

IMMUNOPHENOTYPING USER GUIDE

The Immunophenotyping Laboratory in the Haematology Department at the University Hospital of Wales, Cardiff, provides a flow cytometry and Immunophenotyping service to many hospitals and healthcare organisations in Wales.

The laboratory aims to provide a quality service, and offers a comprehensive portfolio of pathology tests, which use flow cytometry instrumentation to diagnose haematological disease and provide accurate enumeration of specific cell populations in a variety of different sample types and therapeutic products.

There is close collaboration with the following departments:

1. Cytogenetics and Molecular Diagnostics units in Medical Genetics; making a significant contribution towards an integrated diagnostic approach to haematological malignancy testing. In most cases this leads to the production of an integrated report, which incorporates Morphology, Immunophenotyping, Cytogenetics, FISH and PCR (molecular) results.
2. The laboratory also collaborates closely with the Histopathology Department at UHW, to provide integrated diagnosis of tissue lymphoma by the All-Wales Lymphoma Panel.
3. The laboratory also works closely with the Stem Cell Processing Unit (SCPU), providing a timely service for CD34 progenitor estimations which enables efficient time critical service delivery by the SCPU.

The following user guide has been produced to provide basic information about the Immunophenotyping service, and to aid medical staff in requesting the appropriate tests. Please contact the laboratory for further information.

Contact Details

Immunophenotyping

Immunophenotyping Laboratory,
Upper Ground Floor, C Block
University Hospital of Wales
Heath Park
Cardiff, CF14 4XW

Telephone:

02920 742370 (answer machine available)

C&V internal ext.

42370

Tie line:

0172 42370

Fax:

02920 745084 (Haem General Office)

Main Laboratory Contacts

Head of Immunophenotyping Laboratory: Steve Couzens

Tel: 029 20743458, internal ext. 43458

Steve.Couzens@wales.nhs.uk

Deputy: Ian Phillips

Tel: 029 20746720, internal ext. 46720

Ian.Phillips4@wales.nhs.uk

Main Haematology Department Contacts

Head of Haematology Laboratory Services: Dr A.P. Goringe
Telephone secretary on 02920 742033

Directorate Manager: Alun Roderick
Tel: 029 20744202, internal ext. 44202
Alun.Roderick@wales.nhs.uk

Accreditation and Quality

The Haematology Department is currently CPA accredited, and is working towards ISO standards. The Immunophenotyping laboratory operates internal QC procedures to maintain the quality of its results and participates in the following UKAS accredited NEQAS schemes:

1. Leukaemia Diagnostic and Interpretation (parts 1 and 2)
2. CD34 Stem cell
3. Immune Monitoring
4. Paroxysmal Nocturnal Haemoglobinuria
5. Foeto-maternal haemorrhage

Details of the laboratory performance are available on request.

Standard Hours of Operation

Monday to Friday	8.45am to 5.15pm
Weekends and Bank Holidays	No routine service

No routine Immunophenotyping service is provided out of hours, at weekends, or on Bank Holidays. Please contact the laboratory in advance for urgent specimens and/or those requiring special attention (such as Hereditary Spherocytosis screening).

Sampling

Flow cytometry requires a single cell suspension, which can be derived from a number of different tissues or serous fluids where involvement with malignant cells is suspected. Blood and bone marrow aspirates (BMA) should be anticoagulated with EDTA. If it is difficult to provide a BMA for analysis (e.g. packed marrow, or BM fibrosis), an unfixed bone marrow trephine is also suitable. Fluid samples such as CSF, ascitic fluid, and pleural fluid do not require anticoagulation. Spare tissue for flow cytometry e.g. lymph node excisions or core biopsy tissue should be placed into culture medium containing Preservative Free Heparin (provided by the Cytogenetics unit in Medical Genetics), or saline if this is not available. Alternatively, spare tissue for flow cytometry can be disaggregated at source to provide a cell suspension, but this **must** be sent in culture medium to preserve the cells. The tissue or cell suspension should be sent promptly to Cellular Pathology, or directly to the Immunophenotyping laboratory at UHW. Fixed tissue, while suitable for histological assessment, **cannot** be used for flow cytometry.

Flow cytometry

Cells in suspension are stained with a variety of monoclonal antibodies, which are conjugated to fluorescent dyes. Staining is performed in a series of tubes containing specific antibody combinations to provide the maximum information about the different cell populations in the sample. The stained cells are analysed by flow cytometry, where laser light is utilised to characterise the physical structure of the cells, and measure their fluorescent staining. Fluorescence is semi-quantitative, and pattern recognition is required to define malignant cells and distinguish them from background normal cells in the sample. This process is relatively quick – preliminary information on cell lineage and maturation can be provided within a few hours of sample receipt, although a comprehensive cell phenotype will take longer.

Specimen containers and dispatching of samples

Labelling for sample containers and request forms must comply with national guidelines. Cardiff and Vale UHB has recently introduced a zero tolerance “Right First Time” labelling acceptance policy, which may also apply to all external requests at a future date.

All samples being referred from other hospitals should be placed in a suitable, fully labelled, rigid container (such as Hayes DX). To avoid unnecessary delays, Immunophenotyping samples **MUST** be sent in a separate container **directly** to the Immunophenotyping laboratory. Samples requiring FISH or PCR tests must be sent **directly** to Medical Genetics. NEVER send Immunophenotyping and Medical Genetics samples together in the same container – there is a real danger of the samples becoming lost in the system during transfer between laboratories.

All external samples **MUST** be pre-registered on TRAKCare LIMS, and electronically dispatched using the Send Tests module. Samples must be fully labelled, and accompanied by a fully completed request form and packing slip, **both** of which must contain relevant clinical information (see Table 1 below). Note that a request form **MUST** be sent even for samples electronically requested.

Table 1: Essential requirements for samples, request forms, and packing slips:

Request form	Sample container	Packing slip
Full patient name and DOB	Full patient name and DOB	Full patient name and DOB
Hospital number and/or NHS number	Hospital number and/or NHS number	Full patient address
Patient gender	Sample type	Referral reason/clinical details/test required
Full patient address	Date and time of collection	TRAKCare LIMS episode barcode
Referral reason/clinical details/test required	TRAKCare LIMS episode barcode (electronically readable)	Packing slip number
Sample type		Sample type
Consultant name		Requesting hospital
Signature of requesting clinician		
Date and time of collection		
Address to send report to		
High risk status (if appropriate)		
TRAKCare LIMS episode barcode (electronically readable)		

Important Notes:

1. Clinical details should be as complete as possible, as inadequate information may lead to delay/inappropriate processing of the sample. It is unacceptable to simply request “Cell Markers” or “Immunophenotyping”
2. The Cardiff and Vale UHB “Right First Time” Policy discourages the use of consultant initials. If the signature cannot be identified, the report will automatically be sent to the lead Haematology consultant in the appropriate Health Board, leading to possible delays.
3. Any peripheral blood sample referred for investigation must be accompanied by a copy of a full blood count that has been done **on that sample**. Please send the FBC result AND reticulocyte count with samples requiring screening for Hereditary Spherocytosis.
4. Morphological assessment is an important part of the investigative process for acute leukaemia, lymphoproliferative disorders and lymphoma. As samples of blood and bone marrow can take up to 2 days to reach the laboratory we would request that **two labelled unstained smears** are prepared and sent with the sample – this will avoid any EDTA changes to morphological features and aid interpretation when signing out the report.
5. The samples should be fresh and transported to the laboratory with the minimum of delay. Most samples will remain viable for about 2 days if stored at room temperature. However, this is extremely variable and depends on the sample type, and the nature of the malignant cells in the sample. Labile samples such as CSF or samples containing high-grade disease (particularly Burkitt Lymphoma) will deteriorate more quickly and **will require taxi transport**, rather than hospital courier. **Please** contact the laboratory in advance when sending these samples, or if samples require **urgent processing**.
6. Samples requiring results the same day **MUST** be received **in** the Immunophenotyping laboratory by 1.00 pm. Samples received after 3.00 pm on Friday afternoon **MAY** not be processed, as the laboratory is particularly busy at this time.
N.B. Separate restrictions apply for Hereditary Spherocytosis screens; refer to section on HS screening.

Policy for Processing Unlabelled Samples

It is general Cardiff & Vale University Health Board policy that unlabelled samples CANNOT be processed. However, many samples sent for Immunophenotyping are of a “precious” nature - these are frequently obtained by means of an invasive procedure, and the re-taking of the sample would either subject the patient to an additional procedure, or re-sampling may not be possible (e.g. lymph node excision). Therefore, unlabelled samples of a precious nature *will* be processed but **no** results will be released under **any** circumstances until the following criteria have been met:

1. Cardiff and Vale UHB samples: the sample must be labelled by the clinician who took the sample; or if he/she is unavailable, by a clinical colleague willing to accept responsibility for labelling. The clinician labelling the sample must also sign a disclaimer statement.
2. External samples:
 - a. Part A of page 2 of the disclaimer form (LF-HAE-IPUnlabelledIndemnity) will be completed by a senior staff member of the Immunophenotyping laboratory, and the whole form faxed to the source department for the attention of the requesting clinician.
 - b. Part 2 of the form must be fully completed, signed, and sent back by post or internal mail to the Immunophenotyping laboratory.
 - c. Where time is of the essence a preliminary verbal report may be provided, but only **after** return by fax of the completed indemnity form to the Immunophenotyping laboratory. The original form **must** still be returned to the Immunophenotyping laboratory before the final report is issued electronically or by hardcopy.

UHW Fax no: 029 20743397; please also email one of the senior lab contacts to alert the lab about the Fax - the Fax machine is located in the Haematology office off the main Haematology laboratory corridor.

Policy for Processing High Risk Samples

All samples at high risk of infection must be **clearly labelled** as high risk. The request form must also be clearly labelled. For most investigations, samples that are definitely or potentially positive for HIV, Hepatitis B, Hepatitis C, or TB, can be processed using modified protocols, reducing the risk to laboratory staff. However, it is impossible to perform some tests (e.g. cytopins and screening for Hereditary Spherocytosis) on high risk samples.

Testing and Retention of Patient material Policy

By submitting a sample the referring clinician confirms that appropriate consent has been obtained for the testing, use and storage of patient material. It is also assumed that consent is given to send material to appropriate clinical trial laboratories.

The Immunophenotyping laboratory stores slides prepared from patient samples for 2-3 years. Samples are disposed of on a weekly basis.

Prioritisation of Samples

URGENT

All patients with suspected acute leukaemia, high-grade lymphoma, or CSF disease, will be treated as urgent and the samples analysed on the day of arrival whenever possible. A verbal (or email) communication of the results will be made to the requesting clinician. Electronic reports will be authorised as soon as possible (see individual test sections below), but may take longer if additional tests (such as FISH) are required. Requests for the dye-binding screen for Hereditary Spherocytosis are also prioritised because of possible degradation of the sample.

ROUTINE

All other samples are treated as non-urgent and analysed as soon as possible.

Samples Stored Awaiting Confirmation of Diagnosis

Non-urgent samples with an uncertain diagnosis or a diagnosis of MDS or Myeloproliferative Neoplasm will be stored pending morphological assessment. **These will ONLY be processed if there is a communication from the requesting clinician to proceed.** This avoids unnecessary, costly, labour-intensive analysis being performed on patient samples where a haematological neoplasm has not been confirmed, or where flow cytometry will not be helpful in establishing the diagnosis. Samples will deteriorate with time, so **prompt** communication with the laboratory is essential.

Samples **not** processed by the laboratory

Flow cytometry is unhelpful in the following conditions:

1. Chronic Myeloid Leukaemia, in chronic phase.
2. Myeloproliferative Neoplasm, unless in transformation.
3. Staging bone marrows for Hodgkin lymphoma.

Reporting Policy

Authorised reports are available as soon as possible - generally within the declared/published Immunophenotyping TAT; however, this may depend on the cumulative number of individual test(s) requested and the complexity of the investigation for each sample. Any delays in reporting are usually attributable to the non-availability of senior staff, or to general staff shortages. Samples of an urgent nature (see above) will always be prioritised for reporting.

All reports on TRAKCare LIMS are electronic, and the requesting centre is responsible for generating paper copies if required. Currently, LPD Combined Reports are generated using the Telepath LIMS at UHW, and paper reports are sent out by post (internal or first class) to the appropriate consultants. Additional paper reports are available on request. Scanned copies of the paper reports are also sent by email to the consultant. Combined Reporting functionality in TRAK is still under development, so Telepath will still be used for LPD Combined Reports for the foreseeable future.

Verbal or email reports can be given at any time to appropriately qualified personnel, provided that it is within a reasonable timeframe.

It is current Cardiff & Vale University Health Board policy that reports CANNOT be sent via FAX.

Telephone Enquiries and Requests

If information pertinent to a patient diagnosis is available, or sample prioritisation is required, please do not hesitate to contact the Immunophenotyping laboratory by telephone to discuss further. Members of staff are happy to co-operate in dealing with requests for results or information, including provision of appropriate references, where available.

Interpretation/Clinical Advice

If further information is required concerning the interpretation of Immunophenotyping results, or to discuss if sample referral is appropriate, please contact: -

Head of Immunophenotyping Laboratory: Steve Couzens

Tel: 029 20743458

Steve.Couzens@wales.nhs.uk

Deputy: Ian Phillips

Tel: 029 20746720

Ian.Phillips4@wales.nhs.uk

For clinical advice, please contact:

Dr A.P. Goringe (telephone secretary on 029 20742033)

Contingency service planning

In the event of catastrophic loss of the service (e.g. due to fire or flood) the following temporary contingency plan will be put into action. Any samples received by Immunophenotyping will be forwarded on to the relevant laboratory:

CD34 testing: this will be carried out by Immunophenotyping staff using the flow cytometers in the Department of Biochemistry and Immunology at UHW. Send samples to the Haematology Immunophenotyping laboratory as usual.

CD4 and CD3 (renal) testing: this will be done by the staff of the Department of Biochemistry and Immunology at UHW. Send samples **directly** to this laboratory.

Leukaemia and lymphoma Immunophenotyping: this service will be provided by the flow cytometry laboratory in the Department of Haematology at Bristol Royal Infirmary. C&V UHB service users should continue to send samples to Haematology Immunophenotyping at UHW – these will be forwarded to BRI. Non C&V service users should send samples **directly** to BRI (address below), as this will minimise any delay in sample testing.

BRI contact details: Ulrika Johansson / Michelle Crawford

Tel: 0117 3422596

Address for samples: Att. Ulrika Johansson
Flow Cytometry Laboratory
Bristol Royal Infirmary
Queen's Building, Level 8
Upper Maudlin Street
BRISTOL
BS2 8HW

Disease Specific Service Provisions

Acute Leukaemia

Samples should be less than 24 hours old, and will be stained with a basic screening panel containing antibodies against CD7, CD10, CD19, CD13, CD33, CD34, CD117, HLADR and CD45. Peripheral blood samples can be screened in the first instance, and a fuller panel used for the subsequent bone marrow sample which will include important intracellular markers such as TdT, CD79a, myeloperoxidase, cytoplasmic CD3 and lysozyme. Where a bone marrow sample is unlikely to be referred (e.g. the elderly patient) please inform the laboratory so that a complete phenotype can be determined from the peripheral blood sample. The screening panel will include staining for surface kappa/lambda (to exclude Burkitt Lymphoma), and also monocytic markers.

Antigens are also tested for their potential use in minimal residual disease testing (see below). These include CD38/CD56/CD123 (AML), CD38/CD123/CD58/CD200 (B-ALL), and CD99/CD48 (T-ALL).

The laboratory no longer performs PML staining for suspected cases of acute promyelocytic leukaemia, as it duplicates information that is provided by gold standard PML/RARA FISH. Bone marrow slides are best for FISH, but peripheral blood slides can be used, provided that enough abnormal cells are present – these will be forwarded on request to the Regional Medical Genetics unit in UHW.

A verbal or email report will be issued on all new cases of acute leukaemia, and an authorised report provided within 7 days. A paper Combined Report (Cardiff and Vale UHB in-patients **only**) is generally produced within 28 days, following MDT discussion.

Flow cytometry will not automatically be performed on samples from patients with Myelodysplasia, Myeloproliferative Neoplasm, CML in chronic phase, or Aplastic Anaemia, unless there are excess blasts detected on morphology. **It is the responsibility of the requesting clinician to contact the laboratory to progress the test in these circumstances.**

Blast/Monocyte count

A basic antibody panel is available to detect the numbers of myeloid blasts and monocytes in the sample. An authorised report is available within 28 days.

Minimal Residual Disease

Our laboratory uses sensitive 8-colour flow cytometry to measure the extent of disease after treatment has started (Minimal Residual Disease, MRD). Testing depends on the assessment of a full antibody panel at diagnosis to determine aberrant blast phenotypes. These are later used to distinguish low level malignant cells from the background of normal marrow cellular elements.

If the diagnostic blasts do not show sufficient aberrancy, other methods for MRD assessment (such as FISH or PCR) can be considered, although these may not be as sensitive or widely applicable as flow cytometry. Paediatric ALL patients on UKALL2011 are currently phenotyped only at specific time points: diagnosis, Day 8, Day 28, and relapse. However, Immunophenotyping can be requested at any stage of treatment if the clinician has any concerns.

Samples for MRD assessment should **always** be EDTA bone marrow. Peripheral blood is unsuitable unless the patient has obviously relapsed. It is very important to specify the treatment stage on the request form, as this will greatly help the interpretation of the results. An authorised report is provided within 28 days.

Myelodysplasia

The role of flow cytometry in the diagnosis of Myelodysplasia is yet to be fully defined, but several publications have highlighted its usefulness. Our laboratory does not currently offer a full MDS testing service, although this could be a potential future development depending on additional resourcing. We can offer basic testing for absolute numbers of blasts and monocytes with a combination of markers including CD34, CD117, HLADR, CD36, CD64 and CD14. An authorised report is available within 28 days.

Systemic Mastocytosis

The laboratory can detect and enumerate mast cells using a combination of antibodies, including CD117. More detailed phenotypic characterisation of the cells is currently not possible.

Lymphoproliferative Disorders

Samples are usually peripheral blood, bone marrow or serous fluids, and should be less than 48 hours old for optimum results (24 hours for serous fluids). Samples suspected of containing high-grade disease should be transported **urgently** to the laboratory, as the cells will deteriorate very quickly. Screening is done with an 8-colour antibody panel to determine the cell lineages present (B-cell, T-cell or NK-cell). B-cell clonality is assessed using reagents directed against kappa and lambda light chains. Samples containing B-cell clones are stained with additional 8-colour tubes to complete the diagnosis. If there are no clonal B-cells present, the investigation may be concluded, unless the following criteria apply:

1. The absolute lymphocyte count is $> 7.0 \times 10^9$ /litre
2. The cells show abnormal morphology
3. There is a specific request from the clinician
4. There are clinical indications e.g. mediastinal mass
5. The request is for a definite disease e.g. Sezary cells

Samples containing suspected abnormal T-cell populations will be investigated further with an 8-colour panel of additional T-cell and NK-cell markers. T-cell clonality can often be demonstrated by assessment of the TVbeta repertoire. The sample can be sent for PCR if no clonality is demonstrated by flow cytometry.

All new cases of LPD or lymphoma identified/discovered by flow cytometry will be discussed at a weekly MDT Meeting, and further work will be considered if necessary. All cases of CLL with a score of $<3/5$, will receive FISH for the IgH/CCND1 translocation to exclude mantle cell lymphoma, in accordance with BCSH guidelines. Samples suspected of containing follicular lymphoma or DLBCL may receive FISH investigation for IgH/BCL2, BCL6, or MYC as appropriate. CLL samples can be tested for trisomy 12, TP53, ATM and del (13q) by FISH, but this must be requested in advance, or can be done on a subsequent sample preferably sent directly to the Regional Medical Genetics unit.

All patient samples discussed at MDT are currently reported on a paper integrated report generated on the Telepath LIMS, generally within 28 days of sample receipt, depending on the completion of the component parts of the report (flow cytometry, FISH and PCR). Cases of obvious CLL (score 4/5 or 5/5) will be fast tracked and an authorised report will be available within 7-14 days. Verbal results can be given for urgent cases. Copies of LPD Combined Reports in PDF format are emailed to consultants in external hospitals.

LPD Minimal Residual Disease

We offer 8-colour flow cytometry to assess minimal residual disease in patients with known LPD disorders. The sensitivity of this approach will depend on the diagnostic cell phenotype. An authorised report is available within 28 days.

Myeloma

Clonal proliferations of plasma cells occur in Monoclonal Gammopathy of Unknown Significance (MGUS), Plasmacytoma, Amyloidosis, Lymphoplasmacytoid Lymphoma, Multiple Myeloma and Plasma Cell Leukaemia. Plasma cells can be quantitated by flow cytometry and their phenotype and clonality established. However, plasma cell numbers are often higher on the bone marrow aspirate film. This apparent disparity may be due to a number of reasons: patchy disease, haemodilute samples, and loss of cells during sample preparation.

Samples should be less than 48 hours old for best results. An authorised report is usually available within 28 days.

Lymphoma investigations (tissue)

Samples are usually lymph node excisions, biopsies (including core/Trucut) or Fine Needle Aspirates (FNA). Fresh tissue is essential and fixed material **cannot** be used for flow cytometry. Tissue should be placed into culture medium containing Preservative Free Heparin (PFH), or saline if this is not available, and transported urgently to Histopathology for disaggregation. Alternatively, the tissue can be disaggregated at source and the cell suspension transported in PFH culture medium to Cellular Pathology or Immunophenotyping (refer to section on samples). Samples greater than 24 hours old will often show poor viability, particularly if they contain high-grade tumour.

Integrated diagnosis of lymphoma is performed by the All-Wales Lymphoma Panel, and may include flow cytometry, karyotyping, FISH and PCR, besides traditional histological morphology and immunocytochemistry. Flow cytometric screening is very similar to that performed on LPD samples in blood and marrow, but the selected panels used are designed to detect a wider range of malignancies, including non-haemopoietic tumours. There is also greater emphasis on assessment of Ki-67 proliferation to grade the tumour.

Results for flow cytometry **MUST** be interpreted in an MDT setting. Interim Immunophenotyping results can be communicated verbally or by email as soon as the flow cytometry is completed, but the requesting clinician will be encouraged to wait for confirmatory histology. The full (but unauthorised) flow cytometry report is completed on LIMS within 28 days, and a copy of this is entered into the AWLP database at the same time for inclusion in the final histology composite report. Ultimately the turnaround for fully authorised flow cytometry reports **depends** on the progress of the sample through the AWLP diagnostic process, including MDT.

Once the diagnosis has been made, the patient is staged to assess the spread of disease around the body. This process often requires flow cytometric assessment of bone marrow or serous fluids, for which an authorised report is generally available within 28 days.

Non-Haematological disease

Flow cytometry can also be used to characterise non-haematological cells, although the antibody repertoire available is considerably less than that used in Histopathology. The antibody EpCAM is used as a general screen for epithelial cells, and positive cell populations can be confirmed by their expression of intra-cellular cytokeratins. CD56 positivity is useful to diagnose small cell carcinoma. Neuroblastoma staging is mostly done by morphology, and the APAAP method previously used to stain cells on bone marrow slides is no longer available. The flow cytometry authorised report is available within 28 days.

Cell Enumeration

The primary function of the laboratory is to diagnose and monitor haematological disease. However, flow cytometry can also be used to accurately count specific cell populations in a number of different samples and therapeutic products. Cells are quantitated using monoclonal antibodies in a single platform technique. A calibrated suspension of fluorescent microbeads provides a reference source and the concentration of cells is determined by the ratio of the cells to the beads, multiplied by the bead concentration.

The following cell populations can be measured by this method:

1. CD4+ and CD8+ T-cell subsets: for Haematology patients post-BM/PBSC transplant or on treatment, and patients on anti retroviral therapy specifically attending the Haemophilia Reference Centre.
2. CD34+ stem cells in peripheral blood or autologous/allogeneic PBSC harvest or Bone Marrow products. Results are communicated directly to staff in the Stem Cell Processing Unit (SCPU); clinical staff must contact the SCPU for any advice regarding testing or CD34 result interpretation.
3. CD3+ T-cell enumeration in peripheral blood from patients undergoing solid organ transplant and receiving ATG therapy This service is only provided to Renal Medicine **within** Cardiff and Vale UHB.

Requests for the following should be sent directly to the Medical Biochemistry and Immunology Department C&V UHB, and **NOT** to Immunophenotyping:

- a. CD4+ and CD8+ T-cells subsets for all Non-Haematology patients with primary or acquired immunodeficiency (GUM patients, ALPS, Di-Georges syndrome)
- b. Estimation of total B cell numbers in patients receiving B cell immunotherapy (e.g. Rituximab) for Non Haematological disorders such as Rheumatoid Arthritis and Multiple Sclerosis.
- c. Requests on patients receiving Campath immunotherapy.

Samples used for testing are fresh peripheral blood in EDTA, or from apheresis products or bone marrow harvests; results for peripheral blood CD34 tests are authorised within 2 hours of sample receipt, as this will guide the decision to proceed to apheresis. CD34 counting tests done on apheresis or bone marrow harvest products will usually be processed and authorised the same day or possibly the following day on advice from the SCPU. CD3 tests and CD4 tests are authorised within 6 hours and 48 hours, respectively.

Hereditary Spherocytosis (HS) screening

The laboratory can employ an EMA dye-binding test to screen for Hereditary Spherocytosis (HS); however, this test is labour intensive, very time consuming and requires the use of sample time-matched controls. As such, the laboratory **MUST** be contacted well in advance, **before** making arrangements to bleed the patient, so as to ensure adequate staff availability to perform the test.

A pre-transfusion sample is **essential**. Samples can only be processed at the beginning of the week (Mon/Tue/Wed). Also, on Wednesdays, samples **must** be received early to ensure processing that day; a **stringent** latest cut-off time for receipt by 2 pm applies. It is the responsibility of the dispatching laboratory to ensure that the sample arrives *in the Immunophenotyping laboratory* in time to be processed; dispatch by taxi may be preferable, and the transportation package must be labelled as URGENT. Samples **cannot** be accommodated on Thursday or Friday. On occasions, due to workload demands and staff availability, we may not be able to offer the test.

The test should be considered as one of “last resort”, **only** to be requested in patients with an unexplained haemolytic anaemia where an immune cause for the haemolysis, and also common enzymopathies such as G6PD deficiency, has been **previously excluded**. In addition, in those cases where an immune mechanism, or enzymopathy, have been excluded, the BCSH guidelines on Diagnosis and Management of HS (2011) asserts that the use of a screening test (e.g. EMA binding) to establish a diagnosis of HS is **not warranted** where there is a pre-existing family history of HS allied to classical presentation features.

The assay is principally only a screening test for HS, and is not intended to provide a definitive diagnosis; family history, clinical presentation and FBC/reticulocyte count and other tests **must** be considered before requesting the test, and then taken into account before making the final diagnosis. A copy of the FBC and reticulocyte count **MUST** be included with the sample.

Patients with HS show a 20-30% decrease in red cell dye binding fluorescence compared to normal controls. The test shows >99% specificity and >92.7% sensitivity for HS, but does not exclude some Band 4.2 and ankyrin deficiencies. Decreased dye binding is also seen in Southeast Asian Ovalocytosis, Hereditary Pyropoikilocytosis and Cryohydrocytosis, but results are not as clear-cut and the test is most useful for HS screening. For a definitive diagnosis, SDS page electrophoresis should be considered to fully establish the nature of any membrane defect present (not performed by the Immunophenotyping laboratory).

An authorised report is available within 28 days.

Paroxysmal Nocturnal Haemoglobinuria (PNH)

GPI-deficient red cells and granulocytes can be reliably detected by flow cytometry. De-novo PNH is very rare, but small clones can emerge in patients with aplastic anaemia and MDS, suggesting disease evolution. The laboratory measures CD55 and CD59 on red cells, and Type I (normal), Type II (partially deficient) and Type III (fully deficient) red cells can be quantitated. Granulocyte expression is a qualitative assessment via measurement of FLAER, CD24, and CD16. Monocytes are not currently assessed.

PNH testing should only be considered in specific patient groups because of the low incidence of GPI-deficient clones. Please refer to the **Wales PNH referral guidelines**. In particular, PNH testing as part of a **general** Thrombophilia screen should be discouraged, unless there are accompanying clinical indicators such as haemolysis, aplasia or **very unusual** sites of thrombosis (please state the site of thrombosis on the request form).

The sample should be fresh EDTA peripheral blood. Bone marrow should never be tested as expression of some GPI-linked antigens (CD16) is maturation dependent. A pre-transfusion sample is essential, as any red cell clones will be diluted by the transfused cells, leading to underestimation of the clone size. The granulocyte clone is unaffected by transfusion because blood products are leuco-depleted. Samples older than 24 hours will be more difficult to interpret. Neutropenic samples are also problematic.

An authorised report is generated within 28 days of sample receipt.

Platelet Glycoproteins

Testing for platelet glycoproteins is no longer provided by our laboratory. Samples can be referred to the flow cytometry laboratory at Bristol Royal Infirmary; however, please contact BRI **in advance** by telephone to discuss testing and transportation arrangements. Samples must be sent direct to BRI and **not** via Immunophenotyping UHW. The test requires citrated blood, and a control sample is required. Please discuss details with the laboratory prior to sending samples.

BRI contact details: Ulrika Johansson / Michelle Crawford
Tel: 0117 3422596

Address for samples: Att. Ulrika Johansson
Flow Cytometry Laboratory
Bristol Royal Infirmary
Queen's Building, Level 8
Upper Maudlin Street
BRISTOL
BS2 8HW

Immunoplatelet counting

This test is useful when impedance analysers used for routine Haematology full blood counts are unable to accurately define and count the platelet population, due to the presence of small or abnormal red cells in the sample, or large platelets. Platelets are accurately counted by flow cytometry using a fluorescent antibody against CD41. The platelet count is calculated from the ratio of the red cell events to platelet events, then multiplied by the red cell count from the Haematology analyser and scaled up by a factor of 1000 to take into account the relative frequencies of the platelets and red cells.

The sample should be EDTA blood, as fresh as possible. As the red cell count is used in the calculation of the Immunoplatelet result, the reporting set on TRAK LIMS is configured to “pull” the RBC data from the FBC on the same laboratory episode number, so it is **vital** that an FBC is performed *first* on the sample **before** referring it to Immunophenotyping. The results are usually available within 6 hours.

Foeto-maternal haemorrhage (FMH)

Foetal red cells present in the maternal blood circulation as a result of foeto-maternal haemorrhage can be detected by flow cytometry. The test provides a more precise estimate of bleed volume than is otherwise obtainable with the standard acid elution (Kleihauer) test; the flow cytometry method is more sensitive and less subjective.

Flow cytometry is used to detect RhD+ foetal cells in RhD-ve mothers, and quantitates the bleed volume to ensure accurate prophylaxis. This method can be performed on samples of known infection risk, provided that additional precautions are taken.

RhD FMH testing requires a sample of EDTA blood, as fresh as possible. For delivered babies, the Rhesus groups of the mother and baby must be supplied on the request form, along with the date and time of delivery. Testing should take place **BEFORE** anti-D prophylaxis. The blood transfusion laboratory is given a verbal report as soon as the flow cytometry results are known; an authorised report is available on LIMS within 24 hours of testing. The Immunophenotyping laboratory **does not** provide advice on Anti D prophylaxis – please contact the Transfusion Laboratory.

Other flow cytometry methods can be used for FMH estimation. These may be helpful if the mother is RhD+, or if the RhD group of the baby is unknown. Testing is based on the detection of high levels of HbF in foetal red cells compared to adult F cells (“intermediates”) or normal adult red cells. A combination of antibodies against both HbF and carbonic anhydrase can also be used. **These additional FMH tests are not available in our laboratory, and this service is only offered by the Welsh Blood Service.** Please contact the Transfusion laboratory at UHW in the first instance.

Cytospins

Cytospins can be made on a variety of samples fluids and offer an opportunity for morphological cell assessment to complement flow cytometry.

The samples need to be as fresh as possible. Cytospins will **not** be made on samples from known high risk patients or from CSF samples from patients on the Neurology or infectious disease wards. Flow cytometry will be performed instead.

Morphology is often reported in conjunction with flow cytometry, and the authorised report is usually available within 3 days.

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