4/2019/01/886-PDF-Platelet-investigation-request-form.pdf)

Additional requests
Requests for TRALI investigation and additional services can be made using standard haematology forms. The forms must clearly state the required investigation and referral laboratory in addition to the information required below.

WTAIL is a category B laboratory and therefore samples that fall within this classification can be sent to and tested within the laboratory. Known high risk samples must be labelled as such on both the sample tubes and request form.

Please ensure that samples for each patient are packaged separately, with their respective request form, and that packaging conforms with the requirements detailed below.

Labelling
The following information is required on each sample tube:

Details (Please note Patient details on sample & request form must match exactly)	**Requirement**	**Outcome if missing from request form**
Patient name (Surname & Forename)	Mandatory	Discard
Date of birth	Mandatory	Discard
Hospital number/NHS number	Mandatory	Discard

The following information is required on the request forms:

Details (Please note Patient details on sample & request form must match exactly)	**Requirement**	**Outcome if missing from request form**
Patient name (Surname & Forename)	Mandatory	Discard
Date of birth	Mandatory	Discard
Hospital number/NHS number	Mandatory	Discard
Clinical details/ tests required	Mandatory	Discard if we can not ascertain required details
Diagnosis	Desirable	Process
Date and where applicable time (e.g. solid organ rejection cases)	Desirable	Discard if no information on sample tube
Requesting person and place to report.	Desirable	Process and identify requesting clinician/GP, e.g. by contact requesting hospital/GP

Any samples that are not labelled in accordance with the requirements detailed above will be discarded, with the following exceptions:

In the case of individuals whose identity must remain confidential (e.g. GUM patients, research study patients, bone marrow registry donors) samples do not need to meet the requirements listed above but must be labelled with a unique identification number.

In cases of clinical urgency or where it is not possible to obtain repeat samples (i.e. deceased donor samples), the laboratory may agree to process a sample referred without all requested identifiers in accordance with locally documented procedures (copy available on request).

In some cases, prior to processing the sample, the laboratory will need verbal/written clarification that the referring clinician will accept responsibility for processing the requests.

Generated reports will clearly state which identifiers were missing and whether these were from the sample, the request form or both. It is the responsibility of the clinician receiving the report to check the identifiers given to confirm this is the correct patient/donor.

Urgent testing of samples must be by prior arrangement by contacting the laboratory.

Consent

Please note that it is the responsibility of the requester to obtain informed consent for the requested tests. Surplus material may also be stored for further diagnostic testing to benefit the individual and anonymously for quality control, education and training and approved research and development.

Confidentiality

The laboratories have access to the data and information needed to provide a service that meets the needs and requirements of internal and external customers. The laboratory information system (whether computerised or paper-based) provides for the collection, processing, recording, storage, and retrieval of data, and has documented procedures in place to ensure the confidentiality of patient information and the security of the data during each step of the process. WBS laboratories comply with the General Data Protection Regulation (GDPR) (Regulation (EU) 2016/679) and the Caldicott Principles.

Important Factors That May Affect Histocompatibility & Immunogenetics (H&I) Tests

The following factors may affect any of the H & I tests that are performed by the Laboratory:

Factors that may affect testing	How to minimise effects
Sample storage and transportation temperature	Store and transport samples at ambient temperature.
Sample storage time	Ensure samples arrive within 24 hours of collection.
Anticoagulant	Ensure correct blood tubes are used - see the 'Standard Tests and Samples Required' Table
Immunosuppressants, e.g. Rituximab, IVIg	Note medication/treatments on sample request form
Low white cell count	Note low count on sample request form

Please contact the Laboratory for help and advice on any of the above.

Standard Tests and Samples Required

Testing Required		Samples Required		
		EDTA	Clotted	Other
Renal/ Pancreas Recipient HLA-typing	New patients & re-type samples	10ml 6ml	20ml	-
Renal Donor Testing	Recipient	40ml 6ml	20ml	-
	Donor – Live	40ml 6ml	-	-
	Donor – Deceased	60ml	-	Lymph/Spleen
Renal Antibody Monitoring Samples	Routine Transplant list Post Transplant Rejection HLA incompatible	-	20ml	-
ABO Titres	Recipient/Donor	6ml	-	-
HSCT	Recipient	40ml 6ml	10ml	-
	Family member	10-20ml	-	-
Disease Association Investigations	HLA-B27 typing	5-10ml	-	-
	Miscellaneous e.g. Coeliac disease, Abacavir sensitivity	10-20ml		-
Platelet investigation PLEASE NOTE: Samples for GITP and ITP investigations must be received in the laboratory within 24 hours of venopuncture.	Refractory NAIT (mum) GITP ITP Platelet antibody investigation	40ml – hand-written label	10ml– hand-written label	-
	NAIT (partner)	20ml	-	
	NAIT (foetus/baby)	Contact laboratory for sample requirements		
	Drug – ITP	40ml	20ml	Sample of medication implicated
Transfusion reactions	-	-	6ml	-
TRALI Investigations	-	4 x 6ml	4 x 6ml	-

*Please contact WTAIL for sample requirements relating to paediatric patients

Additional Samples and Testing

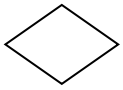
WHAIS also offer a range of other investigations for patients by request. For further information please contact Dr T. Rees, Head of WTAIL.

Blood Sample Transportation

Following collection of samples according to local trust/health board policies and procedures, blood samples should normally be transported at ambient temperature and delivered to the Laboratory in as timely a manner as possible, and wherever possible within 24 hours of collection. Please contact the laboratory for advice if samples cannot be sent within this time-frame.

Please note that sample transport is the responsibility of the test requestor. The requestor must assure themselves that their transport mode is effective and appropriate to maintain the integrity of the referred sample.

All specimens must be packaged in accordance with the current European Agreement concerning Carriage of Dangerous Goods by Road Regulations & packing instructions 650 (ADR) to prevent breakage or spillage in transit. They must be, clearly labelled according to the ADR regulations and display the hazard diamond as illustrated below.



In addition, the statement '**BIOLOGICAL SUBSTANCE CATEGORY B'** must be noted on the packaging. Packaging materials must be suitable for transporting UN3373 Biological substance Category B samples and clearly addressed to:

Welsh Transplantation and Immunogenetics Laboratory
Welsh Blood Service
Ely Valley Rd,
Pontyclun
CF72 9WB

For other arrangements please contact the Laboratory for help and advice.

Procedures for Specimen Handling

Our procedures for handling blood samples and other specimens are available on request.

Reporting Times

The following target response times relate to working days, where applicable, after the sample/request is received at the laboratory. If requested, a copy of the report can be faxed or emailed as soon as it is available.

For urgent requests please contact the laboratory to arrange testing and reporting arrangements.

	Report (written unless otherwise stated)	**WTAIL Operational lead**	**Target Turnaround Times (90% within)**
Renal Transplantation	Live Donor Work-up	Head of Patient Services	10 working days
	Urgent Live Donor Testing	Head of Patient Services	1.5 working days
	Deceased donor testing	Head of Patient Services	4 hours (80% target)
Service	Recipient HLA typing	Head of Molecular Genetics	5 working days
	Potential family donor work-up-matched donors	Head of Molecular Genetics	10 working days from receipt of last sample
	Potential family donor work-up no matched donors	Head of Molecular Genetics	5 working days from receipt of last sample
	Unrelated donor work-up	Head of Molecular Genetics	10 working days from receipt of last sample
Platelet Immunology	Platelet Antibody Investigations – Urgent (verbal report)	Head of Clinical Antibody Testing	1 working day
	Platelet antibody investigations non urgent and written reports for urgent	Head of Clinical Antibody Testing	5 working days
Additional Services	Low resolution HLA type	Head of Molecular Genetics	5 working days
	HLA-B27 typing	Head of Patient Services	10 working days
	HLA antibody investigations - urgent	Head of Clinical Antibody Testing	1 working day
	Day of transplant HLA antibody report	Head of Clinical Antibody Testing	10 working days

The requester will be notified when an examination is delayed that could compromise patient care.

Please note, when successive testing or testing of multiple individuals is required, e.g. typing of potential haemopoietic stem cell transplant donors, the turnaround times may not apply.

Compliments and Concerns

If you have any complaints, or suggestions for improvements, please contact either Dr T. Rees or one of the WTAIL operational leads. Contact can be made via telephone on 01443 622175 and 01443 622178, respectively; by facsimile on 01443 622309; via e-mail using Tracey.Rees2@wales.nhs.uk; or by writing to Dr T. Rees, Welsh Transplantation and Immunogenetics Laboratory, Welsh Blood Service, Ely Valley Road, Talbot Green, Pontyclun. CF72 9WB.

Although we are committed to providing our customers with an excellent service there may be times when we fail to fully meet your requirements. As a learning organisation we firmly believe that every complaint is to be valued as an opportunity to discuss with our customers how the service can be improved.

Terms and Conditions

The WBS standard terms and conditions of service shall apply where a Service Level Agreement has not been signed between WHAIS and the customer. A full set are available on request, the key elements of which are detailed below:

Invoicing

Velindre NHS Trust shall invoice the customer for work undertaken on completion of the individual task, where appropriate. Payment due within 30 days of receipt of invoice.

Any Debts not paid within 30 days shall be considered to be outstanding and may incur interest charges at a daily rate of 0.05%, of the outstanding balance, until the amount has been settled.

Method of payment

The general method of payment shall be in accordance with current policy as stated on the reverse of the Invoice. No receipt will be given unless specifically requested.

Any customer requesting a service supplied by WHAIS shall be deemed to agree to these terms and conditions.

Quality Assurance

WHAIS complies with the WBS Quality Manual, Management Procedures and Standard Operating Procedures (SOPs). A comprehensive Quality Assurance programme monitors and audits all aspects of the service.

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

Quality Assessment and External Audit

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

- **UK NEQAS for Histocompatibility and Immunogenetics**

- **University of California, Los Angeles International HLA DNA Exchange**

- **UK NEQAS for Blood Transfusion Practice**

Accreditation and Regulation

WHAIS is a UKAS accredited Medical laboratory No.9326 (ISO 15189:2012). The full schedule of accreditation may be viewed at UKAS Schedule of Accreditation.

The laboratory is also accredited by the European Federation for Immunogenetics (EFI). See www.efi-web.org/online-directory for details.

Accreditation categories and techniques covered are available on request.

WHAIS, as part of the WBS, is also regulated by the Medicines and Healthcare Products Regulatory Agency (MHRA) License Holder WDA(H)17853.

Further information

More detailed information on any of these services can be obtained, on request.

Please contact Dr T. Rees, Head of WTAIL or one of the following WTAIL operational leads

Miss Emma Burrows, Head of Clinical Antibody Testing
Mrs Sandra Lloyd, Head of Patient Services
Mrs Jennifer Pepperall, Head of Molecular Genetics

Laboratory Contacts for Services Provided

WHAIS Services	Primary Lead	Ext.	Section
Renal and Pancreas Transplantation	Sandra Lloyd	622186	HLA Patient Services
Haemopoietic Stem Cell Transplantation	Jennifer Pepperall	622041	Molecular Genetics
HLA-B27 typing in the spondyloarthropathies	Sandra Lloyd	622186	HLA Patient Services
Miscellaneous Disease Investigations (excluding HLA-B27)	Jennifer Pepperall	622041	Molecular Genetics
HLA Antibody Investigations	Emma Burrows	622031	Clinical Antibody Testing
Platelet Investigations	Emma Burrows	622191	Clinical Antibody Testing
General Service and Research Enquiries	Tracey Rees	622175	Head of WTAIL

Additional Services Hosted by the Laboratory

Service	Primary Lead	Ext.	Website Link
WBMDR	Emma Cook	622025	WBMDR
UK NEQAS for H&I	Deborah Pritchard	622185	UK NEQAS for H&I