OSANTYS SARS-CoV-2 DUO RT-qPCR Kit

INSTRUCTIONS FOR USE

Multiplex real-time RT-PCR test intended for the qualitative detection of SARS-CoV-2 RNA from unpurified saliva and nasopharyngeal specimens

REF **OS-COV2-DUO-100** Σ **100**

REF **OS-COV2-DUO-500** Σ **500**

Version 2.0

CE IVD

For *In Vitro* Diagnostic Use

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The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the instrument. The information in this guide is subject to change without notice.

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Revision History

Revision #	Effective Date	Summary of Revisions
1.0	2022, February	Original Instructions for Use
2.0	2022, May	Additional performance details

Contents

Intended use

OSANTYS SARS-CoV-2 DUO RT-qPCR Kit contains reagents and controls for a real-time reverse transcription-polymerase chain reaction (RT-qPCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in saliva and nasopharyngeal specimens from individuals suspected of COVID-19.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA, but do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Laboratories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the OSANTYS SARS-CoV-2 DUO RT-qPCR Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostics procedures.

Note: The following countries require the CE-marked *In Vitro* Diagnostics: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, UK, Norway, Iceland, Liechtenstein, Switzerland, Turkey.

Summary

An outbreak of pneumonia of unknown etiology in Wuhan City, Hubei Province, China was initially reported to WHO on December 31, 2019. Chinese authorities identified a novel coronavirus (SARS-CoV-2), which has resulted in millions of confirmed human infections globally. Cases of asymptomatic infection, mild illness, severe illness, and deaths have been reported.

OSANTYS SARS-CoV-2 DUO RT-qPCR Kit is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis of COVID-19 and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers and dual-labeled double-quenched hydrolysis probes and control material used in RT-qPCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in saliva and nasopharyngeal specimens.

Test principle

The test is designed for specific detection of SARS-CoV-2 RNA using two primer/probe sets targeting two regions of the virus nucleocapsid (N) gene, N1 and N2. An additional primer/probe set to detect the human RNase P gene (RP) is also used to monitor the presence of PCR inhibitors in clinical specimens and evaluate specimens quality and RNA extraction to avoid a false negative result.

Starting material is a clarified (unpurified) lysate obtained after thermal lysis of a saliva or nasopharyngeal specimen.

RNA is first reverse transcribed to cDNA and subsequently amplified in a one-step process using a real-time PCR instrument. In this process, each probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the real-time PCR instrument. The data are analyzed and interpreted by the analysis software used with the instrument.

Product description

The OSANTYS SARS-CoV-2 DUO RT-qPCR Kit contains the following components:

Reagent Name//Part Number	*Description*	*Quantity & Volume*		*Storage*	*Shelf life*[1]
		100 reactions	*500 reactions*		
SARS-CoV-2 Primer/Probe Mix // OS-COV2-100-1	N1, N2 and RP Combined Primer/Probe Mix	750 µL/tube x 1	750 µL/tube x 5	-25°C to -15°C protected from light	12 months
One-Step RT-qPCR Enzyme Master Mix 2X // OS-COV2-DUO-100-2	reagents for RT-PCR including RT and Taq Polymerase	625 µL/tube x 2	625 µL/tube x 10	-25°C to -15°C	12 months
SARS-CoV-2 Positive Controls // OS-COV2-100-3	plasmids containing SARS-CoV-2 N gene and human RP gene	26 µL/tube x 1	26 µL/tube x 5	-25°C to -15°C	12 months
SARS-CoV-2 Negative Control // OS-COV2-100-4	DNase/RNase free water	100 µL/tube x 1	100 µL/tube x 5	-25°C to -15°C	12 months

[1]The shelf life of the kit is determined by the component with the shortest shelf life.

After 5 thaw / freeze cycles of the entire kit, no reduction in RT-qPCR performance was observed.

- SARS-CoV-2 Positive Control and RNase P Positive Control are used to monitor RT-qPCR reaction setup and reagent integrity.
- SARS-CoV-2 Negative Control is used to monitor reagents contamination and reaction setup.

Symbols

	Contains reagents sufficient for <N> reactions
	Use by date
	In vitro diagnostic medical device
	Catalog number
	Batch number
	Material number
	Components
	Contents
	Number
	Temperature limitations
	Manufacturer
	Instructions for use
	Important note

Required materials not supplied

Sample preparation:

- Saline solution to dilute salivary samples if necessary
- Dry bath (ideally, a 94 wells)
- Centrifuge for microtubes with adjustable speed

Reaction preparation:

- Real-time PCR instrument
- Laboratory freezers -25°C to -15°C
- 96-well and 2 ml microcentrifuge tube cold block or ice
- Vortex mixer
- Racks for 1.5 mL microcentrifuge tubes and 96-well plates or strip tubes
- Microcentrifuge for tubes
- Centrifuge for 96-well plates
- Single channel adjustable micropipettes (10 µL, 20 µL, 200 µL and 1000 µL)
- Multichannel adjustable micropipettes (20 µL or 200 µL)
- Sterile aerosol barrier (filtered) pipette tips
- Disposable pipetting reservoirs (VWR catalog n. 613-1179 or equivalent)
- 1.5 mL microcentrifuge DNase/RNase free tubes
- PCR optical reaction plates or tubes:
  - 0.1 or 0.2 ml PCR optical 96-well reaction plate
  - optical adhesive film
  - adhesive film applicator

  or
  - 0.1 or 0.2 ml PCR strip tubes
  - optical 8-cap Strips
- Detergent/disinfectant (Surfa'Safe Premium Anios, or equivalent)
- Reagents for DNA and RNase removal (DNA-ExitusPlus, RNase-ExitusPlus or equivalent)
- Disposable powder-free gloves and surgical gowns

Warnings and precautions

- Handle all specimens and controls as if infectious in accordance with safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.
- Specimen processing should be performed in accordance with national biological safety regulations.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).
- Heat-inactivation of live virus at 56°C for 30 minutes is not necessary due to the thermal lysis step during the samples preparation.
- Use personal protective equipment (PPE) such as (but not limited to) gloves, eye protection, and lab coats when handling specimen and while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Use separate areas for the preparation of patient samples and controls to prevent false positive results. Samples and reagents must be handled under a laminar airflow hood or PCR hood.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Performance characteristics have been determined with human upper respiratory specimens (saliva and nasopharyngeal swabs) from human patients with signs and symptoms of respiratory infection.
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the *In Vitro* Diagnostic Directive 98/79/EC.
- Do not mix or exchange components from different kit batches.
- Do not use the kit past the expiration date.
- Dispose of waste in compliance with the local regulations.
- Safety Data Sheets are available upon request.
- The quality of the sample and sample preparation may influence the quality of the RT-qPCR test. It is highly recommended to process the sample as soon as possible after collection and store it refrigerated (+ 2°C to + 8°C) before processing. Freezing at -20°C can cause significant degradation of viral RNA.
- It is strongly recommended to carry out the preparation of the RT-qPCR reaction immediately after lysis and in any case within one hour after lysis.

- Lysates can be kept at room temperature during reaction preparation. It is recommended to keep saliva samples refrigerated (+2°C - +8°C) before thermal lysis to ensure stability.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
  - Maintain separate areas for assay setup and handling of samples.
  - Change aerosol barrier pipette tips between all manual liquid transfers.
  - During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
  - Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids and unpurified samples.
  - Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
  - Change gloves between samples and whenever contamination is suspected.
  - Keep reagent and reaction tubes capped or covered as much as possible.
  - Reagents can be thawed at room temperature and maintained at room temperature at all times during preparation and use.
  - Work surfaces, pipettes, centrifuges and other equipment should be cleaned and decontaminated with cleaning products such as Surfa'Safe Premium Anios, DNA-ExitusPlus and RNase-ExitusPlus to minimize risk of nucleic acid contamination.
- Dispose of unused kit reagents and human specimens according to local regulations.

Assay limitations

- This assay is intended to be used for *in vitro* diagnostic purposes only. Follow good laboratory practices and all precautions and established guidelines to avoid cross-contamination between samples.
- Performance of the OSANTYS SARS-CoV-2 DUO RT-qPCR Kit has only been established in saliva and nasopharyngeal specimens.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.

- This kit uses unpurified saliva and nasopharyngeal specimens as a sample for the analysis.

The quality of the samples and of the RNA recovered from biological samples is essential for the quality of the results generated with this kit.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.

- **False-negative results** may arise from:
  - Improper sample collection, transport or handling.
  - Degradation of the SARS-CoV-2 RNA during shipping/storage.
  - Specimen collection after SARS-CoV-2 RNA can no longer be found in the specimen matrix.
  - The presence of RT-PCR inhibitors.
  - Extraction methods other than those indicated in the protocol.
  - Inadequate numbers of organisms present in the specimen.
  - Mutation in the SARS-CoV-2 virus.
  - Failure to follow instructions for use.

- **False-positive results** may arise from:
  - Cross contamination during specimen handling or preparation.
  - Cross contamination between patient samples.
  - Specimen mix-up.
  - RNA contamination during product handling.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

Equipment preparation

Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. Decontamination agents should be used to minimize the risk of nucleic acid contamination and degradation.

Preparation of saliva and nasopharyngeal specimen

This kit is used with unpurified saliva and nasopharyngeal specimen subjected to thermal lysis according to the following protocol:

1. Transfer 50 µL of each saliva or nasopharyngeal specimen to 1.5 mL microtubes.*
2. Place the microtubes in a dry bath at 99°C for 5 minutes. *(Do not incubate the samples for more than 10 minutes at high temperature to avoid any risk of degradation)*.

3. Centrifuge microtubes at 4000 rpm for 5 minutes.

Steps 2 and 3 must be carried out just before the preparation of the reaction to avoid any risk of degradation of the lysate at room temperature. In any case, do not wait more than one hour after thermal lysis to prepare the RT-qPCR reaction.
*If necessary, salivary specimen can be tested diluted up to 1/4 in saline solution with no significant difference in viral detection.

This kit can be used with nasopharyngeal and oropharyngeal swabs collected in the following media:

- Saline solution
- ∑-Virocult (MWE)
- ∑-VCM (MWE)
- ∑-Transwab (MWE)
- DeltaSwab liquid amies (Deltalab)
- Fecal Transwab – liquid Cary Blair (MWE)

Guidelines for RT-qPCR

- Run the plate immediately after preparation. Failure to do so could result in degraded RNA samples.
- To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area.
- Do not use the same pipette for reaction mix and RNA samples.
- Always use aerosol barrier pipette tips. Maintain an RNase-free environment.
- Protect Primer/Probe mix from light.
- For each run plate, include one positive control and one negative control.

Assay set up

Reaction Master Mix and plate set up

1) In the reagent set-up dedicate hood, place the SARS-CoV-2 Primer/Probe Mix and the One-Step RT-qPCR Enzyme Master Mix 2X tubes in a rack.

2) Thaw the reagents at room temperature and maintained them at room temperature during preparation.

3) Gently vortex the SARS-CoV-2 Primer/Probe Mix and mix the One-Step RT-qPCR Enzyme Master Mix by inversion 5 times.

4) Centrifuge reagents briefly to collect contents at the bottom of the tube.

5) Determine the number of reactions (N) to set up per assay. Include excess reaction mix for pipetting error. Use the following guide to determine N:

- If number of samples (n) including controls is 1 through 24, then N = n + 1
- If number of samples (n) including controls is 25 through 48, then N = n + 2
- If number of samples (n) including controls is 49 through 72, then N = n + 3
- If number of samples (n) including controls is 73 through 96, then N = n + 4

6) For each run, in a 1.5 mL microcentrifuge tube combine the following components:

Component	Volume/Reaction
One-Step RT-qPCR Enzyme Master Mix 2X	N x 12.5 µL
SARS-CoV-2 Primer/Probe Mix	N x 7.5 µL
Total Volume	**N x 20.0 µL**

7) Mix reaction mixtures by pipetting up and down. ***Do not vortex***.

8) Centrifuge briefly to collect contents at the bottom of the tube.

9) Set up reaction strip tubes or plates in a 96-well rack.

10) Pipette 20 µL of the reaction mix into each well.

11) Prior to moving to the sample handling hood, prepare the No Template Control (NTC) reaction in the reagent set-up hood. Seal the NTC well with a cap, or a part of an adhesive film

12) Pipette 5 µL of SARS-CoV-2 Negative Control into the NTC well.

13) Cover the entire reaction plate and move the reaction plate to the sample handling hood.

Sample addition

1) Carefully pipette 5.0 µL of each lysate supernatant into each well. *Change tips after each addition.*

2) Pipette 5 µL of SARS-CoV-2 Positive Controls into the positive control well.

3) Seal strip tubes with strip caps, or plate with adhesive film.

4) Briefly centrifuge reaction tube strips or plate for 30 seconds.

Set up the real-time PCR instrument

1. Create the following thermal protocol:

   - Step 1/Denaturation:
     95°C, 30 sec
   - Step 2/Reverse Transcription:
     60°C, 5 min
   - Step 3/PCR reaction:
     Denaturation: 95°C, 5 sec
     Anneal/Extension: 63.1°C, 30 sec (+ plate read)
     45 cycles

2. Select reporter dyes:

Target	Reporter Dye/Channel
N1	FAM/blue
N2	VIC/green
RP	Cy5/red

3. Indicate 25 µl as reaction volume per well.
4. Load the strip tubes or plate into the thermocycler and start the run.
5. After the instrument run is complete, analyze the data.

This kit has been validated for use with:

- CFX96™ Dx System (Bio-Rad) and CFX Dx Manager software version 3.1 (optical plates and tubes with white wells).
- CFX Opus 96 Dx System (Bio-Rad) and CFX Maestro Dx SE Software 2.0 (optical plates and tubes with white wells).
- Any other Real-Time PCR system with channels for FAM, VIC and Cy5 fluorescence reading and not requiring ROX or other reference dye calibration, can be used with this kit.

Data analysis

Refer to your instrument manual for generating the amplification plot and Ct values for each sample, and define baseline threshold and setting.

Result interpretation

Expected performance of Controls included in the OSANTYS SARS-CoV-2 DUO RT-qPCR Kit

Control Type	Used to Monitor	N1	N2	RP	Expected Ct Values
Positive	Substantial reagent failure including primer and probe integrity	+	+	+	< 38
Negative	Reagent and/or environmental contamination	-	-	-	< 38
Endogenous	Failure in lysis and extraction procedure, potential contamination during extraction	-	-	+	< 38

If any of the above controls do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction may have occurred. Invalidate the run and re-test.

Results interpretation for patient samples

N1	N2	RP	Result interpretation [a]	Report	Actions
Ct < 38	Ct < 38	±	SARS-CoV-2 detected	Positive for SARS-CoV-2	Report results to local health authority and sender
If only one of the two targets is positive		±	SARS-CoV-2 detected if Ct < 34	Positive for SARS-CoV-2	Report results to local health authority and sender
			Inconclusive if Ct > 34	Inconclusive	
Ct > 38	Ct > 38	Ct < 38	SARS-CoV-2 not detected	Not Detected	Report results to sender. Consider testing for other respiratory viruses [b]
ND	ND	ND	Invalid Result	Invalid	Repeat thermal lysis and RT-qPCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

ND = not detected

[a] Laboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system.

[b] Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus. The possibility of a false negative result should especially be considered if the patient's recent exposures or clinical presentation suggest that SARS-CoV-2 infection is possible, and diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. If SARS-CoV-2 infection is still suspected, re-testing should be considered in consultation with public health authorities.

If a laboratory obtains unexpected results for assay controls, or if inconclusive or invalid results are obtained and cannot be resolved through the recommended re-testing, please contact Osantys for consultation and possible specimen referral.

RNase P (Endogenous Control)

- All clinical samples should exhibit fluorescence signal for the RP < 38 Ct, thus indicating the presence of the human RNase P gene. Failure to detect RNase P in any clinical specimens may indicate:

- Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation.
- Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.
- Improper assay set up and execution.
- Reagent or equipment malfunction.

- If the RP assay does not produce a positive result for clinical samples, interpret as follows:

- If the SARS-CoV-2 N1 and N2 are positive even in the absence of a positive RP, the result should be considered valid.

It is possible that some samples may fail to exhibit RP growth curves due to low cell numbers in the original clinical sample, or reagent competition. A negative RP signal does not preclude the presence of SARS-CoV-2 virus RNA in a clinical sample.

- If N1, N2 *AND* RP are negative for the specimen, the result should be considered invalid for the specimen.

If residual specimen is available, repeat the lysis procedure and repeat the test.
If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

N1 and N2 SARS-CoV-2 Markers

- When all controls exhibit the expected performance, a specimen is considered ***negative*** if both N1 AND N2 signals are not detected or > 38 Ct AND the RP signal is < 38 Ct.
- When all controls exhibit the expected performance, a specimen is considered ***positive*** for SARS-CoV-2 if both N1 AND N2 signals are < 38 Ct. The RP may or may not be positive as described above, but the result is still valid.
- When all controls exhibit the expected performance, and only one of the SARS-CoV-2 markers (N1 OR N2 but not both markers) shows a signal < 38 Ct, interpret as follows:
  - If N1 or N2 Ct is < 34, a specimen is considered ***positive***. A mutation affecting the other probe could have impacted the detection of the signal.
  - If N1 or N2 Ct is > 34, the result is ***inconclusive***. The sample should be retested. If the same result is obtained, report the inconclusive result and proceed with a new specimen collection.
- When all controls exhibit the expected performance, and Ct for N1, N2 AND the RP markers are not detected, the result is ***invalid***. The sample should be retested. If the re-tested sample is negative for all markers, the result is invalid and collection of a new specimen from the patient should be considered.

Quality Control

- Quality control requirements must be performed in conformance with local regulations or accreditation requirements and the user's laboratory's standard quality control procedures. For further guidance on appropriate quality control practices, refer to your governmental laboratory accreditation system.
- Quality control procedures are intended to monitor reagent and assay performance.
- Always include a negative template control (NTC) and the appropriate SARS-CoV-2 positive control in each amplification run.
- All clinical samples should be tested for human RP gene to control for specimen quality and extraction.

Performance Characteristics

The analytical and clinical performance of the OSANTYS SARS-CoV-2 DUO RT-qPCR Kit was evaluated by comparing performance according to the extraction method, determining limit of detection (LoD), characterizing specificity, cross-reactivity and interfering substances, as described in the following sections.

Analytical performance

Kit performance evaluation for saliva specimen

To evaluate kit performance, saliva specimen previously tested positive or negative with the Osantys SARS-CoV-2 RT-qPCR Kit, were tested upon thermal lysis.

- 59 positive saliva specimens
- 46 negative saliva specimens

Table 1a. Kit performance evaluation for saliva specimen

Results	Osantys SARS-CoV-2 DUO RT-qPCR Kit	Osantys SARS-CoV-2 RT-qPCR Kit
Positive/Expected Positive	58*/59	59/59
Negative/Expected Negative	46/46	45/46
Positive*/Expected Negative	**0/46**	**1/46**

*Reference method: N1 = 40.37, N2 = 35

Analytical sensitivity of the Osantys SARS-CoV-2 DUO RT-qPCR test Kit on saliva samples: 98.3%.

Analytical specificity of the Osantys SARS-CoV-2 DUO RT-qPCR test Kit on saliva samples: 100%.

Kit performance evaluation for nasopharyngeal specimen

To evaluate kit performance, unpurified nasopharyngeal specimen previously tested positive or negative with the Osantys SARS-CoV-2 RT-qPCR Kit, were tested upon thermal lysis.

- 157 positive nasopharyngeal specimens
- 35 negative nasopharyngeal specimens

Table 2a. Kit performance evaluation for nasopharyngeal specimen

Results	Osantys SARS-CoV-2 DUO RT-qPCR Kit	Osantys SARS-CoV-2 RT-qPCR Kit
Positive/Expected Positive	136*/152	152/152
Negative/Expected Negative	35/35	35/35
Positive/Expected Negative	0/35	0/35

*All samples tested negative with the OSANTYS SARS-CoV-2 DUO kit showed a Ct close to the detection limit with the reference method.

Analytical sensitivity of the Osantys SARS-CoV-2 DUO RT-qPCR test Kit on NP samples: 89.5%.

Analytical specificity of the Osantys SARS-CoV-2 DUO RT-qPCR test Kit on NP samples: 100%.

A variable ΔCt is observed between the 2 kits, regardless of viral load. The 2 kits have 2 different target type: the SARS-CoV-2 RT-qPCR kit targets free viral RNA, whereas the SARS-CoV-2 DUO RT-qPCR kit targets the extra- and intra-cellular virus. The relative quantity of the 2 targets may be different in each sample, as shown in Table 2b and 2c.

Table 2b. Evaluation of free RNA, extra- and intracellular virus in NP samples (expressed as Ct values)

NP	N1			N2			RP		
Sample	Extracellular Virus	Intracellular Virus	Free Extracellular RNA	Extracellular Virus	Intracellular Virus	Free Extracellular RNA	Extracellular Virus	Intracellular Virus	Free Extracellular RNA
3048	19.05	21.97	16.59	30.72	35.18	25.32	31.10	33.56	30.84
3028	23.62	25.08	21.18	35.16	35.68	29.13	32.00	31.97	31.09
3013	23.75	19.90	19.32	34.80	33.15	25.07	25.87	27.10	26.18
71	21.61	25.81	22.42	23.13	27.22	22.74	29.64	30.66	30.21
13A	27.9	31.09	27.89	28.91	31.2	27.8	28.9	30.77	28.7
15A	20.02	22.94	18.54	21.67	24.36	18.72	30.64	30.23	29.42
10A	28.89	28.23	23.79	30.07	29.6	23.89	30.69	32.41	29.51
5WT	32.81	31.35	28.8	33.91	32.93	28.67	31.58	32.8	29.89
9WT	39.51	33.75	32.7	35.02	34.13	32.02	29.04	31.31	28.65
4B	33.73	27.49	23.78	32.77	28.3	23.62	28.5	30.39	27.09

Table 2c. Evaluation of free RNA, extra- and intracellular virus in saliva samples (expressed as Ct values)

Saliva	N1		N2		RP	
Sample	Extracellular Virus	Intracellular Virus	Extracellular Virus	Intracellular Virus	Extracellular Virus	Intracellular Virus
16P	20.84	21.44	22.86	23.04	25.83	28.28
7P	33.16	34.60	32.01	33.01	28.37	30.35
40P	28.94	31.12	28.93	31.31	28.27	29.99
6P	27.56	28.15	28.92	29.43	28.68	30.69
33P	28.19	29.75	28.57	29.73	29.29	28.9
25P	28.78	30.8	28.07	29.95	24.28	26.55
8P	24.72	26.35	25.87	27.3	26.77	28.91
14P	26.92	26.4	27.77	27.29	26.73	28.8
11P	31.5	32.09	30.91	31.56	29.06	29.94
15P	32.29	30.1	31.61	30.33	29.28	29.32

Evaluation of the result correlation between saliva and nasopharyngeal swabs

19 patients were subjected to simultaneous saliva and nasopharyngeal sampling.

For saliva, 11 samples were positive and 8 negative for SARS-CoV-2, whereas for nasopharyngeal swabs 8 samples were positive and 11 negative for SARS-CoV-2.

Taking any positive to either of these tests as true positive, the clinical sensitivity of the RT-qPCR test on saliva sample compared to NP sample is 100%. The 95% confidence interval is (0.83 - 1.0).

Limit of detection (LoD)

The LoD study established the lowest SARS-CoV-2 concentration that can be detected by the OSANTYS SARS-CoV-2 DUO RT-qPCR Kit in a particular specimen type at least 95% of time. The LoD was determined by limiting dilution studies.

1. 10 saliva and 10 nasopharyngeal positive specimen were 10-fold serially diluted in a pool of negative saliva or nasopharyngeal matrices and then processed using the kit workflow. 8 replicates were realized for each dilution. Quantified SARS-CoV-2 positive control was used to determine viral copy numbers. The LoD is indicated in the yellow case.

Table 3a. LoD determinations in ten saliva specimen

Targets	N1					N2					
RNA Concentration[1]	10^3	10^2	10^1	10^0	10^{-1}	10^3	10^2	10^1	10^0	10^{-1}	2P
Positives/Total	8/8	8/8	8/8	8/8	2/8	8/8	8/8	8/8	3/8	0/8	
Mean Ct[2]	28.68	31.00	32.43	33.81	34.22	28.74	30.69	33.60	34.58	ND	
Standard Deviation (Ct)	0.273	0.287	0.255	0.781	0.883	0.209	0.530	0.946	0.256	-	

Targets	N1					N2					
RNA Concentration[1]	10^3	10^2	10^1	10^0	10^{-1}	10^3	10^2	10^1	10^0	10^{-1}	14P
Positives/Total	8/8	8/8	8/8	5/8	2/8	8/8	8/8	1/8	0/8	0/8	
Mean Ct[2]	29.90	31.69	33.21	34.34	34.99	29.86	31.66	34.72	ND	ND	
Standard Deviation (Ct)	0.385	0.309	0.445	0.582	0.019	0.303	0.857	-	-	-	

Targets	N1					N2					
RNA Concentration[1]	10^3	10^2	10^1	10^0	10^{-1}	10^3	10^2	10^1	10^0	10^{-1}	32P
Positives/Total	8/8	8/8	8/8	2/8	0/8	8/8	8/8	5/8	1/8	0/8	
Mean Ct[2]	29.91	31.98	34.27	36.24	ND	29.98	32.18	34.72	35.27	ND	
Standard Deviation (Ct)	0.116	0.269	0.346	0.066	-	0.084	1.254	0.434	-	-	

Targets	N1				N2				
RNA Concentration[1]	10^2	10^1	10^0	10^{-1}	10^2	10^1	10^0	10^{-1}	25P
Positives/Total	8/8	8/8	3/8	0/8	8/8	6/8	0/8	0/8	
Mean Ct[2]	32.07	33.63	35.30	ND	31.76	33.67	ND	ND	
Standard Deviation (Ct)	0.174	0.491	0.658	-	1.205	1.178	-	-	

Targets	N1					N2					
RNA Concentration[1]	10^3	10^2	**10^1**	10^0	10^{-1}	10^3	10^2	**10^1**	10^0	10^{-1}	6P
Positives/Total	8/8	8/8	**7/8**	6/8	0/8	8/8	8/8	**7/8**	1/8	0/8	
Mean Ct[2]	30.59	31.77	**33.78**	34.67	ND	30.43	32.15	**34.83**	35.23	ND	
Standard Deviation (Ct)	0.107	0.235	**0.358**	0.192	-	0.221	1.370	**0.377**	-	-	

Targets	N1					N2					
RNA Concentration[1]	10^3	10^2	10^1	10^0	10^0	10^3	10^2	10^1	10^0	10^{-1}	15P
Positives/Total	8/8	8/8	8/8	1/8	2/8	8/8	8/8	2/8	0/8	0/8	
Mean Ct[2]	30.36	32.40	34.44	34.94	35.08	29.96	32.57	35.09	ND	ND	
Standard Deviation (Ct)	0.246	0.400	0.270	-	0.007	0.136	1.047	0.078	-	-	

Targets	N1					N2					
RNA Concentration[1]	10^3	10^2	10^1	10^0	10^{-1}	10^3	10^2	10^1	10^0	10^{-1}	26P
Positives/Total	8/8	8/8	8/8	8/8	3/8	8/8	8/8	3/8	0/8	0/8	
Mean Ct[2]	28.32	31.10	33.17	34.35	35.07	28.89	31.14	34.26	ND	ND	
Standard Deviation (Ct)	0.296	0.877	0.455	0.485	-	0.189	1.408	0.358	-	-	

Targets	N1					N2					
RNA Concentration[1]	10^3	10^2	10^1	**10^0**	10^{-1}	10^3	10^2	**10^1**	10^0	10^{-1}	16P
Positives/Total	8/8	8/8	8/8	**5/8**	0/8	8/8	8/8	**4/8**	0/8	0/8	
Mean Ct[2]	30.35	32.74	34.80	**35.31**	ND	30.63	32.88	**35.88**	ND	ND	
Standard Deviation (Ct)	0.226	0.207	0.556	**0.662**	-	0.104	0.879	**1.166**	-	-	

Targets	N1					N2					
RNA Concentration[1]	10^3	10^2	**10^1**	10^0	10^{-1}	10^3	10^2	**10^1**	10^0	10^{-1}	8P
Positives/Total	8/8	8/8	**7/8**	0/8	0/8	8/8	8/8	**4/8**	0/8	0/8	
Mean Ct[2]	29,31	32,46	**34,00**	ND	ND	28,80	32,31	**34,41**	ND	ND	
Standard Deviation (Ct)	0,183	0,230	**0,378**	-	-	0,083	0,824	**0,695**	-	-	

Targets	N1					N2					
RNA Concentration[1]	10^3	10^2	10^1	10^0	10^{-1}	10^3	10^2	10^1	10^0	10^{-1}	
Positives/Total	8/8	8/8	8/8	2/8	0/8	8/8	8/8	6/8	4/8	0/8	34P
Mean Ct[2]	30.39	32.65	35.02	36.25	ND	30.58	32.03	35.02	36.65	ND	
Standard Deviation (Ct)	0.224	0.242	0.870	0.683	-	0.230	0.224	0.870	0.979	-	

1. Concentration is presented in RNA copies/reaction
2. Mean Ct reported for dilutions that are ≥ 95% positive. Calculations only include positive results. ND = not detected

Table 3b. LoD determinations in ten nasopharyngeal specimen

Targets	N1				N2				
RNA Concentration[1]	10^3	10^2	10^1	10^0	10^3	10^2	10^1	10^0	
Positives/Total	8/8	8/8	8/8	2/8	8/8	8/8	2/8	0/8	10WT
Mean Ct[2]	31.13	34.05	36.50	41.94	31.85	34.39	35.57	ND	
Standard Deviation (Ct)	0.196	0.293	0.474	2.837	0.238	0.586	0.069	-	

Targets	N1					N2					
RNA Concentration[1]	10^3	10^2	10^1	10^0	10^{-1}	10^3	10^2	10^1	10^0	10^{-1}	
Positives/Total	8/8	8/8	8/8	6/8	0/8	8/8	8/8	8/8	0/8	0/8	32
Mean Ct[2]	30.07	31.95	34.33	36.33	ND	31.64	32.91	34.78	ND	ND	
Standard Deviation (Ct)	0.115	0.294	0.607	0.712	-	0.134	0.430	0.313	-	-	

Targets	N1					N2					
RNA Concentration[1]	10^3	10^2	10^1	10^0	10^{-1}	10^3	10^2	10^1	10^0	10^{-1}	
Positives/Total	8/8	8/8	8/8	1/8	0/8	8/8	8/8	7/8	0/8	0/8	173
Mean Ct[2]	30.66	32.60	34.98	35.76	ND	32.19	33.48	37.20	ND	ND	
Standard Deviation (Ct)	0.132	0.320	0.449	-	-	0.310	0.328	3.193	-	-	

Targets	N1					N2					
RNA Concentration[1]	10^3	10^2	10^1	10^0	10^{-1}	10^3	10^2	10^1	10^0	10^{-1}	150
Positives/Total	8/8	8/8	8/8	0/8	0/8	8/8	8/8	4/8	0/8	0/8	
Mean Ct[2]	30.37	32.41	34.41	ND	ND	31.48	33.06	35.01	ND	ND	
Standard Deviation (Ct)	0.404	0.383	0.660	-	-	0.461	0.608	1.275	-	-	

Targets	N1					N2					
RNA Concentration[1]	10^3	10^2	10^1	10^0	10^{-1}	10^3	10^2	10^1	10^0	10^{-1}	37
Positives/Total	8/8	8/8	8/8	3/8	0/8	8/8	8/8	6/8	3/8	0/8	
Mean Ct[2]	30.20	32.58	35.34	36.44	ND	31.90	34.10	35.87	35.89	ND	
Standard Deviation (Ct)	0.104	0.286	0.536	0.709	-	0.199	0.454	0.518	0.887	-	

Targets	N1					N2					
RNA Concentration[1]	10^3	10^2	10^1	10^0	10^{-1}	10^3	10^2	10^1	10^0	10^{-1}	10A
Positives/Total	8/8	8/8	8/8	3/8	0/8	8/8	8/8	6/8	1/8	1/8	
Mean Ct[2]	29.87	32.76	34.77	36.28	ND	30.94	33.35	34.84	34.31	35.48	
Standard Deviation (Ct)	0.122	0.144	0.787	0.448	-	0.122	0.404	0.689	-	-	

Targets	N1				N2				
RNA Concentration[1]	10^3	10^2	10^1	10^0	10^3	10^2	10^1	10^0	4106
Positives/Total	8/8	8/8	5/8	1/8	8/8	8/8	1/8	2/8	
Mean Ct[2]	32.25	33.74	35.87	36.87	33.43	34.45	35.13	35.28	
Standard Deviation (Ct)	0.319	0.432	0.680	-	0.465	0.435	-	0.629	

Targets	N1					N2					
RNA Concentration[1]	10^3	10^2	10^1	10^0	10^0	10^3	10^2	10^1	10^0	10^{-1}	141
Positives/Total	8/8	8/8	5/8	0/8	0/8	8/8	7/8	1/8	0/8	0/8	
Mean Ct[2]	30.87	33.79	35.55	ND	ND	31.87	33.78	36.97	ND	ND	
Standard Deviation (Ct)	0.264	0.250	1.281	-	-	0.221	0.431	-	-	-	

Targets	N1				N2				
RNA Concentration[1]	10^3	10^2	**10^1**	10^0	10^3	**10^2**	10^1	10^0	2A
Positives/Total	8/8	8/8	**6/8**	0/8	8/8	**7/8**	3/8	0/8	
Mean Ct[2]	31.11	34.14	**36.71**	ND	31.69	**34.00**	35.90	ND	
Standard Deviation (Ct)	0.305	0.595	**0.653**	-	0.248	**0.419**	0.415	-	

Targets	N1				N2				
RNA Concentration[1]	10^3	10^2	**10^1**	10^0	10^3	**10^2**	10^1	10^0	4WT
Positives/Total	8/8	8/8	**6/8**	0/8	8/8	**6/8**	2/8	0/8	
Mean Ct[2]	31.53	34.67	**37.05**	ND	32.32	**34.78**	35.68	ND	
Standard Deviation (Ct)	0.269	0.300	**1.024**	-	0.303	**0.530**	0.155	-	

1. Concentration is presented in RNA copies/reaction
2. Mean Ct reported for dilutions that are ≥ 95% positive. Calculations only include positive results. ND = not detected

2. SARS-CoV-2 Virus was 10-fold serially diluted in a pool of negative saliva or nasopharyngeal matrices and then processed using the kit workflow. 8 replicates were realized for each dilution. Quantified SARS-CoV-2 positive control was used to determine viral copy numbers. The LoD is indicated in the yellow case.

Table 4. LoD determination in SARS-CoV-2 Virus in a pool of negative saliva and nasopharyngeal matrices

Saliva	N1						N2					
Virus copy number/ml of *saliva* (copy number/reaction)	200.000 (1000)	100.000 (500)	20.000 (100)	**10.000 (50)**	2.000 (10)	200 (1)	200.000 (1000)	100.000 (500)	20.000 (100)	**10.000 (50)**	2.000 (10)	200 (1)
Positives/Total	8/8	8/8	8/8	**7/8**	3/8	1/8	8/8	8/8	8/8	**6/8**	4/8	1/8
Mean Ct[2]	27.63	28.86	33.27	**33.57**	35.00	36.02	27.37	28.61	32.88	**34.78**	36.07	36.12
Standard Deviation (Ct)	0.032	0.101	0.791	**0.681**	0.671	-	0.105	0.081	1.432	**0.742**	0.522	-

NP	N1						N2					
Virus copy number/ml of *saliva* (copy number/reaction)	200.000 (1000)	100.000 (500)	20.000 (100)	10.000 (50)	2.000 (10)	200 (1)	200.000 (1000)	100.000 (500)	20.000 (100)	10.000 (50)	**2.000 (10)**	200 (1)
Positives/Total	8/8	8/8	8/8	8/8	8/8	3/8	8/8	8/8	8/8	8/8	**4/8**	0/8
Mean Ct[2]	28.04	29.09	32.67	34.04	36.78	37.15	28.27	29.27	32.67	33.91	**35.45**	ND
Standard Deviation (Ct)	0.262	0.143	0.206	0.380	0.973	1.695	0.277	0.158	0.151	0.207	**1.098**	-

1. Concentration is presented in RNA copies/reaction
2. Mean Ct reported for dilutions that are ≥ 95% positive. Calculations only include positive results. ND = not detected

3. SARS-CoV-2 Positive Control and SARS-CoV-2 Virus were 10-fold serially diluted in a pool of negative saliva or nasopharyngeal matrices and then processed using the kit workflow. 2 replicates were realized for each dilution. Quantified SARS-CoV-2 positive control was used to determine viral copy numbers. The LoD is indicated in the yellow case.

Table 5. LoD determination for SARS-CoV-2 positive control and SARS-CoV-2 Virus in a pool of negative saliva and nasopharyngeal matrices

Saliva	RNA Concentration[1]	N1		N2		RP	
		Mean Ct[2]	Ct Std. Dev.	Mean Ct[2]	Ct Std. Dev.	Mean Ct[2]	Ct Std. Dev.
SARS-CoV-2 positive control	10^5	23,28	0,998	23,13	0,962	28,31	1,064
	10^4	27,35	0,425	26,70	0,240	29,00	0,339
	10^3	29,75	0,066	28,80	0,022	28,90	0,010
	10^2	31,53	1,788	29,93	1,384	27,73	2,422
	10^1	34,22	0,551	33,66	1,974	29,02	0,076
	10^0	ND	-	35,15	-	29,21	0,393
	10^{-1}	ND	-	ND	-	29,42	0,095
SARS-CoV-2 Virus	10^3	27,74	0,129	27,42	0,182	28,15	0,086
	10^2	32,75	0,039	32,23	0,314	28,40	0,375
	10^1	35,26	-	ND	-	29,13	0,010
	10^0	35,52	-	ND	-	28,29	0,101
	10^{-1}	ND	-	ND	-	29,45	0,140

NP	RNA Concentration[1]	N1		N2		RP	
		Mean Ct[2]	Ct Std. Dev.	Mean Ct[2]	Ct Std. Dev.	Mean Ct[2]	Ct Std. Dev.
SARS-CoV-2 positive control	10^5	24.21	0.147	24.44	0.105	29.34	0.198
	10^4	27.17	0.110	27.23	0.008	29.52	0.007
	10^3	30.45	0.066	30.06	0.153	29.88	0.032
	10^2	33.08	0.002	32.29	0.050	29.78	0.087
	10^1	34.72	-	33.54	-	29.79	0.026
	10^0	ND	-	ND	-	29.87	0.074
	10^{-1}	ND	-	ND	-	29.86	0.116
SARS-CoV-2 Virus	10^3	27.92	0.210	28.33	0.189	29.67	0.025
	10^2	32.55	0.123	32.79	0.139	29.75	0.079
	10^1	35.28	0.423	35.43	-	29.72	0.028
	10^0	36.21	-	ND	-	29.79	0.055
	10^{-1}	ND	-	ND	-	29.85	0.205

1. Concentration is presented in RNA copies/reaction
2. Mean Ct reported for dilutions that are ≥ 95% positive. Calculations only include positive results. ND = not detected

Conclusion**:** LoD is between 1 and 100 copies/reaction (0.2 and 20 copies/µL) depending on the tested sample. This variability is justified by the use of a lysate (unpurified sample) as starting material.

From the studies reported above, analytical sensitivity is as follows:

- 2 copies/µL for NP specimen
- 2 copies/µL for saliva specimen

Reactivity (Inclusivity)

In silico analysis of primer and probe sequences

The oligonucleotide primer and probe sequences of the OSANTYS SARS-CoV-2 DUO RT-qPCR Kit were evaluated against 31,623 sequences available in the Global Initiative on Sharing All Influenza Data (GISAID, https://www.gisaid.org) database as of June 20, 2020, to demonstrate the predicted inclusivity of the OSANTYS SARS-CoV-2 DUO RT-qPCR Kit. Nucleotide mismatches in the primer/probe regions with frequencies > 0.1% are shown below. With the exception of one nucleotide mismatch with frequency > 1% (2.00%) at the third position of the N1 probe, the frequency of all mismatches was < 1%, indicating that prevalence of the mismatches were sporadic. Only one sequence (0.0032%) had two nucleotide mismatches in the N1 probe, and one other sequence from a different isolate (0.0032%) had two nucleotide mismatches in the N1 reverse primer. No sequences were found to have more than one mismatch in any N2 primer/probe region. The risk of these mismatches resulting in a significant loss in reactivity causing a false negative result is extremely low due to the design

of the primers and probes, with melting temperatures > 65°C and with annealing temperature at 63.1°C that can tolerate up to two mismatches.

Table 6. *In Silico* Inclusivity Analysis of OSANTYS SARS-CoV-2 DUO RT-qPCR Panel among 31,623 genome sequences available from GISAID as of June 20, 2020

Primer/Probe	N1 probe	N1 reverse		N2 probe
Location (5'>3')	3	15	21	13
Mismatch Nucleotide	C>T	G>T	T>C	C>T
Mismatch No.	632	34	71	46
Mismatch Frequency (%)	2.00	0.11	0.22	0.15

In silico and in vitro analysis of SARS-CoV-2 variants

Sequence analysis of the N gene from B1.1.7 (Alpha, UK), B.1.351 (Beta, South Africa), P.1 (Gamma, Brazil), B.1.617.2 (Delta, India) and B.1.427/ B.1.429 (Epsilon, California) variants revealed that N1 and N2 primer/probe sets are not in the regions affected by mutations, thus they can be detected by this assay, excluding any false negative result.

Sequence analysis of the N gene from the Omicron variant (B1.1.529, South Africa) revealed that the set of primers and N2 probe is not in the regions affected by mutations. The mutation present on the 3rd base in 5' of the N1 probe does not impact the detection of the N1 target, as shown by Yanxia Bei *et al*. (5). Thus, the Omicron variant (B1.1.529) can be detected by this assay, excluding any false negative result.

In addition to the *in silico* analysis, saliva and nasopharyngeal specimen positive for validated Delta and Omicron variants were tested with this assay together with NP specimen positive for validated Beta and Gamma variants. The data demonstrate that the expected results were obtained.

Table 7. Detection of SARS CoV-2 variants

Target	N1			N2		
Variant	Beta & Gamma	Delta	Omicron	Beta & Gamma	Delta	Omicron
Positive/Total	10/10	59/59 S 19/19 NP	2/2 S 15/15 NP	10/10	59/59 S 19/19 NP	2/2 S 15/15 NP

Specificity/Exclusivity testing: in silico analysis

BLASTn analysis queries of OSANTYS SARS-CoV-2 DUO RT-qPCR Kit primers and probes were performed against public domain nucleotide sequences. The database search parameters were as follows:
1) The nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100Mb.
2) The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry.
3) Database was updated on 10/03/2019.
4) The search parameters automatically adjust for short input sequences and the expect threshold is 1000.
5) The match and mismatch scores are 1 and -3, respectively.
6) The penalty to create and extend a gap in an alignment is 5 and 2 respectively.

SARS-CoV-2 N1 Assay

N1 Probe sequence showed high sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. However, N1 forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Combining primers and probe, there is no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive RT-qPCR results.

SARS-CoV-2 N2 Assay

The N2 forward primer sequence showed high sequence homology to Bat SARS-like coronaviruses. The N2 reverse primer and N2 probe sequences showed no significant homology with human genome, other coronaviruses or human microflora. Combining primers and probe, there is no prediction of potential false positive RT-qPCR results.

In summary, the N1 and N2 primer/probe sets designed for the specific detection of SARS-CoV-2, showed no significant combined homologies with human genome, other coronaviruses, or human microflora that would predict potential false positive RT-qPCR results.

In addition to the *in silico* analysis, several organisms were extracted and tested in triplicates with the OSANTYS SARS-CoV-2 DUO-qPCR Kit to demonstrate analytical specificity and exclusivity. The study was performed using nucleic acids extracted upon thermal lysis. The data demonstrate that expected results are obtained

Table 8. Specificity/Exclusivity of the OSANTYS SARS-CoV-2 DUO RT-qPCR Kit

Pathogen	Source	N1	N2
Human coronavirus 229E	Viral culture	ND	ND
Human coronavirus OC43	Viral culture	ND	ND
Human coronavirus NL63	Viral culture	ND	ND
MERS-coronavirus	Purified virus*	ND	ND
SARS-coronavirus	Purified virus*	ND	ND
Influenza A (H1N1)	Purified virus*	ND	ND

*combined with either a saliva or NP negative matrix to mimic the biological sample

Endogenous interference substances studies

To evaluate interfering substances in saliva and nasopharyngeal specimens, pooled SARS-CoV-2 negative saliva and nasopharyngeal specimens were spiked with purified and quantified SARS-CoV-2 Virus at 100.000 copies/mL and potential interfering substances at the concentration reported in Table 9. Each substance was tested in triplicate. No false negative results were observed for any of the substances at the concentrations tested as illustrated in Table 9.

Table 9 . Analysis of endogenous interference substances in saliva and nasopharyngeal specimens

Interfering substance	Final concentration in sample	Agreement with expected results			
		Positive saliva specimen	Negative saliva specimen	Positive NP specimen	Negative NP specimen
None	N/A	3/3	3/3	3/3	3/3
Human blood	1% v/v	3/3	3/3	3/3	3/3
Nasal corticosteroid Dymista™	5 µg/mL	3/3	3/3	3/3	3/3
Oral anesthetic and analgesic for sore throat — Xylocaine 5%	1% w/v	3/3	3/3	3/3	3/3
Oseltamivir phosphate – Tamiflu	33 µg/mL	3/3	3/3	3/3	3/3
Antibiotic, nasal ointment — Mupirocin 2%	5 µg/mL	3/3	3/3	3/3	3/3
Antibacterial, systemic — Tobramycin – Nebcin	0.6 mg/mL	3/3	3/3	3/3	3/3
Saline (0.9%)	10% v/v	3/3	3/3	3/3	3/3

Clinical performance

A clinical evaluation study was performed to evaluate the performance of the OSANTYS SARS-CoV-2 DUO RT-qPCR Kit using saliva and nasopharyngeal specimen previously tested positive or negative.

- *60 positive saliva specimens* from purified RNA method using the Bosphore SARS-CoV-2/Flu/RSV Panel Kit (Anatolia geneworks)
- *76 positive nasopharyngeal specimens* from direct RT-qPCR using the OSANTYS-SARS-CoV-2 RT-qPCR Kit
- *33 positive nasopharyngeal specimens* from purified RNA method using the Bosphore SARS-CoV-2/Flu/RSV Panel Kit (Anatolia geneworks)
- *42 positive nasopharyngeal specimens* from direct lysis using the Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit PCR-Fluorescence Probing (Sansure)

- *32 negative saliva specimens* from purified RNA method using the Bosphore SARS-CoV-2/Flu/RSV Panel Kit (Anatolia geneworks)
- *14 negative NP saliva specimens from direct lysis* using the Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit PCR-Fluorescence Probing (Sansure)
- *11 negative nasopharyngeal specimens* from direct RT-qPCR using the OSANTYS-SARS-CoV-2 RT-qPCR Kit
- *10 negative nasopharyngeal specimens* from purified RNA method using the Bosphore SARS-CoV-2/Flu/RSV Panel Kit (Anatolia geneworks)

Table 10a. Saliva clinical evaluation study

Results	
Positive/Total	59*/60
Negative/Total	46/46
Inconclusive/Total	0/106
Invalid/Total	0/106

*Reference method N1 Ct =35.58, N2 Ct =34.26 for the non-detected sample

Table 10b. Nasopharyngeal swabs clinical evaluation study

Results	
Positive/Total	135**/151
Negative/Total	21/21
Inconclusive/Total	0/172
Invalid/Total	0/172

**All samples tested negative with the OSANTYS SARS-CoV-2 DUO kit showed a Ct close to the detection limit with the reference method.

Conclusion:

The clinical specificity of the OSANTYS SARS-CoV-2 DUO RT-qPCR KIT test on NP and salivary samples is 100%.

The clinical sensitivity of the OSANTYS SARS-CoV-2 DUO RT-qPCR KIT test on salivary samples is 98.3%.

The clinical sensitivity of the OSANTYS SARS-CoV-2 DUO RT-qPCR KIT test on NP sample is 89.4%.

References

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Contact Information, Ordering, Customer and Product Support

For additional documentation and information about this kit, visit: www.osantys.com
For technical and product support, send an email to: contact@osantys.com
For orders, send an email to: orders@osantys.com

Protocol At-A-Glance

1. Transfer 50 µL of each saliva or nasopharyngeal specimen to 1.5 mL microtubes
2. Place the microtubes in a dry bath at 99°C for 5 minutes
3. Centrifuge microtubes at 4000 rpm for 5 minutes
4. Thaw reagents at room temperature
5. Mix and spin the tubes briefly before use
6. Set up reaction plate or tubes as follows:

Component	Volume/Reaction
One-Step RT-qPCR Enzyme Master Mix 2X	N x 12.5 µL
SARS-CoV-2 Primer/Probe Mix	N x 7.5 µL
Total Volume	**N x 20.0 µL**

7. Add 5 µL of each lysate supernatant, 5 µL of negative control and 5 µL of positive controls
8. Spin briefly
9. Create the following thermal protocol:
   - Step 1: 95°C, 30 sec
   - Step 2: 60°C, 5 min
   - Step 3: 95°C, 5 sec
     63.1°C, 30 sec (+ plate read) } 45 cycles
10. Select reporter dyes:

Target	Reporter Dye
N1	FAM/blue
N2	VIC/green
RP	Cy5/red

11. Indicate 25 µl as reaction volume per well
12. Load the strip tubes or plate and start the run
13. After the instrument run is complete, analyze the data following instrument manual