FLOW CYTOMETRY USER GUIDE

University Hospitals Bristol and Weston NHS Foundation Trust

The Flow laboratory is located in Haematology, Laboratory medicine, BRI Site.
It forms part of **UHBW Specialist Integrated Haematological Malignancies Diagnostic Service**s (SI-HMDS)
Flow Laboratory: 0117 342 2596
Flow and SI-HMDS Office: 0117 342 0779
General enquiries: UHBWFlowCytometryLaboratory@uhbw.nhs.uk
Section and Clinical Lead: Ulrika.Johansson@UHBW.nhs.uk
Operational lead: Natasha.Futhee@UHBW.nhs.uk
SI-HMDS Clinical Lead: Sarah.Westbury@uhbw.nhs.uk

Operating hours: Mondays –Fridays: 08.30-17.30 No routine service on weekends and Bank Holidays
Samples that arrive after 15.00 on Fridays are processed the following Monday: this is suboptimal.
Avoid sending samples on Friday afternoon
Request samples on ICE/Medway. **If no ICE/Medway access:** Use attached request form.
Results: On ICE/Medway. If no ICE/Medway access: Email/Fax. Urgent results: Telephoned.

Investigation	Sample type	Samples required	Turn-around time
Leukaemia & Lymphoma	Fine needle aspirate (FNA) (non-marrow tissue)	FNA transport media*	1-2 days
	Open biopsy (non-marrow tissue)	FNA transport media* or as last resort, saline.	1-2 days
	Bone Marrow (BM)	EDTA (1×purple top, minimum 1 ml)	1-3 days
	Peripheral Blood (PB)	EDTA (1×purple top)	7 days
	Cerebrospinal Fluid (CSF)	Minimum 600ul, no anticoagulant. Also request Automated WBC and Cytospin.	7 days
	Other Fluid Samples	No anticoagulant required.	7 days
Myeloma	Bone Marrow (BM)	EDTA (1×purple top, minimum 1 ml)	1-3 days
MRD, Follow-up samples	As relevant: ALL, AML, MM: BM. CLL: PB	EDTA (1×purple top)	1-3 days
Bone Marrow failure / MDS	BM and PB	BM: EDTA (1×purple top, minimum 1 ml) PB: EDTA (1×purple top)	1-3 days
Sezary count	PB	EDTA (1×purple top)	7 days
CART19	PB, CSF, BM, FNA.	As for Leukaemia and lymphoma	7 days
PNH	PB	EDTA (1×purple top)	7 days
CD34 count	PB	EDTA (1×purple top)	1.5 hours
CD3 count	PB	EDTA (1×purple top)	1 day
Monocytosis	PB	EDTA (1×purple top)	7 days
Bleeding disorders	PB for platelet glycoprotein test. **See information on next page**	Citrate (1x light blue top)	7 days
URGENT	**Ring the laboratory on 0117 342 2596 ask to speak to senior staff. Leave your contact details and explain the clinical request. We will email/telephone urgent results**		**2 hours-> same day**

* Do you need FNA transport media? Ring the laboratory on 0117 342 2596

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PLATELET GLYOCPROTEIN ANALYSIS FOR BLEEDING DISORDERS

Peripheral blood platelets are labelled with monoclonal antibodies specific for platelet surface glycoproteins that are lost or reduced in Glanzmann's Thrombasthenia and Bernard Soulier Syndrome. Loss of antigen expression supports the diagnosis of these disorders.

Glycoproteins investigated:

GP 3a	CD61, lost in Glanzmann's Thrombasthenia
GP 2b	CD41, lost in Glanzmann's Thrombasthenia
GP 1ba	CD42b, lost in Bernard Soulier Syndrome
CD36	Thrombospondin receptor. Used to gate (identify) platelets.

Specimen Criteria

Peripheral blood anti-coagulated with citrate.

A normal control sample, also fresh, not centrifuged (un-spun) citrated peripheral blood, should always accompany the test sample.

This is an anonymous sample, and may be labelled simply as “control, adult blood”. We do not require paediatric sample for control sample.

It is imperative that samples are handled gently, and for example, not sent by pneumatic tube systems.

Samples must be analysed as soon as possible, and on the same day.

Therefore: Please avoid sending samples on a Friday.

>18 hours old samples will still be investigated but may reduce the assay sensitivity.

Samples **may be sent by taxi or courier** to the address below, please phone the flow laboratory on 0117 342 2596 to let staff know a sample will be sent.

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

Results:

Internally, the results are visible on ICE and issued weekly, for urgent results, contact the laboratory.

External samples; Results may be e-mailed and/or faxed to a safe haven fax.

Ensure your request form contains contact details, including information for whom to send results to.

UHBW Contacts

Flow Cytometry Laboratory: 0117 342 2596;
Ulrika.Johansson@UHBW.nhs.uk; Natasha.Futhee@UHBW.nhs.uk

Clotting Laboratory: 0117 342 2598
Alexander.Macphie@uhbw.nhs.uk; Christopher.Doherty@uhbw.nhs.uk

Consultant Haematologist:
Amanda.Clark@uhbw.nhs.uk ; Sarah.Westbury@uhbw.nhs.uk

University Hospitals Bristol and Weston NHS Foundation Trust

Address/Send samples to:
SI-HMDS
Queen's Building, Level 8
Bristol Royal Infirmary
Bristol, BS2 8HW

Contact Details:
Office Tel: 0117 342 0779
Laboratory Tel: 0117 342 2596

SI-HMDS Referral Form

Hospital No:
Patient Name:
Gender: M / F
DOB:
NHS No:
(Use Label if available)

New Patient / Follow-up

Previously investigated by UHB HMDS: Yes / No
Post-Transplant: Auto / Sib / VUD / Cord / Haplo
Donor: Male/Female BMT Date: _____

Clinical Details / Suspected Diagnosis:
(If diagnosis known, please specify)

(On GCSF: Y / N / unknown)
(Recent Chemotherapy? Y / N/ unknown)

Blood count:
Hb: .....
WBC: .....
Ne: .....
Ly: .....
Plts: .....
Other
.....

Organomegaly:
Spleen Y / N
Liver Y / N
Lymph Nodes Y / N

Paraprotein: Y / N
G / A / M / D / E κ / λ
Quantitation: .....g/l

Specimen taken by (FULL NAME REQUIRED IN ALL CASES):

.....

Contact details:.....

Date / Time of sample: .....

Referring Consultant:.....

Referring Hospital:.....

Infection Risk? Yes / No **If yes, specify:**

Specimens Referred:
Peripheral blood (EDTA)
Peripheral blood air-dried slide
Bone marrow (BM) aspirate (EDTA/heparin)
BM unstained air-dried slides
BM Trephine
Lymph Node
FNA / Core
Other (specify):

Indicate Required Tests

- ☐ **Urgent** Full ? Acute Leukaemia work-up / ? APML
- ☐ Flow Immunophenotyping
- ☐ PNH (Peripheral blood only)
- ☐ Cytogenetics (Heparinised sample)
  - ☐ Store
  - Karyotype: ☐ Myeloid ☐ Lymphoid
- ☐ FISH (Heparinised sample)
  - ☐ CLL: Full CLL Panel / p53del only
  - ☐ Myeloma FISH
  - ☐ BCR/ABL
  - ☐ FGFR1, FIP1L1/PDGFRA, PDGFRB
  - ☐ Urgent PML-Rara
  - ☐ Other: Please specify
- ☐ Histology/Cytopathology and Immunohistochemistry
- ☐ Molecular genetics (EDTA sample)
  - ☐ Store
  - ☐ T/ B cell clonality
  - ☐ MyD 88
  - ☐ BRAF V600E
  - ☐ IgVH mutation ☐ p53 mutation (NGS)
  - ☐ BCR ABL p190 / p210
  - ☐ ABL Kinase mutations for non-response to TKIs
  - ☐ SF3B1
  - ☐ JAK2 V617F

  will proceed to: Exon 12 variants for PRV if JAK2 neg
  will proceed to: CALR/MPL for ET/MF if JAK2 neg
  - ☐ SFSR2 ☐ KIT mutation ☐ Myeloid NGS panel