Assessing Serology Quantitative Values in Infectious Diseases EQAS

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Introduction

Quantitative and/or semi-quantitative infectious diseases serology immunoassays have been routinely used by pathology laboratories to measure the immune response to an infectious agent. Various technologies are commercially available, including the widely used enzyme immunoassays (EIAs). Some EIAs are classified as quantitative and are designed to target specific antigen epitopes or antibody classes[2].

For some infectious disease analytes, quantitative serology immunoassays are calibrated against International Standards (IS) / International Reference Preparations (IRP) to allow comparability and harmonisation of results between different assay platforms. However, there is a history of disparate results when quantifying serological samples, even from assays that are calibrated to an IS[3].

Objective

To investigate comparability of quantitative results between various serology immunoassays for Anti-HBs, Rubella IgG, Toxoplasma IgG and CMV IgG, using NRL EQAS participant results.

A total of 1,448 EQAS participant results obtained using quantitative or semi-quantitative assays were assessed for the four infectious diseases analytes. The results were collated from five Test Events in 2022-2023. All analysed results were obtained from automated EIA-based assays with varying capture/detection formats (CMIA, ECLIA, CLIA and ELFA). Assays with less than three results submitted per sample were excluded from analysis due to low statistical reliability.

Results

1. Anti-HBs

In total, 622 results were analysed across five Test Events using thirteen quantitative Anti-HBs assays (Figure 1). All assays investigated reported in mIU/mL with most adopting 10 mIU/mL as the cut-off based on WHO recommendation for protective concentration against HBV infection[3,5].

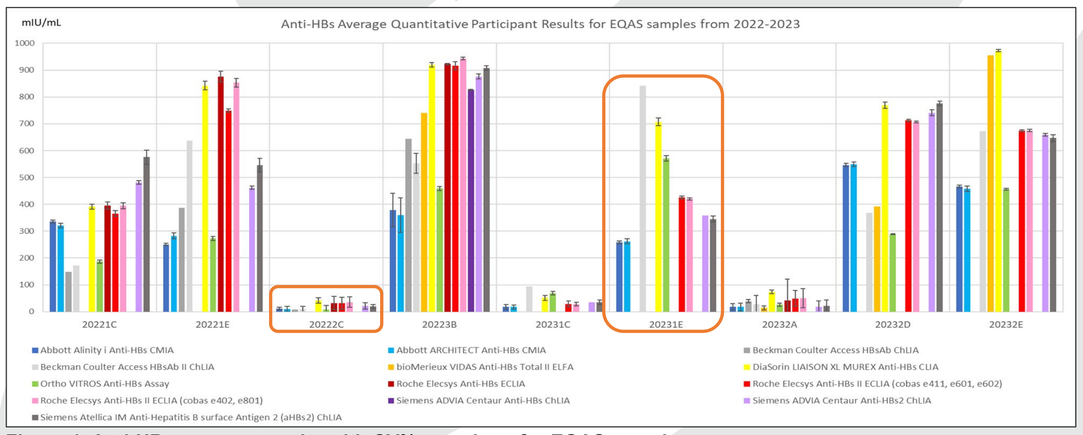

Figure 1. Anti-HBs average results with CV% error bars for EQAS samples.

a) Peer Group Comparison for Low and High Reactive Anti-HBs Samples

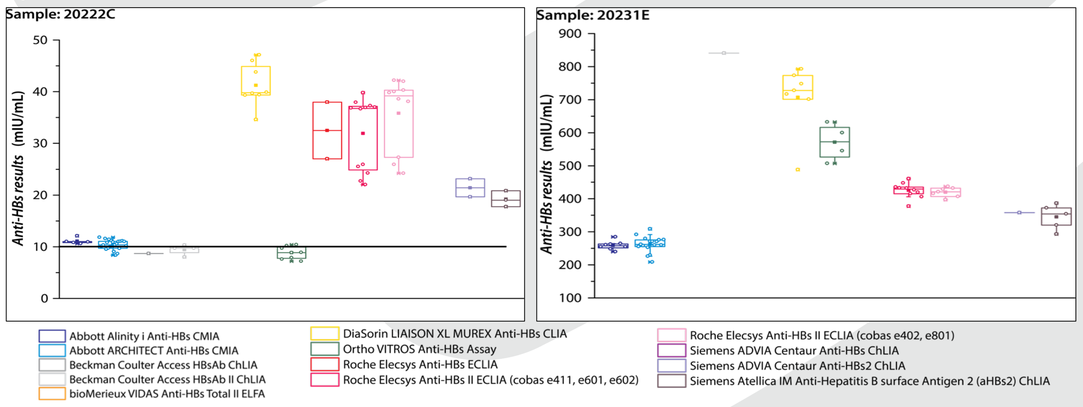

Figure 2. Distribution of results by assay for low reactive (Left: 20222C) and high reactive (Right: 20231E) Anti-HBs samples. The WHO recommended cut-off for protection against HBV infection is 10 mIU/mL.

2. Rubella IgG

In total, 310 results were analysed across five Test Events using eight quantitative Rubella IgG assays (Figure 3). All assays investigated were calibrated against an IS, with most assay Instructions For Use (IFUs) referencing the 1st IS (RUBI-1-94). All assays reported in IU/mL but varied in their reported cut-off. The established protective cut-off is 10 IU/mL for Rubella IgG[2,4].

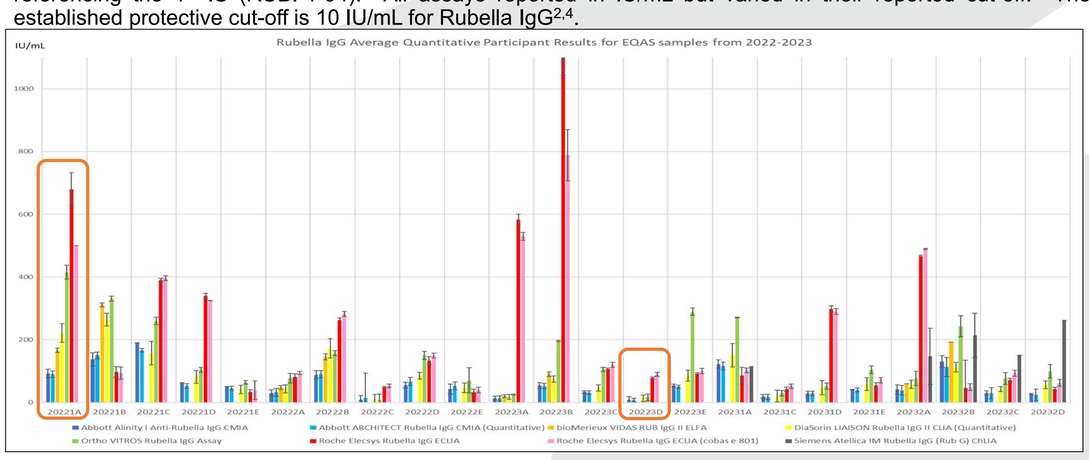

Figure 3. Rubella IgG average results with CV% error bars for EQAS samples.

a) Peer Group Comparison for Low and High Reactive Rubella IgG Samples

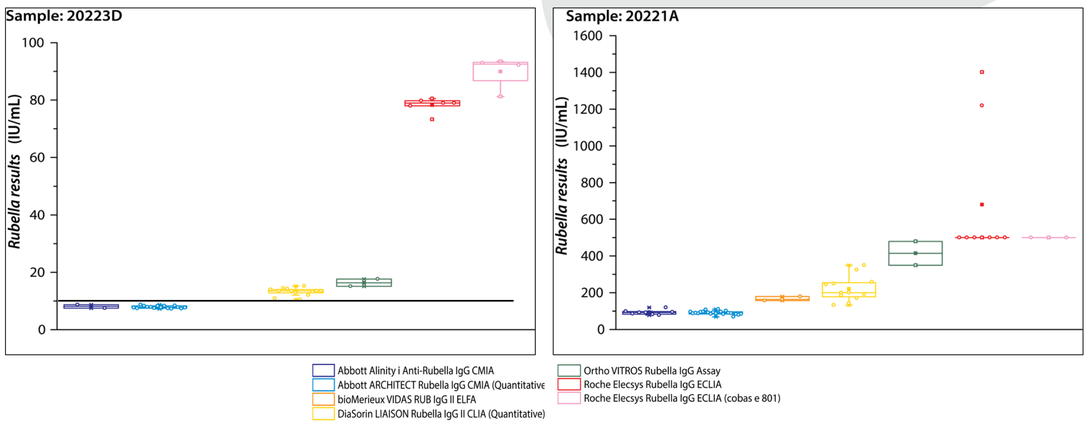

Figure 4. Distribution of results by assay for low reactive (Left: 20223D) and high reactive (Right: 20221A) Rubella IgG samples. The established protective cut-off for Rubella IgG is 10 IU/mL.

3. Toxoplasma IgG

In total, 241 results were analysed across five Test Events using eight quantitative Toxoplasma IgG assays (Figure 5). All assays were calibrated against an IS but varied in the specific generation used. Variable assay cut-offs were observed. Currently, there is no recommended cut-off for protective Toxoplasma IgG level.

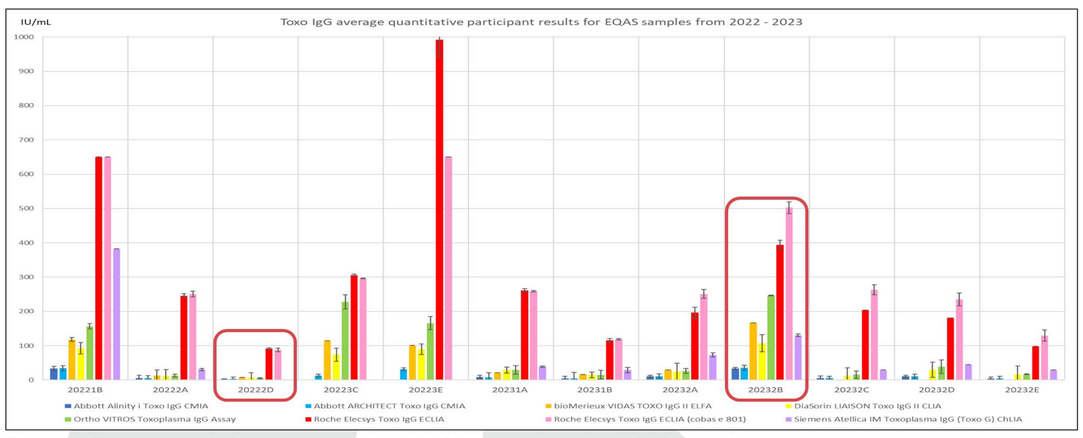

Figure 5. Toxoplasma IgG average results with CV% error bars for EQAS samples.

a) Peer Group Comparison for Low and High Reactive Toxoplasma IgG Samples

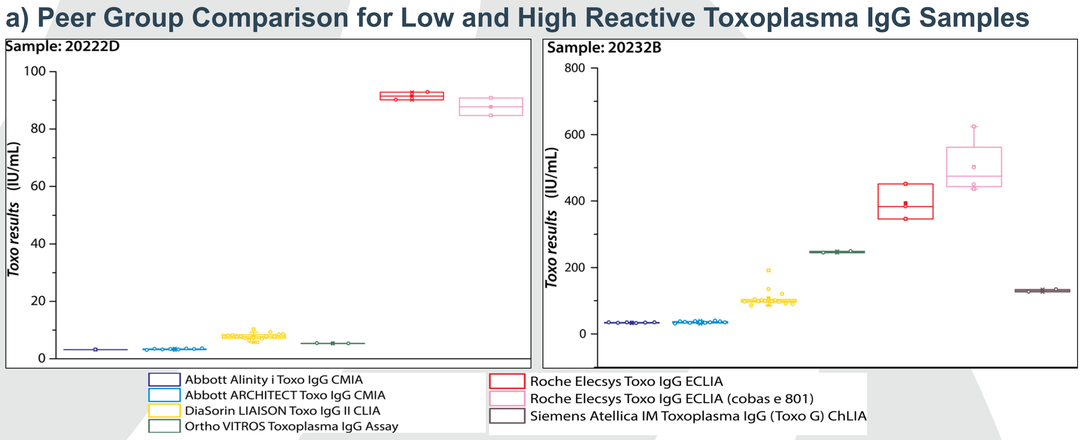

Figure 6. Distribution of results by assay for low reactive (Left: 20222D) and high reactive (Right: 20232B) Toxoplasma IgG samples.

4. CMV IgG

In total, 275 results were analysed across five Test Events using seven quantitative or semi-quantitative CMV IgG assays (Figure 7). No reference in the IFUs of any assay being calibrated against an IS and each assay varied in its reported cut-off as there are no established guidelines for protective level of antibodies.

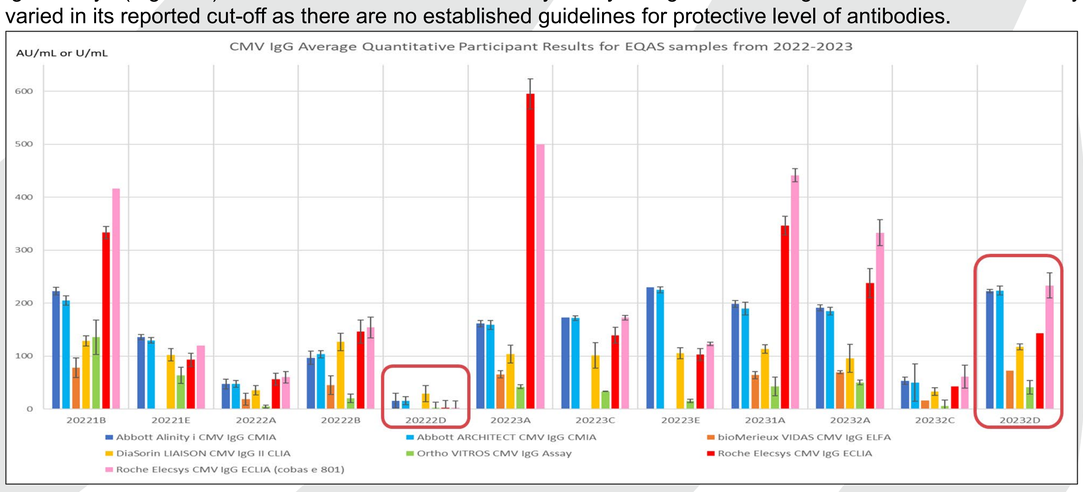

Figure 7. CMV IgG average results with CV% error bars for EQAS samples.

a) Peer Group Comparison for Low and High Reactive CMV IgG Samples

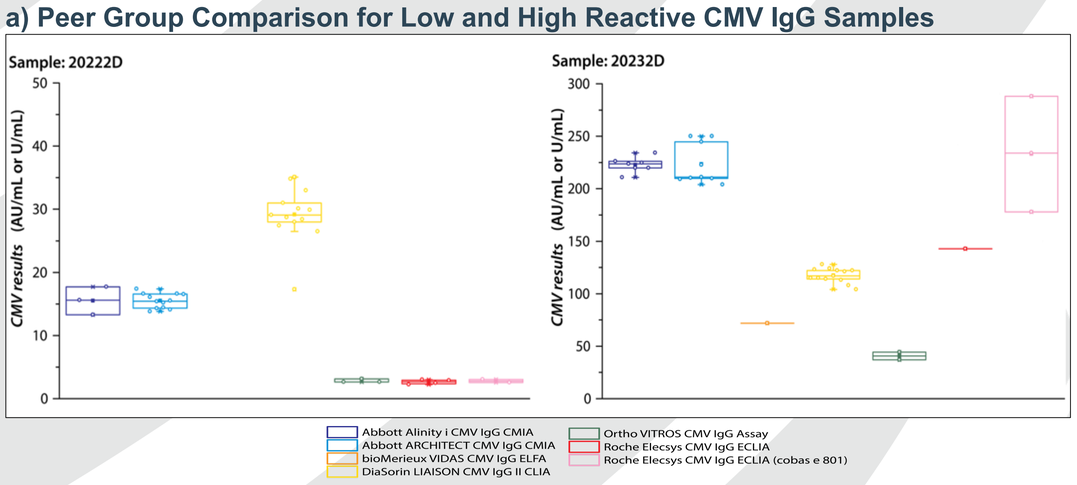

Figure 8. Distribution of results by assay for low reactive (Left: 20222D) and high reactive (Right: 20232D) CMV IgG samples.

Discussion & Conclusion

Reporting in IU and using established cut-offs for protective immunity, suggests that standardised assays are accurate and comparable. However, analysis of the EQAS participant data demonstrated observable differences in quantification between assays.

The overall quantitative values analysed for serology assays calibrated against an IS for Anti-HBs, Rubella IgG and Toxoplasma IgG showed comparable values within a peer group or assay, with low Coefficient of Variation (CV)% for most panel samples. Yet, there were significant differences in the variability of results seen across different peer groups or assays for most panel samples. CMV IgG results displayed similar trends, however, this was expected due to the lack of commercial calibrated assays.

A study of the assay IFUs demonstrated differences in assay cut-offs and variable applications of equivocal ranges for both IS-calibrated and uncalibrated assays. For IS-calibrated assays, some IFUs also contained a disclaimer that results should not be compared with that of other assays. The reported clinical sensitivities and specificities were very high and quite similar for all assays used by participants suggesting assay performance may not be the reason for the disparate values.

Is it therefore reliable to calibrate against IS and apply a universal cut-off for a particular analyte, given the variable nature of assays? Is there clinical significance in quantifying IgG or antibodies? Once the result is over the assay's cut-off and considered reactive, would the values alter the clinical advice given to the patient regarding their immunity? Perhaps it is time to reconsider these guidelines and apply assay-specific recommendations.

References:

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2. Dimech, W., Grangeot-Keros, L. and Vauloup-Fellous, C. (2016) 'Standardization of assays that detect anti-rubella virus IGG antibodies', Clinical Microbiology Reviews, 29(1), pp. 163–174.
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5. World Health Organisation (2009) 'Weekly epidemiological record' No. 40, 2009, 84, pp 405-420.