GRANT AGREEMENT # 115003

Safe-T

Safer and Faster Evidenced-Based Translation

QUALITY CONTROL OF BIOMARKER ENZYMATIC ACTIVITY AND IMMUNOASSAY TESTING
Version 3.5

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1. Scope

The procedures set out in this SOP apply to enzymatic and fluorescent immunoassays which are used for the quantitative determination of biomarkers in samples from human origin within the SAFE-T project.

It should be noted that the experiments outlined in this document represent best practice as defined by the IMI SAFE-T work package (WP) 5. WP5 recognizes that all immunoassays may not be able to achieve the quality standards described, or that it may not be appropriate for technical reasons to perform certain experiments for specific assays. However it is expected that the reasons why specific experiments cannot be performed, or why specific quality standards are not met will be addressed and fully documented in the relevant reports (validation and/or sample testing reports).

2. Introduction

Validation is necessary to demonstrate the performance and the reliability of the assay. However, initial pre-exploratory assay validation of the assay prior to testing of the actual sample of interest does not justify all subsequent use of the assay. An on-going validation in the form of a Quality Control (QC) policy is necessary to ensure that the performance data reported during the initial validation are maintained throughout the actual use of the method. Herein we describe a procedure for the quality control of immunoassays to be applied during the IMI SAFE-T project.

3. Quality control parameters

Maintenance of method performance through routine use of the assay is ensured using evaluation of QC samples according to relevant international standards; namely ISO 5725-6, ISO17025, ISO15189 and largely based on the use of Westgard's rules and Shewhart control charts.

4. Operational implementation

4.1. Quality control samples

QC samples must be selected according to a series of characteristics:

- QC samples at a minimum of 2 to 3 different concentrations:
    - If 3 different QC concentrations are taken: one within 3 times of the LLOQ, one in the mid range, and one approaching the high end of the range (< ULOQ) are incorporated into each run and assessed in duplicate.
    - If 2 QC concentrations are taken: one within 3 times of the LLOQ and one approaching the high end of the range (< ULOQ) are incorporated into each run and assessed in duplicate.

  It is recognized that in the case of multiplex assays it might not be possible to identify or to generate samples which contain all analytes in the preferred concentration range. Attempts should be made to bring as many analytes as possible in the foreseen concentration range.

- QC sample target concentrations are assessed (during the validation experiments) by analyzing the QC samples in at least 10 individual runs.
- QC samples are typically sufficiently stable and homogeneous to give the same result over a given period of time and available in sufficient quantities to allow for repetitive analysis.
- The preferred QC samples are low, medium and high patient samples with known concentrations. If patient samples are not available or of insufficient volume, spiked matrix samples with 3 levels of concentration can be used.
- A batch of QC samples can be prepared, aliquoted and stored for single use. An aliquot is thawed and tested with each assay thereby decreasing the possible variability associated with reoccurring preparation of the QC samples.
- The position of QC samples should be judiciously considered in the run:
    - In a microtiter plate, the QC samples should be preferably placed in the second or third column of wells (not in the first or last column).
    - In methods using serial measurements (e.g. chromatographic methods, clinical chemistry analyzers), the QC samples should be measured after the calibrators.

4.2. Quality control parameters

4.2.1. Intra-assay variation – Repeatability limit

According to the ISO 5725 part 6, the maximum difference between two replicates of a QC sample should be defined as such:

$$\Delta QC_{rep} = 2.8 \times SD_{QC}$$

Where SD_{QC} is the standard deviation observed on a given QC over at least 10 runs.
If QC samples are run in triplicate the following formula applies:

$$\Delta QC_{rep} = 3.3 \times SD_{QC}$$

Acceptance criteria: violation of the rule by one or more QC value will result in rejection of the run.

4.2.2. Intermediate precision (Inter run)-reproducibility limits

4.2.2.1. Target value:

The target value T_{QC} is the value obtained by the measurement of a QC sample over at least 10 runs. The target value for a QC sample should be recalculated at each QC lot change.

4.2.2.2. QC limits

QC limits will be established to allow interpretation of QC data in accordance to Westgard's rules. Limits can be established as follows below. We recommend here the use of a color code to facilitate the interpretation of data:

- Green limits: defined as $[T_{QC} - SD_{QC}; T_{QC} + SD_{QC}]$
- Orange limits: defined as $[T_{QC} - 2 \times SD_{QC}; T_{QC} + 2 \times SD_{QC}]$
- Red limits: defined as $[T_{QC} - 3 \times SD_{QC}; T_{QC} + 3 \times SD_{QC}]$

- Blue limits: Limits provided by the supplier (when applicable)

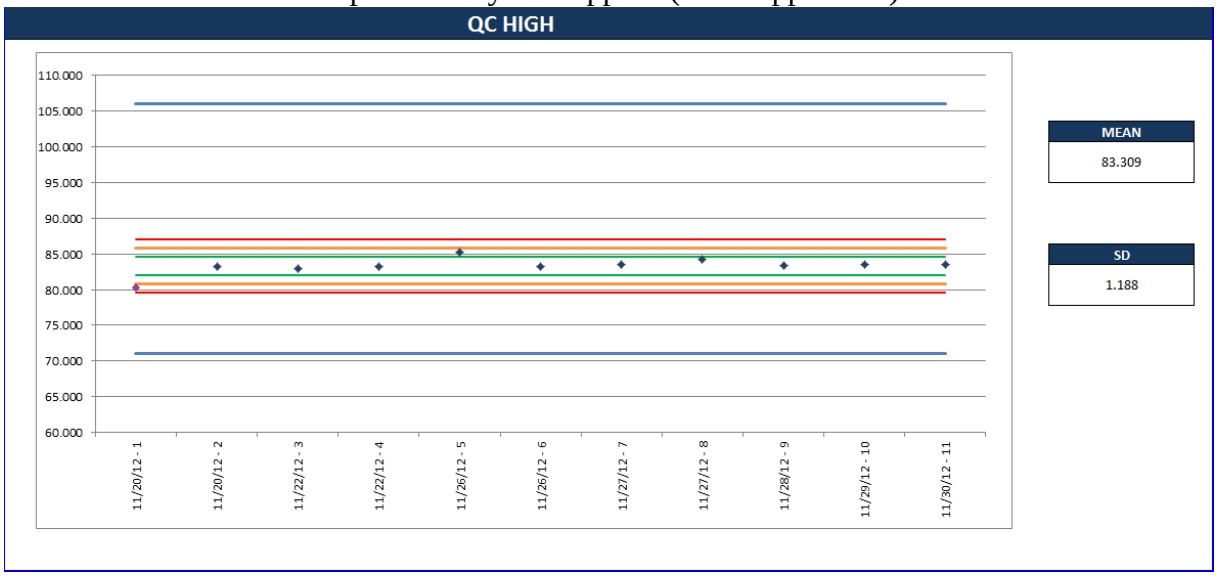

Figure 1: Example of Control chart showing the three different limits

4.2.2.3. Westgard's rules

The traditionally used Westgard's rules provide a basis for the acceptation or the rejection of a run of the assay. A multirole approach is used here to perform QC control of the data. This multirole is based on the application of one alarm that will trigger the application of other Westgard's rules.

- **Alarm rule 1_{2s}:** one QC measurement (mean of replicate) is situated outside +/- 2 SD around the mean. This rule triggers the Westgard rules analysis.
  - **Rejection rule 1_{3s}**: one QC measurement (mean of replicate) is situated outside +/- 3 SD around the mean.
  - **Rejection rule 2_{2s}**: 2 consecutive QC measurements (mean of replicate) on the same side of the mean are situated outside +/-2 SD around the mean.
  - **Rejection rule R-4s**: 2 consecutive QC measurements (mean of replicate) are separated by a difference of 4 SD.
  - One QC measurement (mean of replicate) is situated outside supplier's QC range, if applicable.

Application of any one of these rules will result in rejection of the run. For automated methods, a QC measurement is repeated and no further samples are measured until the situation is controlled.

- **Warning rule 4-1s**: 4 consecutive QC measurements (mean of replicate) on the same side of the mean are situated outside +/-1 SD: The warning rule should trigger investigation on the assay and its QC (eg. recalculation of the target value).