Ortho Clinical Diagnostics

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Performance of the VITROS® Immunodiagnostic Products SARS-CoV-2 Antigen Assay

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Introduction

This study was designed to assess the clinical and analytical performance of the VITROS Immunodiagnostic Products SARS-CoV-2 Antigen assay (VITROS SARS-CoV-2 Antigen) on the VITROS 3600 Immunodiagnostic System and the VITROS 5600/ XT 7600 Integrated Systems.

Methods

Detection of SARS-CoV-2 nucleocapsid protein in the VITROS SARS-CoV-2 Antigen assay is achieved using monoclonal anti-SARS-CoV-2 nucleocapsid antibodies coated onto the well. Sample is added to the coated well in the first stage of the reaction, and SARS-CoV-2 nucleocapsid antigen from the sample is captured. After washing, HRP conjugated monoclonal anti-SARS-CoV-2 nucleocapsid antibodies are added. Following a final wash, bound HRP conjugates are detected using the VITROS signal reagent. The assay cut-off for VITROS SARS-CoV-2 Antigen is 1.00; values above the cut-off are Reactive for SARS-CoV-2 antigen and values below 1.00 are Non-reactive.

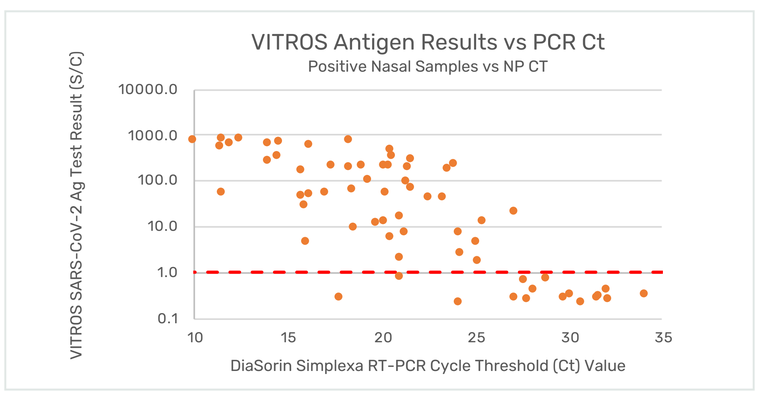
All VITROS testing was performed at the Ortho Clinical Diagnostics R&D lab, located in Rochester, NY, USA. RT-PCR testing of clinical specimen was performed at an external clinical laboratory. Clinical performance was evaluated using 152 paired nasopharyngeal and nasal specimen that were collected in the United States between September and November 2020. Samples were stored frozen between the time of collection and testing and were from patients suspected of having contracted SARS-CoV-2 within seven days of symptom onset. Data were analyzed to calculate the positive percent agreement (PPA) and negative percent agreement to RT-PCR result. Analytical specificity was assessed by testing patient matrix spiked with inactivated organisms known to cause other respiratory infections. Exogenous compounds with potential to be present in upper respiratory specimen collected from patients suffering from upper respiratory infection were also tested for potential interference with the VITROS assay.

Clinical Results (Nasopharyngeal Specimen)

PPA for the VITROS assay in nasopharyngeal specimen was 86.2% overall and 94.8% in samples with RT-PCR cycle threshold (Ct) less than 30.

VITROS	Not Detected (RT-PCR)	Detected (RT-PCR)	Total
Non-reactive	85	9	
Reactive	2	56	
Total	87	65	
PPA	86.2%	95% CI: 75.5 to 93.5%	
NPA	97.7%	95% CI: 91.9 to 99.7%	
Overall	92.8%	95% CI: 87.4 to 96.3%	

VITROS Result	PCR Positive (<30Ct)	PCR Positive (≥30 Ct)
Reactive	55	1
Non-reactive	3	6
PPA	94.6%	14.3%
95% CI	87.5 to 99.6%	0.36 to 57.9%

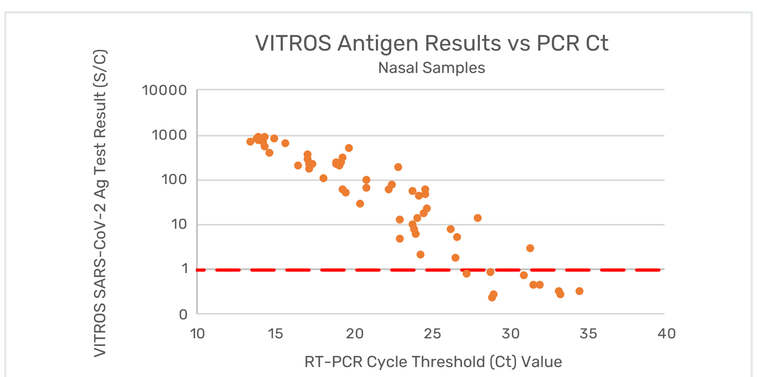


Clinical Results (Nasal Specimen)

PPA for the VITROS Assay in nasal specimen was 83.1% overall and 92.3% in samples with RT-PCR Ct less than 30.

VITROS	Not Detected (RT-PCR)	Detected (RT-PCR)	Total
Non-reactive	93	10	103
Reactive	0	49	49
Total	93	59	152
PPA	83.1%	95% CI: 71.0 to 91.6%	
NPA	100.0%	95% CI: 96.1 to 100.0%	
Overall	93.4%	95% CI: 88.2 to 96.8%	

VITROS Result	PCR Positive (<30Ct)	PCR Positive (≥30 Ct)
Reactive	48	1
Non-reactive	4	6
PPA	92.3%	14.3%
95% CI	81.5 to 97.9%	0.36 to 57.9%



Analytical Specificity (Potentially Cross-Reacting Organisms)

Other respiratory organisms were tested at greater than or equal to 106 CFU/mL of 105 pfu/mL, in the presence and absence of SARS-CoV-2 antigen, to evaluate potential impact on the accuracy of VITROS results if a patient were to be co-infected with the test organism and SARS-CoV-2 at the same time.

Analytical Specificity (Potentially Cross-Reacting Organisms)

Sample Category	Non-Reactive Sample	Spiked Reactive Sample	Cross-Reactivity or Interference? (Y/N)
Human coronavirus 229E	Non-Reactive	Reactive	N
Human coronavirus OC43	Non-Reactive	Reactive	N
Human coronavirus NL63	Non-Reactive	Reactive	N
Influenza A H3N2	Non-Reactive	Reactive	N
Influenza B	Non-Reactive	Reactive	N
Adenovirus (e.g. C1 Ad. 71)	Non-Reactive	Reactive	N
Human Metapneumovirus (hMPV)	Non-Reactive	Reactive	N
Parainfluenza virus 1-4	Non-Reactive	Reactive	N
Enterovirus	Non-Reactive	Reactive	N
Respiratory syncytial virus	Non-Reactive	Reactive	N
Rhinovirus	Non-Reactive	Reactive	N
Hemophilus influenzae	Non-Reactive	Reactive	N
Streptococcus pneumoniae	Non-Reactive	Reactive	N
Streptococcus pyogenes	Non-Reactive	Reactive	N
Candida albicans	Non-Reactive	Reactive	N
Bordetella pertussis	Non-Reactive	Reactive	N
Mycoplasma pneumoniae	Non-Reactive	Reactive	N
Legionella pneumophila	Non-Reactive	Reactive	N
MERS-coronavirus	Non-Reactive	Reactive	N
Chlamydophila pneumoniae	Non-Reactive	Reactive	N
Staphylococcus epidermidis	Non-Reactive	Reactive	N
Staphylococcus aureus	Non-Reactive	Reactive	N

To estimate the likelihood of cross-reactivity with SARS-CoV-2 virus in the presence of organisms that were not available for wet testing, In silico analysis using the Basic Local Alignment Search Tool (BLAST) managed by the National Center for Biotechnology Information (NCBI) was used to assess the degree of protein sequence homology.

- No protein sequence homology was found between M. tuberculosis, P. jirovecii or HCov-HKU1, thus cross-reactivity can be ruled out.
- The comparison between SARS-CoV-2 nucleocapsid protein and SARS-CoV shows homology of 90.52% and suggests that there will be significant cross reactivity in this test.

Analytical Specificity (Potentially Interfering Substances)

Substances that may be found in upper respiratory specimen were tested in the presence and absence of SARS-CoV-2 antigen to evaluate potential impact on the accuracy of VITROS results.

The presence of potentially interfering substances at the concentration shown was shown to not impact test results.

Analytical Specificity (Potentially Interfering Substances)

Proposed Interfering Substance	Active Ingredient	Concentration
Human Blood	Blood	1% and 4%
Hemoglobin	Hemolysate	1000 mg/dL
Biotin	Biotin	3510 ng/mL
Purified mucin protein	Mucin protein	5.0 mg/mL (5%)
OTC Nasal Spray 1	Oxymetazoline	15%
OTC Nasal Spray 2	Fluticasone	5%
OTC Nasal Spray 3	Triamcinolone	5%
OTC Nasal Spray 4	Phenylephrine hydrochloride	15%
OTC Nasal Spray 5	Budesonide (Glucocorticoid)	5%
OTC Nasal Spray 6	Saline	15%
OTC Nasal Spray 7	Cromolyn	15%
OTC Nasal Wash	Alkolol	10%
OTC Nasal Gel	Sodium Chloride (NeilMed)	5%
Sore Throat Phenol Spray	Benzocaine, Menthol, Phenol	0.7 g/mL (70%)
Throat Lozenge	Menthol	0.8 g/mL (80%)
Anti-viral Drug 1	Oseltamivir	5 mg/mL
Anti-viral Drug 2	Zanamivir	282.0 ng/mL
Anti-bacterial, Systemic	Tobramycin	1.25 mg/mL
Homeopathic Cold Remedy	Galphimia glauca, Luffa operculata, Sabadilla	5%
Antibacterial	Mupirocin	10 mg/mL

Conclusions

The VITROS SARS-CoV-2 Antigen assay demonstrates excellent clinical agreement with RT-PCR and can be used as an aid in identifying individuals with active SARS-CoV-2 infection.

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