UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Cerebrospinal Fluid (CSF) Immunophenotyping (Not Accredited)
All Participant Report

Distribution – CSF 252602
Date Issued – 14 October 2025

Participant–
Closing Date – 3 November 2025

PLEASE NOTE – THIS PROGRAMME IS NOT CURRENTLY PERFORMANCE MONITORED

Trial Comments

This trial was issued to 77 participants with 69 (89.6%) returning results. Of the non-returning centres, four had requested an extension to the exercise deadline at the time of report generation.

Please note, this is a pilot programme and is therefore not subject to performance monitoring. All data shown in this report is provided for information purposes only.

Sample Comments

The sample is from a young woman that was referred from the Neurology clinic who had presented with persistent headaches. The sample was made up of an artificial CSF and stabilised white bloods cells. There were no malignant cells present.

Cell counts of the samples issued provided by UK NEQAS LI.

	White Blood Cells (WBC)	Red Blood Cells (RBC)
CSF	10 cells/µL	0 cells/µL

Cytospin image link: https://preci.cloud/slides/fef5ee04-b87a-4513-bab3-016b2bc4dab7

With reference to the appearance of the sample, is any red cell contamination visible?

Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
No	No	No	66 (95.7%)
		Yes	3 (4.3%)

Are a large number of red blood cells present suggesting a traumatic tap?

Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
No	No	No	68 (98.6%)
		Yes	1 (1.4%)

Following examination of the cytospin, were reactive or malignant cells observed?

Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
Reactive cells observed	Reactive cells observed	Reactive cells observed	42 (60.9%)
		No abnormal cells observed	17 (24.6%)
		Malignant cells observed	10 (14.5%)

Following examination of the cytospin, did you carry out immunophenotyping testing?

Your Response	Consensus	Participant Responses	Number of Responses (Percentage values in brackets)
Yes	Yes	Yes	61 (88.4%)
		No	8 (11.6%)

Please note that the total number of responses for some of the following tables may not add up to the total number of participants overall that returned results as not everyone submitted results.

When acquiring the CSF sample on the analyser, when do you stop acquisition?

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
When the tube is empty	When the tube is empty	When the tube is empty	49 (79.0%)
		When the set number of events are acquired	13 (21.0%)

Do you use the cell count provided to anticipate how many cells to acquire?

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
No	No	No	46 (74.2%)
		Yes	16 (25.8%)

Events acquired

	Your Result	Median Value	Interquartile Range	Minimum Value	Maximum Value
Total number of events acquired	2750	2162	3385	0	60000
Total number of CD45+ Events	2700	313	1078	0	7030

Following immunophenotyping, was a discreet population of malignant cells identified?

Your Response	Consensus	Participant Responses	Number of Returns (Percentage value in brackets)
Yes	No	No	41 (67.2%)
		Yes	20 (32.8%)

Conclusion of CSF investigations

Your Response	Participant Responses	Number of Returns (Percentage value in brackets)
Other	Reactive	21 (30.4%)
	Other	20 (29.0%)
	No CNS involvement of disease	14 (20.3%)
	CNS involvement of disease	14 (20.3%)

Trial Summary

- This sample was produced to mimic a non-traumatic lumbar puncture sample from a Neurology patient with **no CNS involvement of disease**. The sample was made using stabilised white blood cells from a waste peripheral blood taken from a patient with **no known malignancies**, in artificial CSF.
- Minimal presence of red blood cells was reported by 98.6% (68/69) of participants in the sample distributed suggestive of a non-traumatic lumbar puncture.
- Reactive cells were reported by 60.9% (42/69) of participants from the cytospin imagery provided.
- Other responses included 17 (24.6%) participants observing no abnormal cells and 10 (14.5%) participants observing malignant cells.
- Following examination of the cytospin, a total of 8 (11.6%) participants did not perform immunophenotyping. Of those 8 participants, 75% had seen reactive cells on the cytospin with the remaining participants not observing any abnormal cells.
- The majority of participants, 88.4% (61/69) proceeded to perform immunophenotyping testing.
- Of those who carried out immunophenotyping, 69.4% (43/69) centrifuged the CSF sample prior to the addition of antibodies. 40.3% (25/69) of participants used 100µl of CSF sample per tube.
- There was variation in the processing steps of the CSF sample between participants with 35.8% of participants using a 'stain, incubate, lyse and wash' method.
- Additionally, there were differences in the number of events acquired. When processing samples on the flow cytometer, 71.0% (49/69) of participants stopped acquisition when the tube was empty. The remainder of participants stopped acquisition once a predefined number of events had been acquired.
- The median for the total number of events acquired was 2162 (IQR=3385), 313 (IQR=1078) for the total number of CD45+ events and 86 (IQR=167) for total number of malignant cells (events).
- **It is the recommendation of the ESCCA/ISCAA group that "*an acquisition of at least 1000 total events is recommended to achieve an optimal cell representation and the detectability of minor cell subsets, as a general rule*" (Del Principe et al, 2021).**
- Of those who reported total events acquired, 27% (17/63) of participants were below the recommendation of minimum events to acquire by ESSCA/ISCAA group. Please note, the sample provided by UK NEQAS LI had an adequate number of cells present to allow participants to adhere with the guidelines. If UK NEQAS LI samples do not pass local QC, please request repeat samples from repeatsamples@ukneqasli.co.uk. If samples still do not pass QC, contact admin@ukneqasli.co.uk for further information. Do not submit results for samples with a failed QC.
- No consensus was reached for the conclusion of CSF investigations. The data was distributed as follows, 30.4% (21/69) of participants gave a conclusion of Reactive, 29% (20/69) selected 'Other', and an equal number of participants (14/69 [20.3%] chose No CNS involvement of disease and CNS involvement of disease.
- One participant provided feedback asking whether the question "CNS involvement of disease" referred specifically to cases of haematological malignancy.
- Based on this and other feedback, we acknowledge that the question and responses for the final CSF Conclusion were ambiguous, resulting in a lack of consensus. This will be made clearer in future exercises.

Reference

Del Principe, M.I., Gatti, A., Johansson, U., Buccisano, F. & Brando, B. (2021) ESCCA/ISCCA protocol for the analysis of cerebrospinal fluid by multiparametric flow-cytometry in hematological malignancies. *Cytometry. Part B, Clinical Cytometry*, 100, 269–281.

Information with respect to compliance with standards BS EN ISO/IEC 17043:2010

4.8.2 a) The proficiency testing provider for this programme is:
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4.8.2 b) The coordinator(s) of UK NEQAS LI programmes: Mr Stuart Scott (acting Director).

4.8.2 c) Person(s) authorising this report: Mr Stuart Scott (acting Director) of UK NEQAS LI.

4.8.2 d) Administration and shipping for this programme is provided by EQA International Limited. No other activities in relation to this EQA exercise were subcontracted.

4.8.2 d) Where externally provided products or services are used in the delivery of EQA, a competent supplier is used, the EQA provider is responsible for this work and participants are informed accordingly.

4.8.2 g) The UK NEQAS LI Privacy Policy can be found at the following link: https://sheffield-ukneqas.ipassportqms.com/document_download/NjRlNTgxYzctMTI4ZS00MTg4LWI2ZDMtZDdkYzJhMTFlZTg3. Participant details, their results and their performance data remain confidential unless we are required by law to share this information. Where required by law or authorised by contractual arrangements to release confidential information, UK NEQAS LI will notify those concerned of the information released, unless prohibited by law. For UK participants, the relevant National Quality Assessment Advisory Panel is informed when a UK participant is identified as having performance issues.

4.8.2 i) All EQA samples are prepared in accordance with strict standard operating procedures by trained personnel proven to ensure homogeneity and stability. Where appropriate/possible EQA samples are tested prior to issue. Where the sample(s) issued is stabilised blood or platelets, pre and post stability testing will have proved sample suitability prior to issue.

4.8.2 l), n), o), r) & s) Please refer to the UK NEQAS LI website at www.ukneqasli.co.uk for detailed information on each programme including the scoring systems applied to assess performance (for BS EN ISO/IEC 17043:2010 accredited programmes only). Where a scoring system refers to the 'consensus result' this means the result reported by the majority of participants for that trial issue. Advice on the interpretation of statistical analyses and the criteria on which performance is measured is also given. Please note that where different methods/procedures are used by different groups of participants these may be displayed within your report, but the same scoring system is applied to all participants irrespective of method/procedure used.

4.8.2 m) We do not assign values against reference materials or calibrants.

4.8.2 q) Details of the programme designs as authorised by The Steering Committee and Specialist Advisory Group can be found on our website at www.ukneqasli.co.uk. The proposed trial issue schedule for each programme is also available.

4.8.2 t) If you would like to discuss the outcomes of this trial issue, please contact UK NEQAS LI using the contact details provided. Alternatively, if you are unhappy with your performance classification for this trial, please find the appeals procedure at www.ukneqasli.co.uk/contact-us/appeals-and-complaints/

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