Using Levey Jennings Plots for Clinical Decision Making

Document Code	Author	Approved
CIS/TK123	Fiona Ashford	

1. SCOPE

Interpretation of Quality Control (QC) results involves both statistical and graphical methods. They are Westgard rules and control charts, in this case, Levey Jennings charts. Levey Jennings charts are a very good way of visually representing QC data providing an indication of the performance of an assay. Westgard rules can be applied to the data presented in Levey Jennings to make decisions based on whether a batch and/or run can be accepted or rejected.

2. RESPONSIBILITIES

The Lab Shift Managers and Principal Scientist are responsible for ensuring all personnel work in accordance with this SOP.

All personnel working at UoB/CIS/TK are responsible for following the information and instructions given in this SOP.

Personnel undertaking the work described in this SOP must be deemed competent through the following criteria, and provide their CV and evidence of completion.

- Completion of the Reporting and Authorising Competency Assessment

- Completion of the Interpretation Software Training

- Completion of the Laboratory Induction

- Completion of the SOP training and Declaration of Understanding.

3. HEALTH AND SAFETY

- Any member of staff who has either a raised temperature, a new persistent cough, a loss of sense of smell or taste or who shares a house with someone exhibiting any of those symptoms MUST self-isolate at home

- In the main laboratory areas, staff must wear full lab PPE including laboratory coat, gloves and laboratory glasses

- **Staff working in the biological safety cabinets with potentially live virus samples must wear either a reusable laboratory coat with elastic cuffs or a disposal laboratory coat with cuffs.**

Clinical Immunology Service	Document code: **TK-Doc- Inc-Sample**
University of Birmingham	Version number: **1.0**
	Date of issue: **September 2020**

- **Staff working in the biological safety cabinets with potentially live virus samples must be double gloved. The inner gloves should be secured to the lab coat and the outer gloves must always be removed in the biological safety cabinets before staff leave the cabinet**

- Any spillages of potentially infective material, however small, must be reported to a senior member of staff and the potentially infected area should be vacated until a decontamination has been carried out according to the relevant work instruction.

4. RISK ASSESSMENTS

- None

5. COSHH ASSESSMENTS

There are no specific COSHH requirements as this document details a dry laboratory activity used for data generation.

6. WESTGARD RULES

Westgard rules are a set of 6 statistical rules which can be used individually, or in combination, to detect both random and systemic errors. Such errors create uncertainty of measurement, which must be taken into account when testing procedures and/or testing results are compared with each other or against specifications.

The Westgard rules are used to define specific performance limits for a particular assay and verify reliability of test results.

The most commonly used Westgard rules are 1_{2SD}, 2_{2SD} and 4_{1SD}, are warning rules who violation should trigger a **review** of the test procedures, reagent performance and equipment calibration.

1_{2SD}	This rule is used as a warning to trigger careful inspection of the control data. If one control measure exceeds the mean ±2SD, control values in the previous run should be considered to rule out a trend.
2_{2SD}	This rule detects systematic errors and is violated when two consecutive control values (on the same side of the mean) exceed the same mean +2SD or mean -2SD limit.
4_{1SD}	This rule detects systematic error. This rule is violated when four consecutive values exceed the same mean +1SD or mean -1SD limit. The run does not need to be rejected if this rule is violated

| | but should trigger recalibration or equipment maintenance. |

Westgard rules 1_{3SD}, R_{4SD} and 4_x are mandatory **'alarm'** rules whose violation should result in the rejection of the results obtained from specimens in that assay run.

1_{3SD}	This control rule detects random error. Violation of this rule may also point to systematic error. The assay run is considered to be out of control when one control value exceeds the mean ±3SD.
R_{4SD}	This is a range rule which detects random error only. This rule is applied only within the current run. The rule is violated when one control measurement in a group exceeds the mean +2SD and another exceeds the mean -2SD.
4x	This rule detects systematic error and is violated when 4 consecutive values fall on the same side of the mean. Its violation often indicates the deterioration of assay reagents.

7. LEVEY JENNINGS PLOTS

Levey Jennings is a more sensitive quality measure than the automatic validation of results that is performed by the Applied biosystems COVID-19 Interpretive Software, and as such should be used to guide the process to ensure optimal quality of results reported.

Levey Jennings charts are drawn, with the target value (mean) and limit values of +1SD, +2SD and +3SD delineated, for each control used. Subsequent values obtained with the assay controls are plotted and the Westgard rules applied to determine the validity of each assay run.

7.1. Setting up Level Jennings plot

MedLab QC is a freeware QC program that can be used to simplify quality control management. An excel spreadsheet for plotting Levey Jennings manually is also available. To set MedLab QC and/or an excel spreadsheet up see SOP CIS/TK92 (Using MedLabQC and Setting up Levey Jennings Plots for Clinical Decision Making).

8. INPUTTING DATA INTO LEVEY JENNINGS PLOTS

The data input into the Levey Jennings plots comes from the Qnostic control sample as it is the closest to a clinical sample with the control material being whole virus lysate, rather than the Thermo kit internal positive, which is a qPCR control.

- Once the Levey Jennings chart has been established, CT values must be entered for every plate.

Clinical Immunology Service
University of Birmingham

Document code:	**TK-Doc- Inc-Sample**
Version number:	**1.0**
Date of issue:	**September 2020**

- CT values for MS2 in the negative control and Orf1ab, N gene and S gene in the Qnostics control should be plotted.

- Ensure the values fall within the Westgard rules.

- If the Westgard rules are violated it will be indicated by a data point outside the predetermined ±SD parameters (MedLab QC), see below
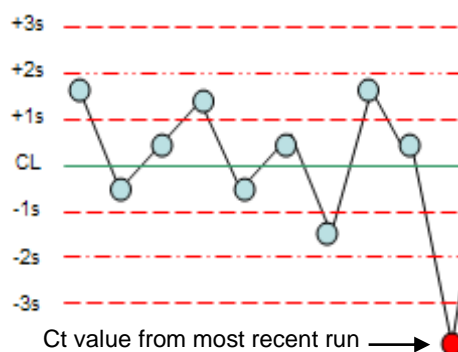
Data point violating the 1_{3SD} Westgard rule

- Using the excel spreadsheet, cells will appear green if the Ct value is in the acceptance range, yellow in warning range and red if the data entry is in the rejection range, see below

Data points indicating acceptance range in excel spreadsheet

8.1. The 1_{2SD} rule has been violated

- If 1_{2SD} rule (see section 6) is violated, a **review** of the process is triggered.

- The plate may be passed once it has been reviewed in the interpretive software and manual plate inspection on Design & Analysis software, as outlined in CIS/TK44 SOP (Reporting, Interpretation and Authorisation of Results in Turnkey Birmingham).

8.2.   The 'alarm' rules have been violated

- If the 2_{2SD}, 1_{3SD}, R_{4SD}, or 4x '**alarm**' rules have been violated, suggestive of a random or systemic error. This could be indicative of a loss of sensitivity of the assay and could therefore increase the risk of false negatives.

- The plates **may** be passed if they are considered valid by the interpretive software, the plate has been manually reviewed and passed the criteria, see SOP CIS/TK44 section 10.4.