Guidance notes for participants – When to request a Competency assessment and instrument validation panel.

1. Introduction and Purpose

This document aims to assist participants in deciding whether to request a competency assessment and instrument validation panel. Please note that this is an additional service for existing members of the immune monitoring EQA programme and is supplied on request only.

The enumeration of lymphocyte subsets is important in a variety of conditions such as primary immunodeficiency (e.g., Severe Combined Immunodeficiency / SCID) or the monitoring of drug therapies such as rituximab in autoimmune disorders. However, the most common use is in the monitoring Human Immunodeficiency Virus / HIV, a secondary immunodeficiency disorder.

To ensure that the programme meets the requirements of all users the programme issues stabilised whole blood with laboratories required to determine the lymphocyte subsets (CD3+, CD3+/CD4+, CD3+/CD8+, CD19+ and CD16+/56+).

Laboratories are requested to report both percentage and absolute values (in cells per microlitre), and performance is monitored using this data, unless a centre chooses to eschew any parameter in which case, they would not be performance monitored for the relevant parameters.

2. Scoring system description

The scoring system is based upon the use of z scores as described in ISO 13528. This involves the calculation of robust statistics from the returned results. Using these values and the participants submitted results, a z score is calculated for each submitted result. These are interpreted as follows:

z score between 2.5 and -2.5 = Satisfactory

z score between >2.5 and 3.5 or <-2.5 and -3.5 = Action

z score >3.5 or <-3.5 = Critical (Two Action notifications in a 3-sample window are also considered critical.

Unsatisfactory Performance (UP) is defined as any occurrence of critical performance.

Persistent Unsatisfactory Performance (PUP) is defined as a critical performance on 3 or more occasions within a 12-month period.

3. Relaying performance status to participants.

Performance status for each sample and over a 12-sample period are initially communicated to the participant on the trial report. This is then followed up with a notification to the participant hub on the website as shown below for unsatisfactory and persistent unsatisfactory performance.

Flow Cytometry - Unsatisfactory Performance for Immune Monitoring xxxxxx *We are writing to inform you that your performance in the above programme has been identified as unsatisfactory. Please refer to the report that has been issued to you for further information on the nature of the performance issue and the samples that are affected. Performance monitoring systems can be found on the EQA/PT Programmes section of the UK NEQAS LI website. If you require assistance, advice or repeat samples during your investigation, please contact us using the details above and we will be happy to help. If no communications are received from your laboratory regarding*

this Unsatisfactory Performance this will also be recorded against your laboratory on the UK NEQAS LI database. Performance monitoring is a requirement of ISO/IEC 17043:2010 accreditation; however, I would like to stress that our role is purely supportive and not punitive.

Persistent Unsatisfactory Performance for Immune Monitoring xxxxxx *We are writing to inform you that your laboratory has been identified as a persistent unsatisfactory performer because of your recent participation in the above trial. Please refer to the report that has been issued to you for further information on the nature of the performance issue and the samples that are affected. Please complete and return an EQA Unsatisfactory Performance form to UK NEQAS LI within 14 days from the date of this letter (a link to the form can be found below). If you require longer, please contact us (admin@ukneqasli.co.uk). Please note, if there is an external quality assessment oversight body operating within your country (e.g., the National Quality Assessment Advisory Panel in the UK) EQA Unsatisfactory Performance form may be provided to them as part of this process.*

If you require assistance, advice or repeat samples during your investigation, please contact us using the details above and we will be happy to help. If no communications are received from your laboratory regarding this persistent unsatisfactory performance this will also be recorded against your laboratory on the UK NEQAS LI database. Performance monitoring is a requirement of ISO/IEC 17043:2010 accreditation; however, I would like to stress that our role is purely supportive and not punitive.

In both notifications participants are advised that repeat samples are available to assist in their investigations to performance issues.

4. Repeat samples/Competency Assessment and Instrument Validation Panel Requests

Should repeat samples be required these can be requested via the website http://www.ukneqasli.co.uk/contact-us/temp-request-a-repeat-sample/ specifying unsatisfactory performance as reason for repeat sample request.

If analysis of repeat samples is satisfactory the EQA unsatisfactory performance form can be completed accordingly, and the incident closed.

If the repeat testing is again unsatisfactory, then at this point it is worth considering a competency assessment and instrument validation panel.

The Competency Assessment and Instrument Validation Panel for Immune Monitoring is a panel consisting of 6 samples (comprising of singlets and/or duplicates and/or triplicates) of predefined CD3+, CD3+/CD4+ and CD3+/CD8+ counts. The data entry form can be tailored to the individual parameter testing needs of the participant (absolute values, percentages, or both). Performance monitoring via the use of z scores will be shown immediately following result entry, providing immediate feedback to the participant. Please note that each panel is supplied on request only and will vary.

This panel can be used in several ways:

- To support competency assessment within a laboratory. Panels may be processed by individuals either as part of their initial training or as part of their ongoing competency assessment. Results and educational feedback can be shared with the relevant training officer or section leads for discussion at appraisal providing objective evidence of training and competency in line ISO 15189:2012 requirements.

- Troubleshooting assistance for poor performance. If laboratories have an incident of unsatisfactory or persistent unsatisfactory performance within the Immune Monitoring programme, then, depending on the nature of the issue identified during the root cause analysis process, panels may be useful in helping to assist identification of the source of the error and/or validate any changes to their protocol that may be required as part of any corrective actions.

- Part of instrument validation. When a new instrument/assay is being introduced into a clinical laboratory setting it a requirement of ISO 15189:2012 that a comprehensive validation is performed and characteristics such a limit of detection, limit of quantification and specificity are defined. With counts spanning the range that would be expected clinically, the CAIV panel would augment the validation process, ensuring that the instrument/assay is applicable to EQA samples as well as clinical samples, allowing any issues identified to be rectified without causing any poor performance in the EQA programme. Please note, processing of these samples alone would not constitute a complete validation.

Requests for these panels or requests for further information in relation to the panels can be made by email admin@uknegasli.co.uk

5. Processing samples

As with all other EQA programmes please treat the samples as you would routine samples, adhering to local quality controls/guidelines as stated in your Standard Operating Procedures. Because the material is stabilised, some minor adjustments may be required to the Forward Scatter (FSc) and Side Scatter (SSc) Photo Multiplier Tube (PMT) voltages. This is normal and does not affect the staining characteristics. Owing to the stabilisation process, the cells are not viable. UK NEQAS LI therefore recommends that viability dyes are either not used or, if used, all cells are included in the viable cells gate. In addition, the stabilisation process allows for haemoglobin to leach out of the red blood cells. As a result of this the samples may have a haemolysed appearance. This is normal and the samples can be tested.

6. Results Submission

Data entry webpages for these exercises are hosted by Jotform and can be accessed online using the link that will be provided to you. The data entry form can be tailored to the individual parameter testing needs of the participant (absolute values, percentages, or both). Participants may then enter results as outlined in the document "UK NEQAS Leucocyte Immunophenotyping Competency Assessment and Instrument Validation Panel-Instructions for the entry of results", this will be provided after requesting a panel. Performance monitoring via the use of z scores will be shown immediately following result entry, providing immediate feedback to the participant.

If unsatisfactory performance is identified when the panel results are uploaded, generic advice is provided on the website or bespoke feedback can be provided by UK NEQAS for Leucocyte Immunophenotyping staff.

It is possible to upload FCS files to the data entry form to assist UK NEQAS LI staff in identifying the source of error.