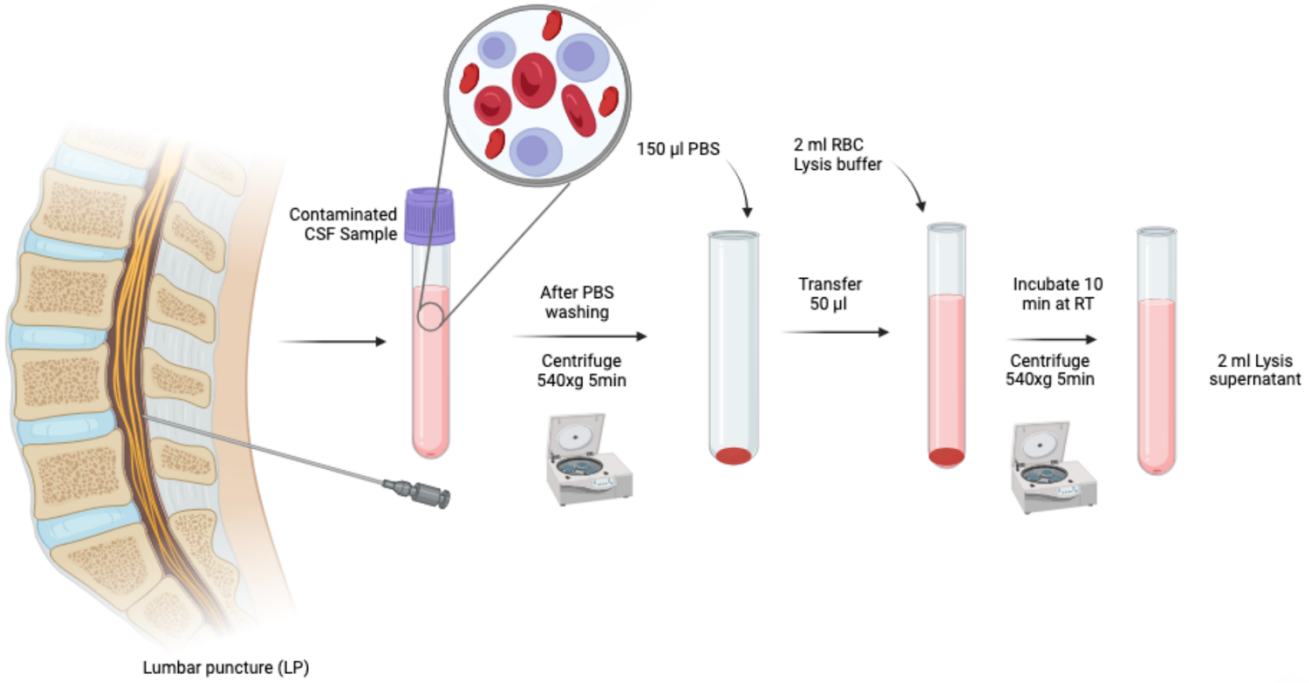
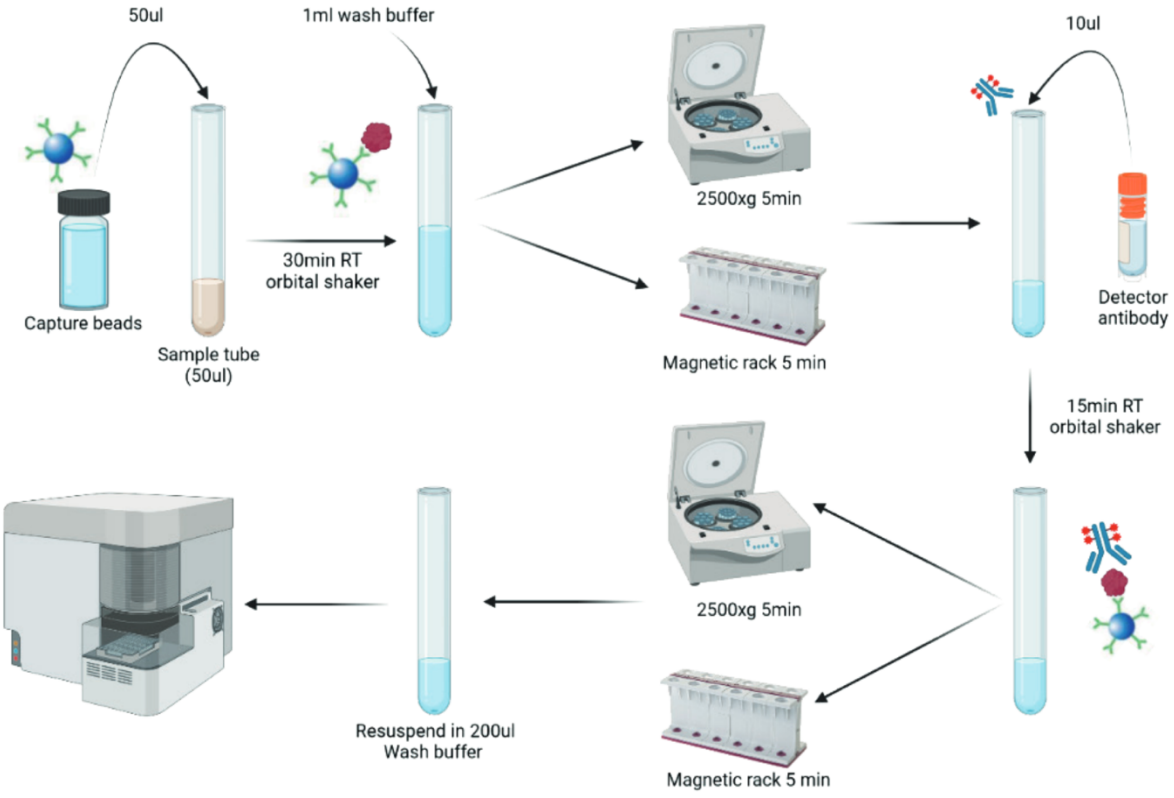
PROTOCOLS

ANNEX I. CSF Sample Preparation

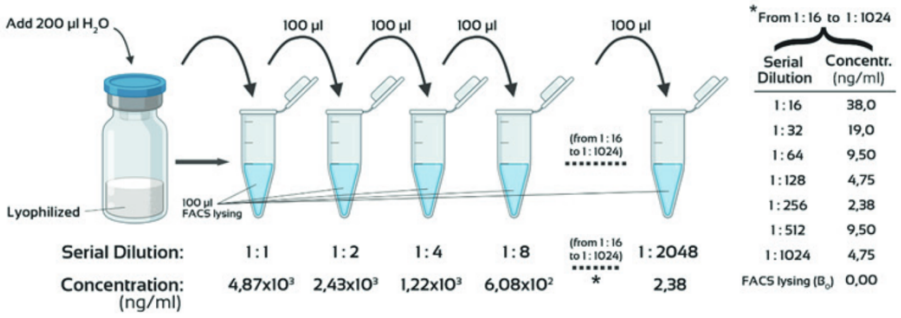


*Annotation: The CSF sample preparation is accord to Euroflow Standard Operating Procedure (SOP).

ANNEX II. Cytometer tuve workflow



ANNEX III. Serial dilution (1:2) of the standard for the construction of the standard curve



ANEXO IV. Interpretation of results

[Hb - ng/ml] lysis supernatant

Calculate CSF/lysis supernatant volume ratio (%)

Calculate [Hb - ng/ml] CSF, according to the following formula:

$$[\text{Hb - ng/ml}]\ \text{LCR} = \frac{[\text{Hb - ng/ml supernatant lisis x 6}]}{a}$$

(a): CSF/ supernatant lysis volume ratio (%)

I Transform [Hb ng/ml] interpolation result to g/dL, dividing by $1x10^7$

$g/dL = (ng/ml)\ 1x10^7$

II Estimate the dilution factor of the PB in the CSF as follows:

Dilution factor = a/b

(a)=[Hb - g/dL] from haemogram or CoA

(b)=[Hb - g/dL] result from step 1

III Calculate the concentration [RBC/µl] present in the CSF sample according to the following formula:

$$[\text{RB C/µl}] = \left(\frac{a}{b}\right)$$

(a) = Nr. RBC x 10^6 /µl of haemogram or CoA

(b) = Dilution factor

IV Calculate the number of WBC present in the CSF sample according to the following formula:

N° WBC= a/b X Vol. LCR

(a)= Nr. WBC X 10^3/[Hb - g/µl of haemogram or CoA

(b)= Dilution factor