INSTRUCTIONS FOR USE

CV2Ag

VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Controls

REF 619 9943

Rx ONLY, IVD, EUA Only

Intended Use

For *in vitro* diagnostic and laboratory professional use.

For use in monitoring the performance of the VITROS 3600 Immunodiagnostic System and the VITROS 5600/XT 7600 Integrated Systems for the qualitative detection of the SARS-CoV-2 nucleocapsid antigen.

This test has not been FDA cleared or approved; but has been authorized by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories certified under CLIA that meet the requirements to perform moderate or high complexity tests.

This test has been authorized only for the detection of proteins from SARS-CoV-2, not for any other viruses or pathogens.

The emergency use of this test is authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

Warnings and Precautions

WARNING: *Potentially Infectious Material*

Treat as if capable of transmitting infection.

Handle, use, store and dispose of solid and liquid waste from samples and test components, in accordance with procedures defined by appropriate national biohazard safety guideline or regulation (e.g. CLSI document M29). [1]

WARNING: *Contains 2-methyl-3(2H) isothiazolone (MIT) (CAS 2682-20-4)* [2]

The VITROS SARS-CoV-2 Antigen Controls contain 0.0475% 2-methyl-3(2H) isothiazolone (MIT). H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P302 + P352: IF ON SKIN: Wash with plenty of water and soap. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P363: Wash contaminated clothing before reuse. P501: Dispose of contents/container to an approved waste disposal plant.

Refer to www.orthoclinicaldiagnostics.com for the Safety Data Sheets and for Ortho contact information.

WARNING

Materials Provided

3 sets of VITROS SARS-CoV-2 Antigen Controls 1 and 2 (recombinant SARS-CoV-2 nucleocapsid antigen in buffer with bovine serum albumin and antimicrobial agent, 3 mL)

Materials Required but Not Provided

- Pipette, sample containers
- VITROS Immunodiagnostic Products SARS-COV-2 Antigen Extraction Buffer

Control Storage, Preparation and Handling

Control	Storage Condition		Stability
Unopened	Frozen	≤-20 °C (≤-4 °F)	expiration date
Opened	Refrigerated	2–8 °C (36–46 °F)	≤5 days

- VITROS SARS-CoV-2 Antigen Controls are supplied frozen. DO NOT REFREEZE.
- VITROS SARS-CoV-2 Antigen Controls are suitable for use until the expiration date on the carton when stored and handled as specified. Do not use beyond the expiration date.
- Thoroughly mix controls by inversion and bring to 15–30 °C (59–86 °F) before use.
- Handle controls in stoppered containers to avoid contamination and evaporation. To avoid evaporation, limit the amount of time controls are on the system. Refer to the operating instructions for your system.
- Return to 2–8 °C (36–46 °F) as soon as possible after use, or load only sufficient volume for a single determination.
- Baseline statistics for controls should be entered onto the system. For further information, refer to the operating instructions for your system.
- The expiration date for the controls must be entered onto the system. For further information, refer to the operating instructions for your system.

Testing Procedure

For each control:

- Combine 100 µL VITROS SARS-CoV-2 Extraction Buffer and 400 µL of control into a sample container.
- Mix well (e.g. cover sample container with cap/plug and vortex approx. 3-5 seconds).

Load each control onto the system by transferring an aliquot into a sample container, if needed (taking account of the volume required by the test and the minimum fill volume of the container). Process in the same manner as samples, according to the instructions in the appropriate VITROS Immunodiagnostic Products Reagent Pack and Calibrator instructions for use.

Note: Do not use visibly damaged product.

For further information on quality control procedures refer to the operating instructions for your system.
Not all products and systems are available in all countries.

Baseline Statistics

VITROS SARS-CoV-2 Antigen Control 1 should generate Non-reactive results.
VITROS SARS-CoV-2 Antigen Control 2 should generate Reactive results.

References

1. CLSI *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Fourth Edition.* CLSI document M29-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
2. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

Glossary of Symbols

The following symbols may have been used in the labeling of this product.

Do Not Reuse	Upper Limit of Temperature	Range
Use by or Expiration Date (Year-Month-Day)	Lower Limit of Temperature	Range of Means
LOT Batch Code or Lot Number	Temperature Limitation	Midpoint
SN Serial Number	Consult Instructions for Use	Revised
REF Catalog Number or Product Code	Attention: The Instructions for Use (IFU) has been updated	Supersedes
Caution	S1 For use in Slide Supply 1	Σ Contains Sufficient for "n" Tests
Keep Dry (Protect from Moisture/Humidity)	S2 For use in Slide Supply 2	IVD *in vitro* Diagnostic Medical Device
Manufacturer	SI SI Units	Der Grüne Punkt (the Green Dot). Manufacturer follows certain packaging material waste disposal management regulations
Date of Manufacture	CONV Conventional Units	σ Estimated within-lab SD
EC REP Authorized Representative in the European Community	Value	
Corrosive	Flammable	Serious Health Hazards
Health Hazards	Acute Toxicity	Environmental or Aquatic Toxicity

Revision History

Date of Revision	Version	Description of Technical Changes*
2021-11-30	2.0	Warnings and Precautions: updated Hazard and Precaution statements to align with the new Safety Data Sheets

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

Signature

Obsolete Date

Conditions of supply: all supplies are made subject to the standard terms and conditions of Ortho Clinical Diagnostics or its distributors. Copies of these are available on request.

Distributed in the US by:
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Ortho Clinical Diagnostics

INSTRUCTIONS FOR USE

VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Extraction Buffer

REF 619 9944

Rx ONLY, IVD, EUA Only

Intended Use

For *in vitro* diagnostic and laboratory professional use.

For use to extract the viral materials from nasopharyngeal and anterior nasal swab samples placed in CDC's formulation of VTM, WHO's formulation of VTM, COPAN Universal Transport Media (UTM)®, Hardy R99 VTM, FlexTrans™ Transport Media, or Saline for use on the automated VITROS 3600 Immunodiagnostic System and the VITROS 5600/XT 7600 Integrated Systems.

This test has not been FDA cleared or approved; but has been authorized by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories certified under CLIA that meet the requirements to perform moderate or high complexity tests.

This test has been authorized only for the detection of proteins from SARS-CoV-2, not for any other viruses or pathogens.

The emergency use of this test is authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

Warnings and Precautions

WARNING:	*Potentially Infectious Material* *Handle, use, store and dispose of solid and liquid waste from samples and test components, in accordance with procedures defined by appropriate national biohazard safety guideline or regulation (e.g. CLSI document M29).*[1]
WARNING:	*Contains Mixture, 3(2H)-isothiazolone, 5-chloro-2-methyl- with 2-methyl-3(2H)-isothiazolone (CAS 55965-84-9)*[2] *The VITROS SARS-CoV-2 Antigen Extraction Buffer contains ≥0.0015–<0.06% of Mixture, 3(2H)-isothiazolone, 5-chloro-2-methyl- with 2-methyl-3(2H)-isothiazolone. H317: May cause an allergic skin reaction. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash before reuse.* *Refer to www.orthoclinicaldiagnostics.com for the Safety Data Sheets and for Ortho contact information.*

WARNING

Safe Disposal

Follow local disposal regulations based on your location along with recommendations and content in the Safety Data Sheet to determine the safe disposal of this product.

Materials Provided

1 extraction buffer pack containing:

- 4 bottles of VITROS SARS-CoV-2 Antigen Extraction Buffer (28 mL) with antimicrobial agent

Materials Required but Not Provided

- Appropriate volume pipette and sample containers for extraction

Refer to the VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Reagent Pack and Calibrator instructions for use.

Storage, Preparation and Handling

Extraction Buffer	Storage Condition		Stability
Unopened	Refrigerated	2–8 °C (36–46 °F)	Expiration date
Opened	Refrigerated	2–8 °C (36–46 °F)	≤4 weeks

- VITROS SARS-CoV-2 Ag Extraction Buffer is supplied ready to use.
- Do not freeze.

Testing Procedure

Refer to the VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Reagent Pack and Calibrator instructions for use.

Note: Do not use visibly damaged product.

Not all products and systems are available in all countries.

Serious Incident

For a patient/user/third party in the European Union and in countries with identical regulatory regime (Regulation 2017/746/EU on IVD Medical Devices); if, during the use of this device or as a result of its use, a serious incident has occurred, please report it to the manufacturer and/or its authorized representative and to your national authority.

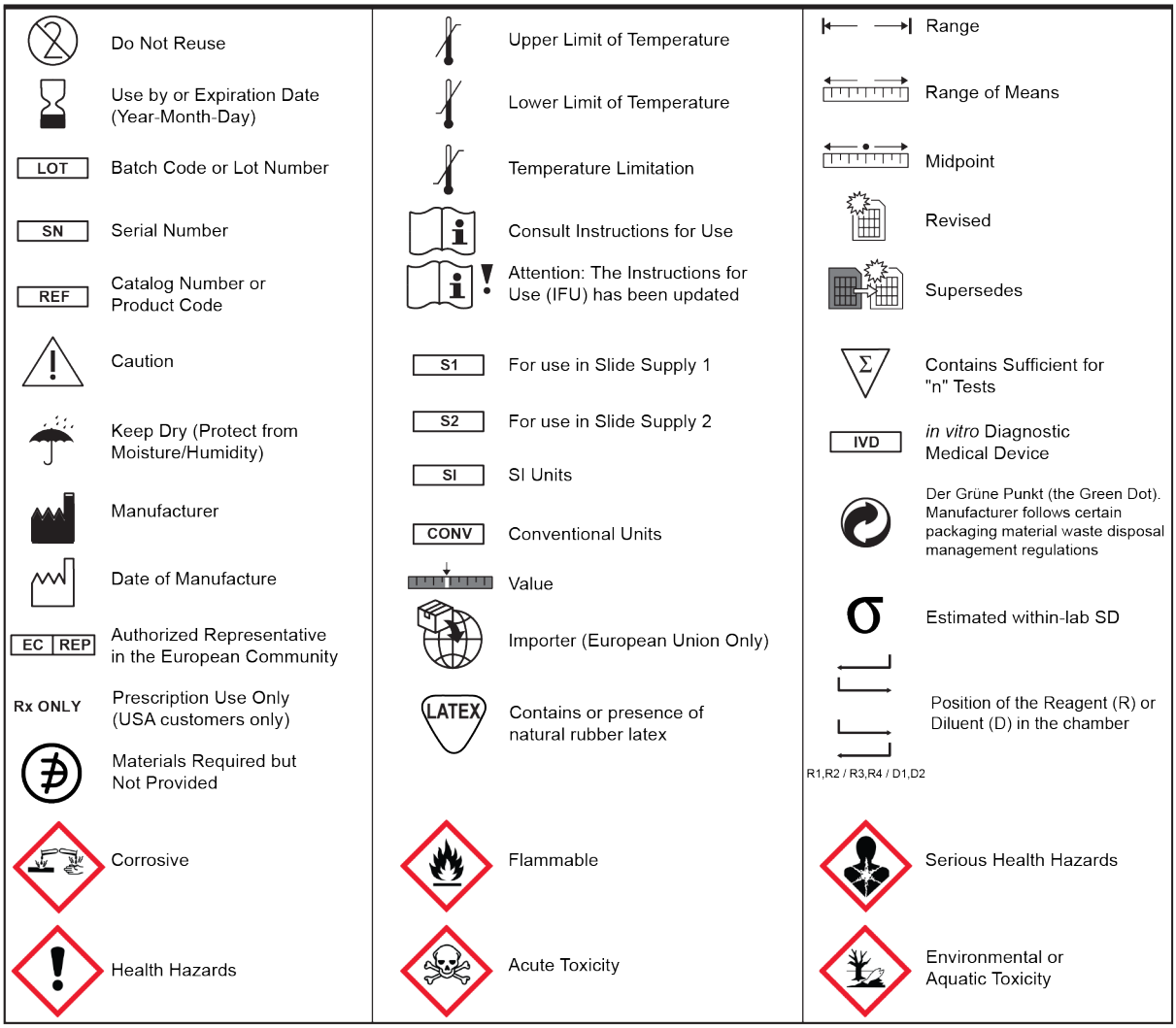
The manufacturer can be contacted via the company website address: www.orthoclinicaldiagnostics.com or by phoning the Ortho Care™ Technical Solutions Center number, which can be found on the website.

References

1. CLSI. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline - Fourth Edition*. CLSI document M29-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
2. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

Glossary of Symbols

The following symbols may have been used in the labeling of this product.



Revision History

Date of Revision	Version	Description of Technical Changes*
2022-02-23	3.0	Updated to comply with IVDR 2017/746 - Annex I Chapter III (20.0 to 20.4)

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

Signature

Obsolete Date

Conditions of supply: all supplies are made subject to the standard terms and conditions of Ortho Clinical Diagnostics or its distributors. Copies of these are available on request.

Distributed in the US by:
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Ortho Clinical Diagnostics

INSTRUCTIONS FOR USE CV2Ag

VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Reagent Pack	REF	619 9949
VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Calibrator	REF	619 9950

Rx ONLY
For *in vitro* diagnostic and laboratory professional use. For emergency authorization use only.

Intended Use

The VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Reagent Pack, when used in combination with the VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Calibrator, is a chemiluminescent immunoassay intended for the qualitative detection of nucleocapsid protein antigens from SARS-CoV-2 in direct nasopharyngeal (NP) and anterior nasal swab specimens collected by a healthcare provider in CDC's formulation of VTM, WHO's formulation of VTM, COPAN Universal Transport Media (UTM)®, Hardy R99 VTM, FlexTrans™ Transport Media, saline or phosphate buffered saline (PBS) from individuals who are suspected of COVID-19 within the first 7 days of symptom onset when tested at least twice over three days with at least 48 hours between tests using the VITROS 3600 Immunodiagnostic System and the VITROS 5600/XT 7600 Integrated Systems. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform moderate or high complexity tests.

The VITROS SARS-CoV-2 Antigen test does not differentiate between SARS-CoV and SARS-CoV-2.

Results are for the identification of SARS-CoV-2 nucleocapsid antigen. This antigen is generally detectable in nasopharyngeal swab and anterior nasal swab specimens during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definitive cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

All negative results are presumptive and confirmation with a molecular assay, if necessary, for patient management, may be performed. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control measures such as isolating from others and wearing masks. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19.

The VITROS SARS-CoV-2 Antigen test is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of *in vitro* diagnostic procedures, and proper infection control procedures. In the United States, the VITROS SARS-CoV-2 Antigen test is only for *in vitro* diagnostic use under the Food and Drug Administration's Emergency Use Authorization. This product has not been FDA cleared or approved.

Summary and Explanation of the Test

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a novel beta coronavirus and is the causative agent of Coronavirus Disease 2019 (COVID-19) and the pandemic. SARS-CoV-2 is mainly transmitted through droplets and contact routes, and people who are infected with SARS-CoV-2 may express signs and symptoms of acute respiratory illness, such as fever, cough, shortness of breath, etc., but can also be asymptomatic. [1-2] The median incubation time is estimated to be 5.1 days with symptoms expected to be present within 12 days of infection. [3] Symptomatic, pre-symptomatic and asymptomatic SARS-CoV-2 carriers all can be potential sources for viral transmission. [4]

Real-time reverse transcription polymerase chain reaction (rRT-PCR) detecting viral genes is the current gold standard for the diagnosis of COVID-19. Upper respiratory specimen, such as nasopharyngeal swab and anterior nasal swabs, are commonly used for diagnostic testing. [2] SARS-CoV-2 produces multiple viral antigens with the nucleocapsid antigens being the most abundant. [5] Immunoassays detect the SARS-CoV-2 nucleocapsid antigen and are also used for the diagnosis of active infection. [6]

Principles of the Procedure

The VITROS Immunodiagnostic Products SARS-CoV-2 Antigen test is performed using the VITROS SARS-CoV-2 Antigen Reagent Pack and the VITROS SARS-CoV-2 Antigen Calibrator on the VITROS 3600 Immunodiagnostic System and the VITROS 5600/XT 7600 Integrated Systems. An immunometric technique is used; this involves a two stage reaction. In the

first stage SARS-CoV-2 nucleocapsid antigen present in the sample binds with monoclonal anti-SARS-CoV-2 coated on the well.

Unbound sample is removed by washing. In the second stage horseradish peroxidase (HRP)-labeled monoclonal anti-SARS-CoV-2 is added in the conjugate reagent. The conjugate binds specifically to any SARS-CoV-2 nucleocapsid captured on the well in the first stage. Unbound conjugate is removed by the subsequent wash step.

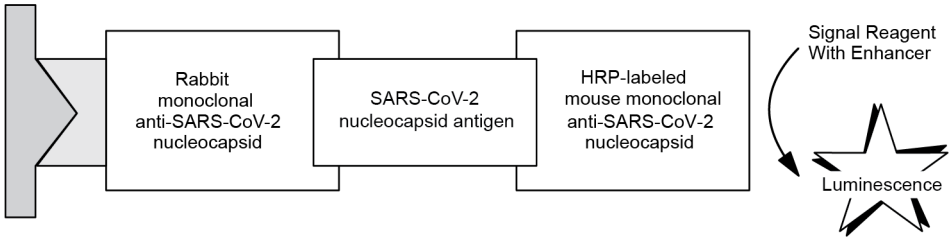
The bound HRP conjugate is measured by a luminescent reaction. [7] A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. Signal to cutoff numerical values will increase as the amount of SARS-CoV-2 antigen present in the sample increases.

Test Type	System*	Incubation Time	Time to first result	Test Temperature	Reaction Sample Volume
Immunometric	3600, 5600, XT 7600	37 minutes	48 minutes	37 °C	Extracted 80 µL**

* Not all products and systems are available in all countries.

** 80 µL of extracted sample (see Specimen Collection and Preparation)

Reaction Scheme



Warnings and Precautions and Safety Information

1. For *in vitro* diagnostic use. For prescription use only.
2. In the USA, this product has not been FDA cleared or approved, but has been authorized by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories certified under CLIA that meet the requirements to perform moderate or high complexity tests. This product has been authorized only for the detection of proteins from SARS-CoV-2, not for any other viruses or pathogens. The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated, or authorization is revoked sooner.
3. **Serial testing should be performed in individuals with negative results at least twice over three days (with 48 hours between tests) for symptomatic individuals. You may need to purchase additional tests to perform this serial (repeat) testing.**
4. Do not use if any of the test kit contents or packaging is damaged.
5. Test components are single-use. Do not re-use.
6. Do not use kit past its expiration date.
7. For more information on EUAs please visit: https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization
8. For the most up to date information on COVID-19, please visit: www.cdc.gov/COVID19
9. Calibration is lot specific; reagent packs and calibrator are linked by lot number.
10. Laboratories within the United States and its territories are required to report all results to the appropriate public health laboratories.
11. Treat all specimens as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
12. All components of this kit should be discarded as Biohazard waste according to Federal, State and local regulatory requirements.
13. Proper laboratory safety techniques should be followed at all times when working with SARS-CoV-2 patient samples. Proper handling and disposal methods should be established by the laboratory in accordance with local, state and federal regulations.
14. For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) [8] located at www.orthoclinicaldiagnostics.com.

WARNING: ***Potentially Infectious Material***

	Treat as if capable of transmitting infection. *Handle, use, store and dispose of solid and liquid waste from samples and test components, in accordance with procedures defined by appropriate national biohazard safety guideline or regulation (e.g. CLSI document M29).*[9]
WARNING:	*Contains EDTA (CAS 10378-23-1) and ProClin 300*[8] *The VITROS SARS-CoV-2 Antigen Reagent Pack contains 1.9% EDTA and 1.0% ProClin 300. H317: May cause an allergic skin reaction. H319: Causes serious eye irritation. P280: Wear protective gloves, Eye Protection. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash before reuse.*
WARNING:	*Contains 2-methyl-3(2H) isothiazolone (MIT) (CAS 2682-20-4)*[8] *The VITROS SARS-CoV-2 Antigen Calibrator contains 0.0475% 2-methyl-3(2H) isothiazolone (MIT). H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P302 + P352: IF ON SKIN: Wash with plenty of water and soap. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P363: Wash contaminated clothing before reuse. P501: Dispose of contents/container to an approved waste disposal plant.* *Refer to www.orthoclinicaldiagnostics.com for the Safety Data Sheets and for Ortho contact information.*

WARNING

Safe Disposal

Follow local disposal regulations based on your location along with recommendations and content in the Safety Data Sheet to determine the safe disposal of this product.

Reagents

Reagent Pack Contents

1 reagent pack containing:

- 100 coated wells (rabbit monoclonal anti-SARS-CoV-2 nucleocapsid, 1.0 µg/mL)
- 6.0 mL assay reagent (buffer with bovine protein stabilizers and antimicrobial agent)
- 16.2 mL conjugate reagent (HRP-mouse monoclonal anti-SARS-CoV-2 nucleocapsid, 2.0 µg/mL) in buffer with protein stabilizers and antimicrobial agent

Reagent Pack Handling

- The reagent pack is supplied ready for use.
- The reagent pack contains homogeneous liquid reagents that do not require shaking or mixing prior to loading onto the system.
- Handle the reagent pack with care. Avoid the following:
  - allowing condensation to form on the pack
  - causing reagents to foam
  - agitation of the pack

Reagent Pack Storage and Preparation

Reagent	Storage Condition		Stability
Unopened	Refrigerated	2–8 °C (36–46 °F)	expiration date
Opened	On system	System turned on	≤4 weeks
Opened	Refrigerated	2–8 °C (36–46 °F)	≤4 weeks

- The VITROS SARS-CoV-2 Antigen Reagent Pack is suitable for use until the expiration date on the carton when stored and handled as specified. Do not use beyond the expiration date.
- Do not freeze reagent packs.
- Load reagent packs directly from refrigerated storage to minimize condensation.
- Opened reagent packs are moisture/humidity sensitive. Store opened refrigerated reagent packs in a sealed VITROS Immunodiagnostic Products Reagent Pack Storage Box with desiccant.

Calibrator Contents

- 2 vials of VITROS SARS-CoV-2 Antigen Calibrator (recombinant SARS-CoV-2 nucleocapsid antigen in buffer with bovine serum albumin and antimicrobial agent, 1.0 mL)
- 8 calibrator bar code labels

Calibrator Handling

- Use only with reagent packs of the same lot number. Mix thoroughly by inversion and bring to 15–30 °C (59–86 °F) before use.
- Handle calibrators in original stoppered containers to avoid contamination and evaporation. To avoid evaporation, limit the amount of time calibrators are on the system. Refer to the operating instructions for your system. Return to 2–8 °C (36–46 °F) as soon as possible after use or load only sufficient volume for a single determination.

Calibrator Storage and Preparation

Calibrator	Storage Condition		Stability
Unopened	Frozen	≤-20 °C (≤-4 °F)	expiration date
Opened	Refrigerated	2–8 °C (36–46 °F)	≤24 hours

- VITROS SARS-CoV-2 Antigen Calibrator is supplied frozen. DO NOT REFREEZE.
- The VITROS SARS-CoV-2 Antigen Calibrator is suitable for use until the expiration date on the carton when stored and handled as specified. Do not use beyond the expiration date.
- The VITROS SARS-CoV-2 Antigen test uses 80 µL of calibrator for each determination. Transfer an aliquot of each calibrator into a sample container (taking account of the minimum fill volume of the container). For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.

Caution: **Do not add extraction buffer to calibrator.**

- The VITROS SARS-CoV-2 Antigen Calibrator is automatically processed in duplicate.

Specimen Collection, Preparation and Storage

Patient Preparation

No special patient preparation is necessary.

Specimens Recommended

Nasopharyngeal and anterior nasal swab specimens stored in:

- CDC viral transport media
- Hardy R99 viral transport media
- COPAN* UTM® Universal transport media
- Bartels FlexTrans™ transport media [Trinity Biotech]
- WHO viral transport media
- Saline (Phosphate Buffered Saline (PBS) or 0.9% NaCl)

*COPAN UTM® is also distributed as Becton Dickinson UVT and other brands.

Special Precautions

IMPORTANT: *Certain transport media, including the Remel M4RT, have been reported to affect other analytes and tests.* [10] *False positive results for SARS-CoV-2 have been*

observed in some tests with use of some transport media, including the Remel M4RT viral transport media.

Specimen Collection and Preparation

- Collect nasopharyngeal (NP) or anterior nasal swab specimens following CDC guidelines with proper infection control and personal protective equipment (PPE). [9, 10, 11]
- Follow the instructions provided with your transport media for use and processing of the sample. [10]
- The VITROS SARS-CoV-2 Antigen test uses 80 µL of extracted sample for each determination. This is in addition to the minimum fill volume of the chosen sample container. For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.
- Sample Preparation and Testing:

1. Prepare Sample for Extraction
   - Receive sample swab in transport media.
   - Mix transport tube well (e.g., vortex approx. 3-5 seconds).
2. Add Extraction Buffer to Sample
   - Transfer 100 µL VITROS SARS-CoV-2 Antigen Extraction Buffer into a labeled new sample tube.
   - Add 400 µL viral sample into the same sample tube.
   - Mix well (e.g. cover sample container with cap/plug and vortex approx. 3-5 seconds).

IMPORTANT: *Care should be taken when handling sample tubes as the sample should be considered potentially infectious.*

3. Load to Instrument
   - Load and process samples in the same manner as other testing on your VITROS System.
   - Refer to the operating instructions for your system.

Note: An alternate sample volume may be used if desired, using 4 parts media to1 part VITROS SARS-CoV-2 Antigen Extraction Buffer. It is recommended to have a minimum of 250 µL of sample/extraction buffer loaded on the VITROS system.

If programming samples on the system manually, process samples by selecting the CV2Ag test button on system.

Handling and Storage Conditions

Extraction	Storage Condition	Stability
Pre- or Post-	Room Temperature (up to 30 °C [86 °F])	24 hours
Pre- or Post-	Refrigerated 2–8 °C (36–46 °F)	48 hours

- Handle samples in stoppered containers to avoid contamination and evaporation.
- Follow procedures within your laboratory to avoid cross contamination of patient specimens.
- The amount of time samples are on the system prior to analysis should be limited to avoid evaporation. Refer to the operating instructions for your system.
- Return unused sample (pre- or post-extraction) to 2–8 °C (36–46 °F) as soon as possible after use or load sufficient volume for a single determination.
- Samples (pre- or post-extraction) that will not be tested within the time frames outlined above should be stored at ≤-20 °C [≤-4 °F].

Testing Procedure

Materials Provided

- VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Reagent Pack
- VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Calibrator

Materials Required but Not Provided

- VITROS Immunodiagnostic Products Signal Reagent
- VITROS Immunodiagnostic Products Universal Wash Reagent
- Quality control materials such as VITROS Immunodiagnostic Products VITROS SARS-CoV-2 Antigen Controls

- VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Extraction Buffer
- VITROS Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant
- Appropriate volume pipette and sample containers for extraction

Operating Instructions

Check the inventory regularly to aid the management of reagents and ensure that sufficient VITROS Signal Reagent, VITROS Universal Wash Reagent and calibrated reagent lots are available for the work planned. When performing panels of tests on a single sample, ensure that the sample volume is sufficient for the tests ordered.

For detailed information refer to the operating instructions for your system.

Note:	Do not use visibly damaged product.

Default Test Name

The default test name which will appear on patient reports is SARS-CoV-2 Ag. The default short name that will appear on the test selection menus and laboratory reports is CV2Ag. These defaults may be reconfigured, if required. For detailed information refer to the operating instructions for your system.

Calibration

Calibration Procedure

- Calibration is lot specific; reagent packs and calibrator are linked by lot number. Reagent packs from the same lot may use the same calibration.
- A Master Calibration is established for each new reagent lot by performing multiple tests. This is the process by which a lot-specific parameter [a] which links the signal at the cutoff (cutoff value) to the calibrator signal is determined.
- Cutoff value = (a x Signal of Cal 1)
- Ensure that the Master Calibration for each new reagent lot is available on your system.
- Load sufficient volume for the automatic duplicate determination. Calibration need not be programmed if bar code labels are used; calibration will be initiated automatically.
- When the calibrator is processed, the validity of the calibration is assessed against quality parameters which compare the actual signal of the calibrator with the expected signal. If the calibration is acceptable the cutoff value is calculated and stored for use with any reagent pack of that lot.
- The quality of calibration cannot be completely described by a single parameter. The calibration report should be used in conjunction with acceptable control values to determine the validity of the calibration.
- Recalibration is required after a pre-determined calibration interval, or when a different reagent lot is loaded.
- Calibration results are assessed against a range of quality parameters. Failure to meet any of the defined quality parameter ranges will be coded in the calibration report. For actions to be taken following a failed calibration refer to the operating instructions for your system.
- Refer to the operating instructions for your system for detailed instructions on the calibration process.

When to Calibrate

- Calibrate when the reagent pack and calibrator lot changes.
- Calibrate every 28 days.
- After specified service procedures have been performed.
- If quality control results are consistently outside of your acceptable range.

For additional information on when to calibrate, refer to the operating instructions for your system.

Traceability of Calibration

Calibration of the VITROS SARS-CoV-2 Antigen test is traceable to an in-house reference calibrator which has been value assigned to optimize clinical sensitivity and specificity.

Calibration Model

Results are calculated as a normalized signal, relative to a cutoff value. During the calibration process a lot-specific parameter is used to determine a valid stored cutoff value for the VITROS Immunodiagnostic and VITROS Integrated Systems.

Quality Control

Quality Control Material Selection

VITROS SARS-CoV-2 Antigen Controls are recommended for use with the VITROS Immunodiagnostic and VITROS Integrated Systems. There are 2 VITROS SARS-CoV-2 Antigen Controls (SARS-CoV-2 Ag negative and SARS-CoV-2 Ag positive).

Appropriate quality control value ranges must be established for all quality control materials used with the VITROS SARS-CoV-2 Antigen test.

Quality Control Procedure Recommendations

- Good laboratory practice requires that controls be processed to verify the performance of the test.
- To verify system performance, analyze control materials:
  - After calibration
  - If the system is turned off for more than 2 hours
  - After reloading reagent packs that have been removed from the MicroWell Supply and stored for later use
  - According to local regulations or at least once each day that the test is being performed
  - After specified service procedures are performed

If quality control procedures within your laboratory require more frequent use of controls, follow those procedures.

- Analyze quality control materials in the same manner as patient specimens.
- If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.
- Refer to published guidelines for general quality control recommendations. [12]

For more detailed information, refer to the operating instructions for your system.

Quality Control Material Preparation and Storage

Refer to the manufacturer's product literature for preparation, storage, and stability information.

Results

Results are automatically calculated by the VITROS Immunodiagnostic and VITROS Integrated Systems.

Result Calculation

$$\text{Result} = \frac{\text{Signal for test sample}}{\text{Signal at Cutoff (Cutoff value)}}$$

Interpretation of Results*

Sample results will be displayed with a numerical signal to cutoff (S/C) value and a "Non-reactive" (negative) or "Reactive" (positive) label.

Result (S/C)	<1.00	≥1.00
Result Text	Non-reactive (negative)	Reactive (positive)

*Quantitative values should not be reported to health care providers.

Repeat testing is needed to improve test accuracy. Please follow the table below when interpreting test results.

Status on first day of Testing	First Result Day 1	Second Result Day 3	Interpretation
With Symptoms	Positive	N/A	Positive for COVID-19
	Negative	Positive	Positive for COVID-19
	Negative	Negative	**Negative** for COVID-19

Results should be considered in the context of an individual's recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19.

COVID-19 Positive (+)

Repeat testing does not need to be performed if patients have a positive result at any time.

A positive test result means that the virus that causes COVID-19 was detected in the sample, and it is very likely the individual has COVID-19 and is contagious. Please contact the patient's doctor/primary care physician (if applicable) and the local health authority immediately and instruct your patient to adhere to the local guidelines regarding self-isolation. There is a very small chance that this test can give a positive result that is incorrect (a false positive).

Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Individuals who test positive with the VITROS SARS-CoV-2 Ag assay should self-isolate and seek follow up care with their physician or healthcare provider as additional confirmatory testing with a molecular test for positive results may also be necessary, if there is a low likelihood of COVID-19, such as in individuals without known exposures to COVID-19 or residing in communities with low prevalence of infection.

COVID-19 Negative (-)

To increase the chance that the negative result for COVID-19 is accurate, you should:

- Test again in 48 hours if the individual has symptoms on the first day of testing.

A negative test result indicates that the virus that causes COVID-19 was not detected in the sample. A negative result does not rule out COVID-19. There is a higher chance of false negative results with antigen tests compared to laboratory-based tests such as PCR tests. If the test is negative but COVID-19-like symptoms, e.g., fever, cough, and/or shortness of breath continue, follow up testing for SARS-CoV-2 with a molecular test or testing for other respiratory disease should be considered. If applicable, seek follow up care with the primary health care provider.

All negative results should be treated as presumptive and confirmation with a molecular assay may be necessary if there is a high likelihood of SARS-CoV-2 infection, such as in an individual with a close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with high prevalence of infection. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions.

Limitations of the Procedure

Known Interferences

The VITROS SARS-CoV-2 Antigen test was evaluated for interference. Commonly encountered substances were tested on one lot of reagent. Of the compounds tested, none was observed to interfere with the clinical interpretation of the test. SARS-CoV was not tested using the VITROS assay however it does cross-react in the VITROS SARS-CoV-2 Antigen assay. Refer to "Substances that do not Interfere" for a list of compounds tested that did not show interference.

Other Limitations

- The contents of this kit are to be used for the qualitative detection of SARS-CoV-2 antigens from nasopharyngeal or anterior nasal swab specimens only.
- Anterior nasal swabs are less sensitive, when compared to paired nasopharyngeal swabs.
- There is a higher chance of false negative results with antigen tests than with laboratory-based molecular tests due to the sensitivity of the test technology. This means that there is a higher chance this test will give a false negative result in an individual with COVID-19 as compared to a molecular test, especially in samples with low viral load.
- All COVID-19 antigen test negative results are presumptive and confirmation with a molecular assay may be necessary.
- Performance has only been established with nasopharyngeal and anterior nasal specimens. Other specimen types have not been evaluated and should not be used with this assay. Performance in fresh specimens has not been established and may differ.
- Remel M4RT should not be used in with the VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Reagent Pack and Calibrator in either the VITROS 3600 Immunodiagnostic System or the VITROS 5600/XT 7600 Integrated Systems. Some lots of Remel M4RT have been shown to cause false positive results when used with the VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Reagent Pack.
- For claimed VTMs, performance was established using a limited number of VTM lots and may differ due to lot-to-lot variability within each claimed VTM.
- Only qualitative results should be reported. Semi-quantitative numerical results have not been clinically or analytically validated and may not correlate with patient disease status, duration of illness or severity of illness. Semi-quantitative results have not been demonstrated to correlate with the success or failure of any therapeutic interventions and should not be used to guide clinical management.
- Failure to follow the instructions for use may adversely affect test performance and/or invalidate the test result.
- Test results should be considered in the context of all available clinical and diagnostic information, including patient history and other test results.
- A negative test result may occur if the level of antigen in a sample is below the detection limit of the test.
- Positive test results do not rule out co-infections with other pathogens.

- Positive test results do not differentiate between SARS-CoV and SARS-CoV-2.
- Negative test results are not intended to rule in other non-SARS viral or bacterial infections.
- If the differentiation of specific SARS viruses and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- A false-negative test result may occur if the level of viral antigen in a sample is below the detection limit of the test or if the sample was collected or transported improperly; therefore, a negative test result does not eliminate the possibility of SARS-CoV-2 infection.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens collected between September 2020 and November 2020. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- This test detects both viable (live) and nonviable SARS-CoV-2. Test performance depends on the amount of virus (antigens) in the sample and may or may not correlate with viral culture results performed on the same sample.
- The performance of this test has not been evaluated for use in patients without signs and symptoms of respiratory infection. Decreased clinical sensitivity was observed for mid-turbinate swabs collected from asymptomatic individuals.
- If the patient continues to have symptoms of COVID-19, and both the patient's first and second tests are negative, the patient may not have COVID-19, however additional follow-up may be needed.
- If the test is positive, then proteins from the virus that causes COVID-19 have been found in the sample and the individual likely has COVID-19.
- Incorrect test results may occur if a specimen is incorrectly collected or handled.

Conditions of Authorization for the Laboratory

The VITROS Immunodiagnostic Products SARS-CoV-2 Antigen test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Recipients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas-antigen-diagnostic-tests-sars-cov-2. However, to assist clinical laboratories using the VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Reagent Pack test ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

- Authorized laboratories* using your product must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using your product must use your product as outlined in the VITROS SARS-CoV-2 Antigen Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
- Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories must collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Ortho-Clinical Diagnostics, Inc. at OrthoCareTechnicalSolutions@orthoclinicaldiagnostics.com or via phone by contacting Ortho Customer Support Services at 1-800-421-3311 any suspected occurrence of false reactive or false non-reactive results and significant deviations from the established performance characteristics of your product of which they become aware.
- All operators using your product must be appropriately trained in performing and interpreting the results of your product, use appropriate personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.
- Ortho-Clinical Diagnostics, Inc., authorized distributors, and authorized laboratories using your product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

*The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a," that meet the requirements to perform high or moderate complexity tests.

Performance Characteristics

Limit of Detection

The Limit of Detection (LoD) was determined by evaluating different dilutions of heat inactivated SARS-CoV-2 virus added to pooled nasal wash. 50 µL of the viral particle solution was added to dry swabs and the swab was then placed into 2 mL of transport media. The transport media with eluted viral particles was tested repeatedly using the VITROS SARS-CoV-2 Antigen test (n=20). LoD is defined as the lowest virus concentration at which a minimum of 19 replicates out of 20

generate a Reactive result. Testing was performed across seven transport media types and the resulting LoD ranged from 5.0 x 10^2 $TCID_{50}$ per mL to 3.0 x 10^3 $TCID_{50}$ per mL.

LoD Determinations

Transport Medium	$TCID_{50}$ per mL	$TCID_{50}$ per swab
CDC Viral Transport Medium	5.0 x 10^2	25
COPAN Universal Transport Medium	5.0 x 10^2	25
Hardy Viral Transport Medium	1.5 x 10^3	76
FlexTrans Transport Medium	3.0 x 10^3	151
WHO Viral Transport Medium	7.6 x 10^2	38
Saline (PBS and 0.9% NaCl)	1.5 x 10^3	76

Clinical Performance Characteristics - Nasopharyngeal Specimens

Clinical performance characteristics of the VITROS SARS-CoV-2 Antigen test was evaluated using residual samples from patients suspected of having contracted the SARS-CoV-2 virus within seven days of symptom onset. Samples were collected between September 2020 and November 2020 from 69 female patients and 36 male patients. Seven samples were from patients less than 21 years of age, 47 were from patients 21 to 60 years of age and 51 were from patients over the age of 60. Nasopharyngeal samples were stored frozen between the time of collection and the time of testing. FDA Emergency Use Authorized high sensitivity real-time Polymerase Chain Reaction (RT-PCR) assays for the detection of SARS-CoV-2 were utilized as the comparator methods for this study.

Testing was performed by operators who were blinded to the RT-PCR test result. External control testing, using VITROS SARS-CoV-2 Antigen Controls was performed on each day of VITROS testing.

The performance of VITROS SARS-CoV-2 Antigen test was established with 105 nasopharyngeal specimens collected from individual symptomatic patients (within 7 days of onset) who were suspected of COVID-19 and compared to RT-PCR on a paired NP swab.

VITROS SARS-CoV-2 Antigen Performance in RT-PCR Positive and Negative Nasopharyngeal Samples Collected Within 7 Days of Symptom Onset Against the Comparator Method

VITROS SARS-CoV-2 Antigen Test	RT-PCR Comparator Method		
	Detected	Not Detected	Total
Reactive	24	0	24
Non-reactive	6	75	81
Total	30	75	105
Positive Percent Agreement: 80.0% (95% CI: 56.6–88.5%)			
Negative Percent Agreement: 100.0% (95% CI: 95.2–100.0%)			

Positive results broken down by days since symptom onset.

Days Since Symptom Onset	Cumulative VITROS PCR Positive (+)	Cumulative VITROS Reactive (+)	PPA
0	8	4	50.0%
1	10	6	60.0%
2	14	10	71.4%
3	20	16	80.0%
4	20	16	80.0%
5	22	18	81.8%
6	26	21	80.8%
7	30	24	80.0%

A second clinical performance study was conducted using residual samples from patients suspected of having contracted the SARS-CoV-2 virus within seven days of symptom onset. Samples were collected between September 2020 and November 2020 from 41 female patients and 111 male patients. Six samples were from patients less than 21 years of age, 116 were from patients 21 to 60 years of age and 30 were from patients over the age of 60. Nasopharyngeal samples were stored frozen between the time of collection and the time of testing. FDA Emergency Use Authorized high sensitivity real-time Polymerase Chain Reaction (RT-PCR) assays for the detection of SARS-CoV-2 were utilized as the comparator methods for this study.

Testing was performed by operators who were blinded to the RT-PCR test result. External control testing, using VITROS SARS-CoV-2 Antigen Controls was performed on each day of VITROS testing.

The performance of VITROS SARS-CoV-2 Antigen test was established with 152 nasopharyngeal specimens collected from individual symptomatic patients (within 7 days of onset) who were suspected of COVID-19 and compared to RT-PCR on a paired NP swab.

VITROS SARS-CoV-2 Antigen Performance in RT-PCR Positive and Negative Nasopharyngeal Samples Collected Within 7 Days of Symptom Onset Against the Comparator Method

VITROS SARS-CoV-2 Antigen Test	RT-PCR Comparator Method		
	Detected	Not Detected	Total
Reactive	56	2**	58
Non-reactive	9*	85	94
Total	65	87	152
Positive Percent Agreement: 86.2% (95% CI: 75.3–93.5%)			
Negative Percent Agreement: 97.7% (95% CI: 91.9–99.7%)			

* One non-reactive result was also negative on an alternate RT-PCR method

** One reactive result was also positive on an alternate RT-PCR method.

Positive results broken down by days since symptom onset.

Days Since Symptom Onset	Cumulative VITROS PCR Positive (+)	Cumulative VITROS Reactive (+)	PPA
0	2	2	100.0%
1	8	8	100.0%
2	19	19	100.0%
3	34	31	91.2%
4	40	36	90.0%
5	55	47	85.5%
6	63	54	85.7%
7	65	56	86.2%

Clinical Performance Characteristics - Anterior Nasal Specimens

Clinical performance characteristics of the VITROS SARS-CoV-2 Antigen test was evaluated using residual samples from patients suspected of having contracted the SARS-CoV-2 virus within seven days of symptom onset. Samples were collected from 41 female patients and 111 male patients. Six samples were from patients less than 21 years of age, 116 were from patients 21 to 60 years of age and 30 were from patients over the age of 60. Nasal samples were stored frozen between the time of collection and the time of testing. FDA Emergency Use Authorized high sensitivity real-time Polymerase Chain Reaction (RT-PCR) assays for the detection of SARS-CoV-2 were utilized as the comparator methods for this study.

Testing was performed by operators who were blinded to the RT-PCR test result. External control testing, using VITROS SARS-CoV-2 Antigen Controls was performed on each day of VITROS testing.

The performance below of VITROS SARS-CoV-2 Antigen test was established with 152 nasal specimens collected from individual symptomatic patients (within 7 days of onset) who were suspected of COVID-19 and compared to RT-PCR on a paired anterior nasal swab. Performance compared to a paired RT-PCR on an NP swab is also presented in the table below.

VITROS SARS-CoV-2 Antigen Performance in RT-PCR Positive and Negative Nasal Samples Collected Within 7 Days of Symptom Onset Against the Comparator Method

VITROS SARS-CoV-2 Antigen Test	RT-PCR Comparator Method		
	Detected	Not Detected	Total
Reactive	49	0	49
Non-reactive	10*	93	103
Total	59	93	152
Positive Percent Agreement: 83.1% (95% CI: 71.0–91.6%)			
Negative Percent Agreement: 100.0% (95% CI: 96.1–100.0%)			

* Two non-reactive results were also negative on an alternate RT-PCR method.

Positive results broken down by days since symptom onset.

Days Since Symptom Onset	Cumulative VITROS PCR Positive (+)	Cumulative VITROS Reactive (+)	PPA
0	2	1	50.0%
1	8	7	87.5%
2	18	17	94.4%
3	31	29	93.5%
4	35	32	91.4%
5	49	42	85.7%
6	57	47	82.5%
7	59	49	83.1%

Clinical Concordance: VITROS Nasal vs. RT-PCR Nasopharyngeal Results

VITROS SARS-CoV-2 Nasal Swab Antigen Performance Compared to RT-PCR Positive and Negative Nasopharyngeal Samples Collected Within 7 Days of Symptom Onset Against the Comparator Method

ALL	RT-PCR Nasopharyngeal		
VITROS Nasal	Detected	Not Detected	Total
Reactive	49	0	49
Non-reactive	16	87	103
Total	65	87	152
PPA*		75.4% (95% CI: 63.1–85.2%)	
NPA		100.0% (95% CI: 95.8–100.0%)	

* The decreased PPA may be attributed to lower viral loads present in anterior nasal swabs when compared to NP swabs. Paired RT-PCR specimens demonstrated a PPA of 90.8% when comparing RT-PCR anterior nasal sample results to RT-PCR nasopharyngeal samples.

VITROS SARS-CoV-2 Antigen Reactivity in Nasal Samples by Days Since Symptom Onset

Days Since Symptom Onset	Cumulative RT-PCR NP Positive	Cumulative VITROS Nasal Reactive	PPA
0	2	1	50.0%
1	8	7	87.5%
2	19	17	89.5%
3	34	29	85.3%
4	39	32	82.1%
5	54	42	77.8%
6	62	47	75.8%
7	65	49	75.4%

Clinical Performance Characteristics - Performance of SARS-CoV-2 antigen test with serial testing

A prospective clinical study was conducted between January 2021 and May 2022 as a component of the Rapid Acceleration of Diagnostics (RADx) initiative from the National Institutes of Health (NIH). [13] A total of 7,361 individuals were enrolled via a decentralized clinical study design, with a broad geographical representation of the United States. Per inclusion criteria, all individuals were asymptomatic upon enrollment in the study and at least 14 days prior to it and did not have a SARS-CoV-2 infection in the three months prior to enrollment. Participants were assigned to one of three EUA authorized SARS-CoV-2 OTC rapid antigen tests to conduct serial testing (every 48 hours) for 15 days. If an antigen test was positive, the serial-antigen testing result is considered positive.

At each rapid antigen testing time point, study subjects also collected a nasal swab for comparator testing using a home collection kit (using a 15-minute normalization window between swabs). SARS-CoV-2 infection status was determined by a composite comparator method on the day of the first antigen test, using at least two highly sensitive EUA RT-PCRs. If results of the first two molecular test were discordant a third highly sensitive EUA RT-PCR test was performed, and the final test result was based upon the majority rule.

Study participants reported symptom status throughout the study using the MyDataHelps app. Two-day serial antigen testing is defined as performing two antigen tests 36–48 hours apart. Three-day serial antigen testing is defined as performing three antigen tests over five days with at least 48 hours between each test.

Out of the 7,361 participants enrolled in the study, 5,609 were eligible for analysis. Among eligible participants, 154 tested positive for SARS-CoV-2 infection based on RT-PCR, of which 97 (62%) were asymptomatic on the first day of their infection, whereas 57 (39%) reported symptoms on the first day of infection.

Performance of the antigen test with serial testing in individuals is described in the table below.

Data establishing PPA of COVID-19 antigen serial testing compared to the molecular comparator single day testing throughout the course of infection with serial testing. Data is from all antigen tests in study combined.

DAYS AFTER FIRST PCR POSITIVE TEST RESULT	Ag Positive/PCR Positive (Antigen Test Performance %PPA)		
	SYMPTOMATIC ON FIRST DAY OF TESTING		
	1 Test	2 Tests	3 Tests
0	34/57 (59.6%)	47/51 (92.2%)	44/47 (93.6%)
2	58/62 (93.5%)	59/60 (98.3%)	43/43 (100%)
4	55/58 (94.8%)	53/54 (98.1%)	39/40 (97.5%)
6	27/34 (79.4%)	26/33 (78.8%)	22/27 (81.5%)
8	12/17 (70.6%)	12/17 (70.6%)	7/11 (63.6%)
10	4/9 (44.4%)	3/7 (42.9%)	–

1 Test = one (1) test performed on the noted days after first PCR positive test result Day 0 is the first day of documented infection with SARS-CoV-2.
2 Tests = two (2) tests performed an average of 48 hours apart. The first test performed on the indicated day and the second test performed 48 hours later.
3 Tests = three (3) tests performance an average of 48 hours apart. The first test performed on the indicated day, the second test performed 48 hours later, and a final test performed 48 hours after the second test.

Potentially Cross-reacting Subgroups

The VITROS SARS-CoV-2 Antigen test was evaluated for potential microbial cross-reactivity and interference using contrived samples in the absence and presence of SARS-CoV-2. Potentially cross-reactive organisms were spiked into solution at concentrations of greater than or equal to 10^6 CFU/mL for bacteria and greater than or equal to 10^5 pfu/mL for viruses. The results are summarized in the table below.

Sample Category	Non-Reactive Sample	Spiked Reactive Sample	Cross-Reactivity (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

Sample Category	Non-Reactive Sample	Spiked Reactive Sample	Cross-Reactivity (Y/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
Pooled human nasal wash	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.

Specificity

Substances that do not Interfere

The VITROS SARS-CoV-2 Antigen test was evaluated for interference. Of the compounds tested, none was found to interfere with the clinical interpretation of the test in Non-reactive and weakly Reactive samples at the concentrations indicated.

Interfering Substance	Active Ingredient	Concentration
Human Blood	Blood	4%
Hemoglobin	Hemolysate	1000 mg/dL
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	5.0 µg/mL
Anti-viral Drug 2	Zanamivir	282.0 ng/mL
Anti-bacterial, Systemic	Tobramycin	1.25 mg/mL
Hemeopathic Cold Remedy	Galphimia glauca, Luffa operculata, Sabadilla	5%
Antibacterial	Mupirocin	10 mg/mL

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13. *Performance of Screening for SARS-CoV-2 using Rapid Antigen Tests to Detect Incidence of Symptomatic and Asymptomatic SARS-CoV-2 Infection: findings from the Test Us at Home prospective cohort study* - (https://www.medrxiv.org/content/10.1101/2022.08.05.22278466v1).

Glossary of Symbols

The following symbols may have been used in the labeling of this product.

Do Not Reuse	Upper Limit of Temperature	Range
Use by or Expiration Date (Year-Month-Day)	Lower Limit of Temperature	Range of Means
LOT Batch Code or Lot Number	Temperature Limitation	Midpoint
SN Serial Number	Consult Instructions for Use	Revised
REF Catalog Number or Product Code	Attention: The Instructions for Use (IFU) has been updated	Supersedes
Caution	S1 For use in Slide Supply 1	Contains Sufficient for "n" Tests
Keep Dry (Protect from Moisture/Humidity)	S2 For use in Slide Supply 2	IVD *in vitro* Diagnostic Medical Device
Manufacturer	SI SI Units	Der Grüne Punkt (the Green Dot). Manufacturer follows certain packaging material waste disposal management regulations
Date of Manufacture	CONV Conventional Units	Estimated within-lab SD
EC REP Authorized Representative in the European Community	Value	
Corrosive	Flammable	Serious Health Hazards
Health Hazards	Acute Toxicity	Environmental or Aquatic Toxicity

Revision History

Date of Revision	Version	Description of Technical Changes*
2023-02-28	4.0	Added new Conditions of Authorization aligned to FDA's Policy Change • Updated Intended Use to add serial testing • Updated Warnings and Precautions • Added additional information on Interpretation of Results • Updated Limitations • Updated Limit of Detection Table • Updated Performance Section to add NIH Prospective Clinical Study Data • References: Added reference

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

Signature

Obsolete Date

Conditions of supply: all supplies are made subject to the standard terms and conditions of Ortho Clinical Diagnostics or its distributors. Copies of these are available on request.

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