MATERIAL REQUEST FORM / A

I-BFM ALL CSF BIOMARKER RESEARCH

Hospital or Clinic

1. PATIENT DATA – for clinical staff to fill in

First name | Last name

Date of birth (YYYY-MM-DD) | Any other ID

2. CSF SAMPLE COLLECTION DATA – for clinical staff to fill in

Time of collection (YYYY-MM-DD HH:MM) | Chemo cycle

Estimated volume (mL) | Day No. (from start of cycle)

Macroscopic look ☐ Clear & colourless ☐ Macroscopic blood ☐ Other:

Departure to lab (YYYY-MM-DD HH:MM) | Storage temp. before transfer (°C)

Comments

3. CSF SAMPLE PROCESSING DATA – for laboratory staff to fill in

Sample arrival at lab (YYYY-MM-DD HH:MM) | Transport temp. en route to lab (°C)

Supernatant ☐ check this box if no such material is available

Start of centrifuging (YYYY-MM-DD HH:MM) | Storage temp. ☐ liquid N_2 ☐ °C

Start of freezing (YYYY-MM-DD HH:MM) | Total vol. mL | No. of aliquots:

TRIzol ☐ check this box if no such material is available

Start of freezing (YYYY-MM-DD HH:MM) | Storage temp. °C

Cytospin ☐ check this box if no such material is available

Start of centrifuging (YYYY-MM-DD HH:MM) | Initial vol. / spin µL | No

Comments

4. REPORTS OF THIS SAMPLE BY STANDARD METHODS – to fill in in retrospect (unless ready in registry)

Cell counts ☐ No manual ☐ No automated

Manual		Automated	
RBC	/µL	RBC	/µL
WBC	/µL	FVS	/µL

Cytospin ☐ No data

Initial vol. /µL

No. of RBCs n=

No. of blasts n=

Flow cytometry ☐ No data

Initial vol. ml

% of blasts %

No. of blasts n=

Same day peripheral blood count – if both RBCs & blasts detectable by any method WBC G/L RBC T/L

Comments

5. PSEUDONYMIZATION DATA – for laboratory staff to fill in.

Surname initial | First name initial | Hospital/Clinic

Year of birth | Year of diagnosis | Date of sample (YYYY-MM-DD)

Biobank patient ID | **Biobank sample ID**

INFORMATION FOR BIOBANKING

AIM – Standardised storage and biobanking of CSF for leukaemia CNS biomarker research (I-BFM initiative).
TARGET POPULATION – Frontline and relapsed (1st, 2nd, etc. relapse of) ALL and lymphoblastic lymphoma.

FOR CLINICAL STAFF

MANDATORY TIME POINTS OF SAMPLING

- From all patients, samples should be collected: (1) initial LP at time of diagnosis or relapse (2) on 8th-15th day of induction (3) at end of induction or before 2nd cycle (4) at 1st LP of maintenance or last LP before transplantation.
- From CNS 2/3 patients and relapsed patients: all LPs in first 3 months of therapy and at 1st LP of maintenance or last LP before transplantation.

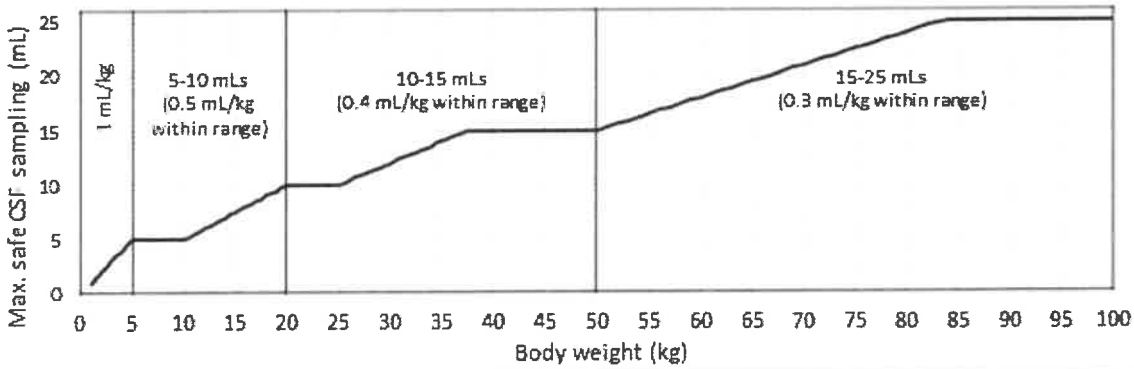
SAMPLE VOLUMES

- Main project: aim to take ≥2 mLs of CSF for research above the volume needed for routine diagnostics. (Note: very young babies may not be suitable for this much extra CSF sampling.)
- Optional: take more CSF, max. as per graph.

GENERAL POINTS

- Routine diagnostic tests are of highest priority, only the extra volume should be used for research.
- Use universal containers to collect CSF, do not add additional reagents before centrifugation.
- If bloody tap, aim to collect later clearer drops.
- Perform LP in lying patient position if taking large volumes. Allow passive dripping.
- CSF to reach lab ideally within 30 mins from sampling; tolerable for up to 6 hrs. Note: quality of routine diagnostic CSF tests will also decrease beyond 30 mins.
- If early transfer is not possible, best to keep samples on +2 to +8 °C.

SAFE MAXIMUM CSF SAMPLING VOLUMES (see references in SOP)



FOR LABORATORY STAFF

MANDATORY FOR LOCAL PROCESSING

- Use native CSF for banking, before any contamination with reagents, e.g., Transfix.
- Separate the supernatant from cells ideally within 30 mins (tolerable within 6 hrs) from sampling.
  → Centrifuge at 300 g for 10 mins, no active break.
  → Store 0.5 ml aliquots of the supernatant, ideally in liquid nitrogen, or as cold as possible
- If centrifugation and deep freezing are not possible before further transfer, the sample will still be accepted.

SAMPLE TRANSFER

If transfer is needed to another lab, do it ideally in as cold circumstances as possible (dry ice, if not possible, then -20°C or 4°C etc.). Consider freezing samples at the local hospital and ship as a batch every few months to a national centre together in dry ice.

OPTIONAL EXTRAS FOR LOCAL PROCESSING

Only from samples which may contain leukemic cells e.g., diagnostic and early follow up CSF samples:

1. Adding TRIzol to the pellet: after removing the supernatant (see left), resuspend the cells (invisible usually) in 800 µl TRIzol and store at -80°C.
2. Storing fixated cytospins: (1) Encircle the cells on the slide by a fat pen. (2) Pipette 150 µl fresh PFA 4% (cold, stored at 4°C before) on the cells, incubate for 10 mins under fume hood, gently take away the PFA using a pipette. (3) Cover the cytospins with 150 µl PBS 2x for 10 mins, gently rinse the PBS on a lab tissue. (4) Carefully rinse with distilled water using a pipette or a wash bottle. (5) Dry slides in standing position. (6) Store at room temperature.

Format of patient IDs and sample IDs: [group specific,please add]

MATERIAL REQUEST FORM / B

I-BFM ALL CSF BIOMARKER RESEARCH

Hospital or Clinic

PATIENT ID **SAMPLE ID**

DATA OF SUPERNATANT-ALIQUOTS

Aliquot ID	Aliquot volume (µl)	Aliquot ID	Aliquot volume (µl)	Aliquot ID	Aliquot volume (µl)	Aliquot ID	Aliquot volume (µl)	Aliquot ID	Aliquot volume (µl)
1		11		A_21		31		41	
2		12		A_22		32		42	
3		13		A_23		33		43	
4		14		A_24		34		44	
5		15		A_25		35		45	
6		16		A_26		36		46	
7		17		A_27		37		47	
8		18		A_28		38		48	
9		19		A_29		39		49	
10		20		A_30		40		50	

COMMENTS:

THAWING / FREEZING

Supernatant or TRIzol aliquot ID	Thawing/ freezing No.	Start of thawing YYYY-MM-DD HH:MM	Start of freezing YYYY-MM-DD HH:MM	Storage temp.	Remaining volume

COMMENTS: