Choosing between two Varicella Zoster IgG assays

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INTRODUCTION

The clinical need for VZV IgG testing

Varicella zoster virus (VZV) is a highly contagious herpes virus that causes chickenpox (varicella; primary infection) and shingles (herpes zoster; reactivation).

Primary infection or vaccination causes the production of VZV IgG antibodies which confer immunity in immunocompetent individuals.

Primary infection can be severe in adults, particularly in pregnant women where it may also cause infection of the foetus or newborn. Post-exposure prophylaxis with antivirals and/or immunoglobulin may therefore be prescribed for non-immune patients. If immune status (from vaccination or previous infection) is unknown, serology testing is needed to quantify the exposed patient's VZV IgG.

Why we needed a new VZV IgG assay

The DiaSorin LIAISON VZV IgG indirect chemiluminescence immunoassay (CLIA) previously used by the UKHSA Public Health Laboratory Birmingham was withdrawn by the manufacturer and replaced with the LIAISON VZV IgG HT kit.

Initial local evaluation of the replacement kit did not demonstrate the performance expected. Of particular concern was sample that was found to be repeatably positive on the new assay (>200mIU/ml) but consistently negative (<20mIU/ml) on the old assay (see results). This kind of result potentially poses a significant clinical risk if a patient who is at risk and exposed to VZV is misidentified as immune, and therefore not offered post-exposure prophylaxis with an antiviral and/or varicella immunoglobulin. An alternative assay for VZV IgG was therefore sought.

The SERION ELISA Classic Varicella Zoster Virus IgG assay was identified as potential alternative assay which would meet the laboratory's need and be automatable on the laboratory's existing Dynex DS2 platform.

METHODS

Both Liaison VZV IgG kits and the Serion ELISA Classic VZV IgG assay were used as per the manufacturer's instructions. The Serion assay was automated in the Dynex DS2 instrument.

The following were run on all three assays:

- Previously tested serum samples (n=114)
- NEQAS EQA samples (Immunity Screen Distribution 5601)
- Dilutions of the WHO International Standard for varicella zoster immunoglobulin
- An independent quality control (Optitrol Paediatric G control)

Results from the two new assays were compared with original test results (LIAISON VZV IgG) as the "gold standard".

Results >100 mIU/ml were considered positive (see "Reporting VZV IgG Levels" below).

RESULTS

Independent Positive Quality Control

On both evaluated platforms, independent QC samples gave expected results (data not shown).

NEQAS EQA Samples

All assays gave good performance against the expected results (Table 1).

NEQAS No.	NEQAS Expected Qualitative Result	LIAISON VZV IgG		LIAISON VZV IgG HT		SERION ELISA Classic VZV IgG (replicate 1)		SERION ELISA Classic VZV IgG (replicate 2)	
		mIU/mL	Qualitative Result	mIU/mL	Qualitative Result	mIU/mL	Qualitative Result	mIU/mL	Qualitative Result
8510	Neg	82.70	Equiv / Neg	69.25	Equiv / Neg	73.84	Equiv / Neg	68.05	Equiv / Neg
8511	Pos	980.60	Pos	900.00	Pos	654.01	Pos	620.86	Pos
8512	Pos	1625.00	Pos	1407.00	Pos	718.70	Pos	651.43	Pos
8513	Pos	685.70	Pos	803.90	Pos	864.49	Pos	810.44	Pos
8514	Neg	42.88	Neg	57.80	Equiv / Neg	42.00	Neg	41.40	Neg
8515	Pos	1250.00	Pos	864.50	Pos	768.38	Pos	715.99	Pos

Table 1: NEQAS EQA Sample results Neg = negative, Pos = positive, Equiv = equivocal

Clinical Serum Samples

Both assays demonstrated good correlation of IgG levels when compared with the old LIAISON VZV IgG assay (Figure 1)

However, one sample tested repeatably negative (<20 mIU/ml) on the old Liaison and Serion ELISA assay (also replicated when tested by another local laboratory) but clearly, repeatably positive (>200 mIU/ml) on the LIAISON VZV IgG HT assay.

When considering sensitivity and specificity, <u>for VZV IgG assays, specificity is more clinically critical than sensitivity</u>, as false positives may result in not giving prophylaxis when it is needed.

Discounting the one outlier sample, the differences in sensitivity between the two assays when evaluated against the old LIAISON VZV IgG assay (see tables 2 and 3) were attributable to samples with values very near the cut-off of 100 mIU/mL but within the expected measurement uncertainty.

		SERION ELISA Classic VZV IgG	
		Negative	Positive
LIAISON VZV IgG	Negative	82	0
	Positive	7	25

Sensitivity 78.13%
Specificity 100.00%

		LIAISON VZV IgG HT	
		Negative	Positive
LIAISON VZV IgG	Negative	80	2
	Positive	5	25

Sensitivity 83.33%
Specificity 97.56%

Tables 2 and 3: Evaluated assays: sensitivity and specificity
Note: Two samples tested on the Serion ELISA were not tested on the LIASON VZV IgG HT

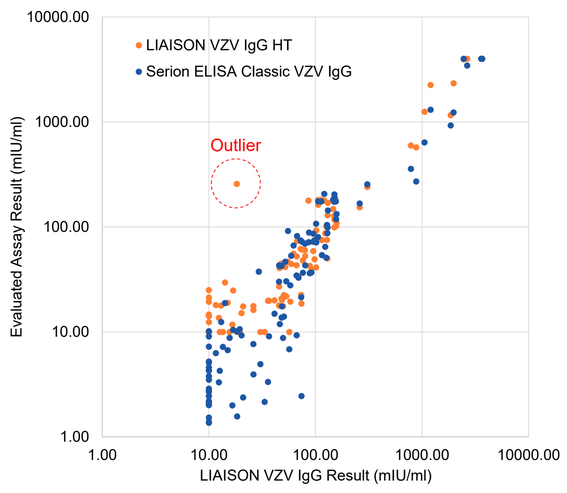

Figure 1: Quantitative correlation of evaluated assays with LIAISON VZV IgG
Note: The limits of the LIAISON assays are 10 to 4000 mIU/ml

WHO Standards

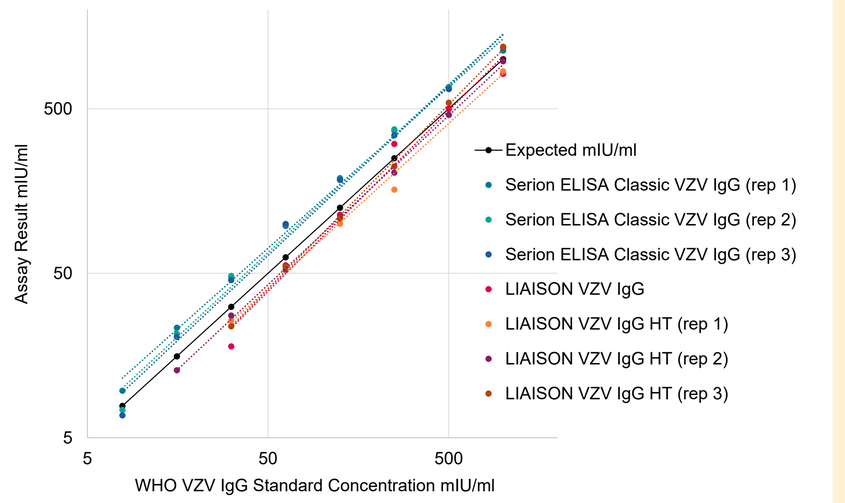

Figure 2: Correlation with expected WHO quantitative standard dilution (doubling dilution from 1000 to 7.8 mIU/mL)
Note: The lower limit of the LIAISON assay is 10 mIU/ml so values of ≤ 10 mIU/ml have not been plotted for the LIAISON assays. "rep" = replicate

Replicates of the WHO standard on the two new assays under evaluation showed high consistency between runs (Figure 2).

All assays showed good linearity between 10 and 1000 mIU/ml. R^2 values for the trendlines were all >0.96 for the LIAISON assays and >0.99 for the Serion ELISA assay.

Compared with expected, the quantitative values were on average:

0.13 log above expected for the Serion ELISA

0.06 log below expected for the LIAISON VZV IgG HT

The differences in log values from the expected values are all at or below 0.20 log different from the standard. This would normally be considered within acceptable variation.

CONCLUSIONS

- In general, both the new LIAISON VZV IgG HT and the Serion ELISA Classic VZV IgG assay perform well when compared with the LIAISON VZV IgG assay and externally validated test materials.
- Replacing the old LIAISON VZV IgG assay with the new LIAISON VZV IgG HT assay would have been be the simplest practical choice for the laboratory.
- **However**, our consultant virologists assessed the clinical risk from the outlier sample from the new LIAISON VZV IgG HT assay to be too great. False positives could lead to false assurance of immunity, potentially resulting in severe primary VZV infection in vulnerable patients.
- Discussion with other UKHSA and NHS clinical diagnostic laboratories in England informed us that similar findings had also been identified in other laboratories evaluating the new Liaison assay
- The Serion ELISA Classic VZV IgG assay has therefore been implemented at the UKHSA Public Health Laboratory, Birmingham.

REPORTING VZV IgG LEVELS

At UKHSA Public Health Laboratory, Birmingham, samples with VZV IgG are reported as follows:

- <50 mIU/mL are reported as negative
- between 50 and 100 mIU/mL are graded equivocal, but reported as negative
- >100 mIU/mL are reported as positive
- Between 100 and 150 mIU/mL, reports also have the comment "Guidelines suggest that VZV IgG levels < 150 IU/ml may not be protective in the immunocompromised'

This reporting is based on the following references:

- UK Standards for Microbiology Investigations V30 "Investigation of exposure to vesicular and non-vesicular rash in pregnancy" Issue no: 6.1
- UKHSA "Guidelines on post-exposure prophylaxis (PEP) for varicella or shingles" (July 2025)

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