Ortho Clinical Diagnostics

Because Every Test Is A Life™

An Evaluation of Performance of the VITROS® Immunodiagnostic Products Anti-SARS-CoV-2 IgG Quantitative Assay#

Paul Contestable, Brett Hirsch, Amanda Gardner
Ortho Clinical Diagnostics, Rochester, NY

Introduction

This study was designed to assess the clinical and analytical performance of the VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG Quantitative Reagent Pack (VITROS IgG Quant) on the VITROS family of analyzers. The assay is for the quantitative detection of SARS-CoV-2 IgG antibodies with calibration traceable to the First WHO International Standard for Anti-SARS-CoV-2 Immunoglobulin (20/136). Results are reported in both qualitative (reactive/non-reactive) and quantitative values (Binding Antibody Units/mL (BAU/mL)).

Assay Measuring Range

- The Limit of Quantitation (LoQ) was determined to be 1.82 BAU/mL as determined by the lowest concentration at which precision and accuracy design requirements are still met and within the linear range of the test.

Parameter	BAU/mL
Lo/Q	1.82

- All linearity panels demonstrated acceptable linearity from a result of 0.80 BAU/mL up to 225.8 BAU/mL. Linearity was demonstrated throughout the proposed measuring interval of 2.00 – 200 BAU/mL and at least 10% beyond the upper and lower limits of this interval.

Assay measuring range is 2.00 to 200 BAU/mL without dilution and 2.00 to 4000 BAU/mL with a 1:20 dilution. (see below)

Sample Dilution

Study was conducted according to EP34. Five samples were prepared by diluting either the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (20/136) or the 1st International Reference Panel for Anti-SARS-CoV-2 Immunoglobulin (20/268) into five separate non-reactive plasma specimens to achieve levels that are above the analytical measuring interval, but within the proposed extended measuring interval. 1:20 dilutions of each of the five samples were generated off-board using VITROS High Sample Diluent B as the diluent. The dilution of each sample was tested with five replicates on a VITROS 3600 system and a VITROS 5600 system. The mean dilution recovery was calculated for each sample and dilution ratio and compared to the known concentration of the neat sample.

Sample ID	Neat (BAU/mL) Known Value	Dilution Ratio 1:20				
		20				
		Predicted Dose (BAU/mL)	Recovery (%)	Predicted Dose (BAU/mL)	Recovery (%)	Within-Lab %CV
		5600 System		3600 System		
1	1000	983	98.3	961	96.1	2.3
2	766	778	101.6	744	97.1	3.5
3	350	397	113.4	365	104.3	4.4
4	268	302	112.5	276	102.9	5.1
5	246	227	92.4	217	88.1	3.5
AVERAGE RECOVERY			103.6		97.7	

This data demonstrates accurate recovery of samples diluted up to a 1:20 ratio which establishes an extended measuring interval of 2.00 – 4000 BAU/mL.

Recovery

Reactive or non-reactive clinical samples were spiked with WHO standard 20/136 at known concentrations to demonstrate accurate recovery across the assay range. The average recovery of the two samples at each analyte level were within the acceptance criteria of 80-120%.

Sample Preparation with			Spiked Sample	Un-Spiked Sample	Expected Increase in Concentration (BAU/mL)	Recovery (%)
			Average of Results	Average of Results		
Sample	Base Sample Matrix	Measured Increase in Concentration (BAU/mL)	(BAU/mL)	(BAU/mL)		
Sample 1	Pre-COVID	21.9	21.9	N/A	20.0	109.5
Sample 2	Pre-COVID	21.5	21.5	N/A	20.0	107.5
Average of 1&2	Pre-COVID	21.7	21.7	N/A	20.0	108.5
Sample 3	Antibody Reactive clinical Specimen	46.0	91.4	45.4	58.0	79.3
Sample 4	Antibody Reactive clinical Specimen	48.2	92.9	44.7	57.5	83.8
Average of 3&4	Antibody Reactive clinical Specimen	47.1	92.2	45.1	57.8	81.6
Sample 5	Antibody Reactive clinical Specimen	100.6	181	80.4	100	100.6
Sample 6	Antibody Reactive clinical Specimen	97.5	179	81.5	97.7	99.8
Average of 5&6	Antibody Reactive clinical Specimen	99.1	180	81.0	98.9	100.2
Average of all samples						96.8

N/A: Not applicable since the un-spiked sample is a pre-pandemic sampe and the result is 0 BAU/mL

Precision

Precision was evaluated consistent with CLSI document EP5. Two replicates each of six samples were tested on two separate occasions per day on 20 different days. The experiment was performed using two reagent lots on two different systems with one reagent lot on each system. The data presented are a representation of the product performance.

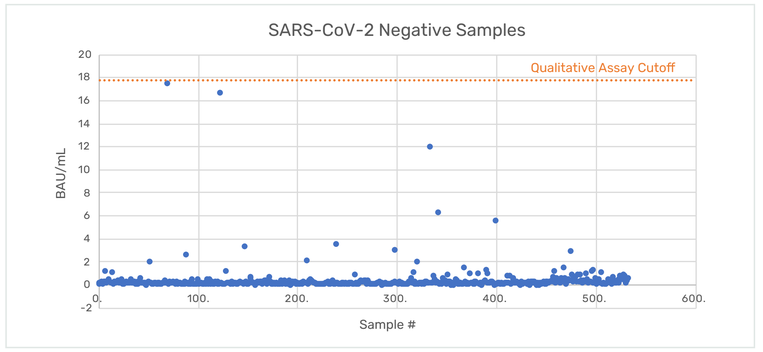
Units = BAU/mL								
Mean Concentration	Repeatability*		Within Calibration**		Within Laboratory***		Number of Obs.	Number of Days
	SD	%CV	SD	%CV	SD	%CV		
5.49	0.074	1.3	0.118	2.1	0.163	3.0	80	20
38.9	0.53	1.4	0.68	1.7	0.97	2.5	80	20
132	3.1	2.3	6.8	5.2	8.5	6.4	80	20
2.99	0.042	1.4	0.068	2.3	0.079	2.6	80	20
26.3	0.40	1.5	0.56	2.1	0.53	2.0	80	20
78.0	1.78	2.3	2.62	3.4	2.8	3.6	80	20

* Repeatability (within run). Between Duplicate precision averaged over all runs
** Within-calibration. Total precision with weighted components of within-run, between-run and between-day variation
*** Within-lab. A measure of the effect of recalibration on total precision, calculated within reagent lot

Clinical Specificity

Qualitative clinical specificity was evaluated with 533 samples collected prior to December 2019 tested across four days and a single calibration event. Of the 533 samples, 196 were K2-EDTA plasma, 114 were Lithium Heparin plasma, and 223 were serum. Of the 533 samples, none gave a reactive result (≥ 17.8 BAU/mL).

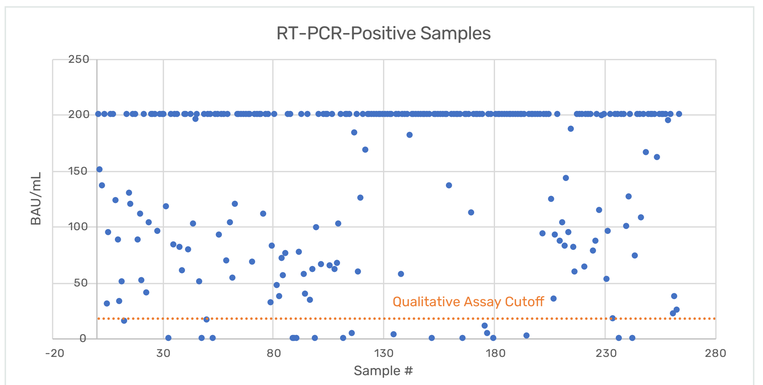
The VITROS IgG Quant assay has a specificity of 100.0% with a 95% confidence interval of 99.3 – 100.0%



Red line indicates the qualitative reactivity cut-off of 17.8 BAU/mL

Positive Percent Agreement with RT-PCR

To evaluate qualitative clinical sensitivity or positive percent agreement (PPA) with RT-PCR 264 samples reported as RT-PCR-reactive were collected and tested on the VITROS assay. Of the 264 samples, all were ≥ 15 days since reported symptom onset. Of the 264, 48 were < 15 days since the reactive RT-PCR result, the remainder were ≥ 15 days since reactive RT-PCR test. Of the 264 samples, 62 were lithium heparin plasma, 136 were serum, and 66 were K2-EDTA plasma. Of the 264 samples, 244 were determined to be reactive on the VITROS assay (result ≥ 17.8 BAU/mL). 20 of the samples gave a non-reactive result. The VITROS IgG Quant assay had an observed PPA of 92.4% with a 95% confidence interval of 88.5 – 95.3% with RT-PCR positive individuals on samples collected ≥ 15 days post symptom onset.

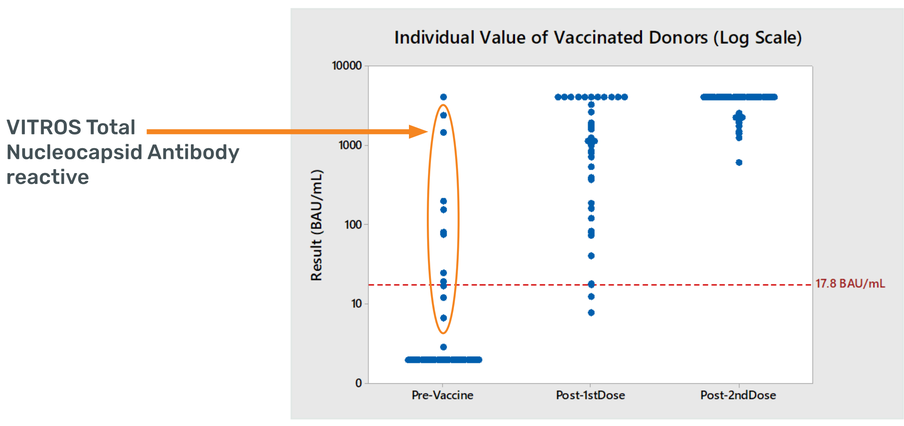


Red line indicates the qualitative reactivity cut-off of 17.8 BAU/mL

Reactivity with Vaccinated Individuals

Samples were collected from 45 individuals prior to vaccination, after the 1st Moderna vaccine dose (22-59 days post vaccine), and after the 2nd Moderna vaccine dose (12-17 days post vaccine dose). Some donors may have also had a prior COVID-19 infection. Each sample was analyzed on the VITROS IgG Quant assay in singleton. If a result was above the analytical measuring range (>200 BAU/mL), the sample was diluted 1:20 in VITROS High Sample Diluent B and run again in singleton.

All vaccinated donors yielded a reactive result on the VITROS IgG Quant assay after the second dose of the Moderna COVID-19 vaccine. The test demonstrated a quantitative increase in antibody titer throughout the course of vaccination. 32 of the 45 donors generated results above the extended measuring interval of 2-4000 BAU/mL after the second vaccine dose as illustrated in the figure below. In addition, 11 of the pre-vaccine samples that had elevated BAU/mL values were reactive with the VITROS Total Nucleocapsid antibody assay indicating natural infection prior to vaccination.



Note: In this figure, results < 2.00 BAU/mL were given a result of 2.00 BAU/mL and results > 4000 BAU/mL were given a result of 4000 BAU/mL

Conclusions

The VITROS IgG Quantitative assay demonstrates excellent clinical and analytical performance and can detect quantitative antibody response to COVID-19 infection or vaccination.

Dilution and vaccine performance not authorized by FDA.

Presented at 2021 AABB Virtual Annual Meeting, Oct. 17 -19, 2021 | PR-11971