



Rx Only

cobas[®] SARS-CoV-2 Qualitative

Nucleic acid test for use on the cobas[®] 6800/8800 Systems

For in vitro diagnostic use

cobas[®] SARS-CoV-2 Qualitative 192T

P/N: 09446109190

cobas[®] SARS-CoV-2 Qualitative 480T

P/N: 09448870190

cobas[®] SARS-CoV-2 Qualitative Control Kit

P/N: 09446117190

cobas[®] Buffer Negative Control Kit

P/N: 09051953190

Table of contents

Intended use	5
Summary and explanation of the test	5
Reagents and materials	7
cobas® SARS-CoV-2 Qualitative reagents and controls.....	7
cobas omni reagents for sample preparation	9
Reagent storage requirements	10
Reagent handling requirements.....	11
Additional materials required.....	12
Alternative collection kits for swab specimens.....	12
Instrumentation and software required.....	13
Precautions and handling requirements	14
Warnings and precautions	14
Reagent handling	14
Good laboratory practice	15
Sample collection, transport, and storage	15
Sample collection – swab specimen types	15
Nasal (anterior nares) swab specimen collection - clinician or self-collected on site	16
Sample collection – saliva	17
Transport and storage – swab specimen types	17
Transport and storage – saliva	18
Instructions for use	19
Procedural notes.....	19
Running cobas® SARS-CoV-2 Qualitative utilizing swab specimens	19
Specimens collected in cobas® PCR Media, 0.9% physiological saline, UTM-RT or UVT	19
Specimens collected using cobas® PCR Media Uni or Dual Swab Sample Kit or cobas® PCR Media together with cobas® Uni Swab 100 Kit	20
Running cobas® SARS-CoV-2 Qualitative utilizing saliva specimens	20

Sample pooling for SARS-CoV-2 testing	22
Pooling methods.....	22
Pool result reporting and follow-up testing	23
Running cobas® SARS-CoV-2 Qualitative	23
Results	25
Quality control and validity of results	25
Interpretation of results.....	25
Procedural limitations.....	28
Use of pooling based on prevalence	28
Non-clinical performance evaluation	30
Analytical sensitivity (Limit of Detection) – swab specimen types.....	30
Analytical sensitivity (Limit of Detection) – saliva specimen types	30
Inclusivity	31
Precision	32
Analytical specificity / cross-reactivity.....	33
Interference	35
Matrix equivalency – UTM-RT/UVT, cobas® PCR Media and 0.9% physiological saline.....	36
Whole system failure.....	36
Cross contamination.....	36
Performance in sample pools.....	36
Clinical performance evaluation.....	38
Performance with clinical specimens – swab specimen types.....	38
Performance with clinical specimens – saliva specimen	39
Reproducibility.....	40
Additional information.....	43
Key test features	43
Symbols.....	44
Technical support	45
Manufacturer and importer.....	45

Trademarks and patents.....	45
Copyright.....	45
References.....	46
Document revision	47

Intended use

cobas® SARS-CoV-2 Qualitative for use on the **cobas® 6800/8800 Systems** is a real-time RT-PCR test intended for the qualitative detection of nucleic acids from SARS-CoV-2 in healthcare provider-instructed self-collected anterior nasal (nasal) swab and saliva specimens (collected on site), and healthcare provider-collected nasal, nasopharyngeal, and oropharyngeal swab specimens collected from any individuals, including those suspected of COVID-19 by their healthcare provider, and those without symptoms or other reasons to suspect COVID-19.

This test is also intended for the qualitative detection of nucleic acids from SARS-CoV-2 in pooled samples containing up to and including six individual samples from healthcare provider-instructed self-collected nasal swab specimens (collected on site), or healthcare provider-collected nasal, nasopharyngeal, and oropharyngeal swab specimens. Negative results from pooled samples should be treated as presumptive and, if inconsistent with clinical signs and symptoms or necessary for patient management, pooled samples should be tested individually. Specimens included in pools with a positive or presumptive positive result must be tested individually prior to reporting a result. Specimens with low SARS-CoV-2 RNA concentrations may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Results are for the detection of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA but may not represent the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.

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

cobas® SARS-CoV-2 Qualitative is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Summary and explanation of the test

Explanation of the test

cobas® SARS-CoV-2 Qualitative is a qualitative nucleic acid test for use on the **cobas® 6800 System** or **cobas® 8800 System** for the detection of the 2019 novel coronavirus (SARS-CoV-2) RNA in individual saliva specimens collected in an empty collection container and individual or pooled nasal, nasopharyngeal and oropharyngeal swab samples collected in Copan Universal Transport Medium System (UTM-RT), BD™ Universal Viral Transport System (UVT), **cobas® PCR Media**, or 0.9% physiological saline. The RNA Internal Control, used to monitor the entire sample preparation and PCR amplification process, is introduced into each specimen during sample processing. In addition, the test utilizes external controls (low titer positive control and a negative control).

Principles of the procedure

cobas® SARS-CoV-2 Qualitative is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas® 6800/8800 Systems** consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the

cobas® 6800/8800 Systems software(s), which assigns test results for all tests. Results can be reviewed directly on the system screen, and printed as a report.

Nucleic acid from patient samples and added internal control RNA (RNA IC) molecules are simultaneously extracted. Nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors, are removed with subsequent wash steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way.

Selective amplification of target nucleic acid from the sample is achieved by the use of target-specific forward and reverse primers for ORF1 a/b non-structural region that is unique to SARS-CoV-2. Additionally, a conserved region in the structural protein envelope E-gene were chosen for pan-Sarbecovirus detection. The pan-Sarbecovirus detection sets will also detect SARS-CoV-2 virus.

Selective amplification of RNA Internal Control is achieved by the use of non-competitive sequence specific forward and reverse primers which have no homology with the coronavirus genome. A thermostable DNA polymerase enzyme is used for amplification.

The **cobas®** SARS-CoV-2 Qualitative master mix contains detection probes which are specific for the coronavirus type SARS-CoV-2, members of the Sarbecovirus subgenus, and the RNA Internal Control nucleic acid. The coronavirus and RNA Internal Control detection probes are each labeled with unique fluorescent dyes that act as a reporter. Each probe also has a second dye which acts as a quencher. When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Each reporter dye is measured at defined wavelengths, which enables simultaneous detection and discrimination of the amplified coronavirus target and the RNA Internal Control. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicons from previous PCR runs are destroyed by the AmpErase enzyme [uracil-N-glycosylase], which is included in the PCR mix, when heated in the first thermal cycling step. However, newly formed amplicons are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

Reagents and materials

The materials provided for cobas® SARS-CoV-2 Qualitative can be found in Table 1. Materials required, but not provided can be found in Table 2, Table 3, Table 4, Table 6, Table 7, and Table 9.

Refer to the **Reagents and materials** section and **Precautions and handling requirements** section for the hazard information for the product.

cobas® SARS-CoV-2 Qualitative reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4.

Table 1 cobas® SARS-CoV-2 Qualitative

cobas® SARS-CoV-2 Qualitative Store at 2-8°C 192 test cassette (P/N 09446109190) 480 test cassette (P/N 09448870190)			
Kit components	Reagent ingredients	Quantity per kit 192 tests	Quantity per kit 480 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase, glycerol EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin from <i>Bacillus subtilis</i> . May produce an allergic reaction.	22.3 mL	38 mL
RNA Internal Control (RNA IC)	Tris buffer, < 0.05% EDTA, < 0.001% non-Sarbecovirus related armored RNA construct containing primer and probe specific primer sequence regions (non-infectious RNA in MS2 bacteriophage), < 0.1% sodium azide	21.2 mL	38 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL	38 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 mL	14.5 mL
SARS-CoV-2 QL Master Mix Reagent 2 (SARS-CoV-2 QL MMX-R2)	Tricine buffer, potassium acetate, < 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% upstream and downstream SARS-CoV-2 and Sarbecovirus primers, < 0.01% Internal Control forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for SARS-CoV-2, Sarbecovirus, and the RNA Internal Control, < 0.01% oligonucleotide aptamer, < 0.1% Z05D DNA polymerase, < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL	17.5 mL

Table 2 cobas® SARS-CoV-2 Qualitative Control Kit


cobas® SARS-CoV-2 Qualitative Control Kit Store at 2–8°C (P/N 09446117190)		
Kit components	Reagent ingredients	Quantity per kit
SARS-CoV-2 QL Positive Control (SARS-CoV-2 QL (+)C)	Tris buffer, < 0.05% Sodium azide, < 0.005% EDTA, 0.003% Poly rA, < 0.01% Non-infectious plasmid DNA (microbial) containing SARS-CoV-2 sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing pan-Sarbecovirus sequence	16 mL (16 x 1 mL)

Table 3 cobas® Buffer Negative Control Kit

cobas® Buffer Negative Control Kit Store at 2–8°C (P/N 09051953190)		
Kit components	Reagent ingredients	Quantity per kit
cobas® Buffer Negative Control (BUF (-) C)	Tris buffer, < 0.1% sodium azide, EDTA, 0.002% Poly rA RNA (synthetic)	16 mL (16 x 1 mL)

cobas omni reagents for sample preparation

Table 4 cobas omni reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	43% (w/w) guanidine thiocyanate***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol***, dihydro sodium citrate	4 x 875 mL	 <p>DANGER H302 + H332: Harmful if swallowed or if inhaled. H314: Causes severe skin burns and eye damage. H411: Toxic to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. P391: Collect spillage. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol</p>
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* These reagents are not included in the cobas® SARS-CoV-2 Qualitative kits. See listing of additional materials required (Table 7).

** Product safety labeling primarily follows EU GHS guidance.

***Hazardous substance.

Reagent storage requirements

Reagents shall be stored and will be handled as specified in Table 5 and Table 6.

When reagents are not loaded on the cobas® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® SARS-CoV-2 Qualitative 192T	2–8°C
cobas® SARS-CoV-2 Qualitative 480T	2–8°C
cobas® SARS-CoV-2 Qualitative Control Kit	2–8°C
cobas® Buffer Negative Control Kit	2–8°C
cobas omni Lysis Reagent	2–8°C
cobas omni MGP Reagent	2–8°C
cobas omni Specimen Diluent	2–8°C
cobas omni Wash Reagent	15–30°C

Reagent handling requirements

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas**® 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 6 Reagent expiry conditions enforced by the **cobas**® 6800/8800 Systems

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas ® SARS-CoV-2 Qualitative 192T	Date not passed	90 days from first usage	Max 40 runs	Max 40 hours ^b
cobas ® SARS-CoV-2 Qualitative 480T	Date not passed	90 days from first usage	Max 20 runs	Max 20 hours ^b
cobas ® SARS-CoV-2 Qualitative Control Kit	Date not passed	Not applicable ^a	Not applicable	Max 8 hours ^b
cobas ® Buffer Negative Control Kit	Date not passed	Not applicable ^a	Not applicable	Max 10 hours ^b
cobas omni Lysis Reagent	Date not passed	30 days from loading ^b	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading ^b	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading ^b	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading ^b	Not applicable	Not applicable

^a Single use reagents.

^b Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

Additional materials required

Table 7 Materials and consumables for use on the **cobas®** 6800/8800 Systems

Material	P/N
cobas omni Processing Plate	05534917001
cobas omni Amplification Plate	05534941001
cobas omni Pipette Tips	05534925001
cobas omni Liquid Waste Container	07094388001
cobas omni Lysis Reagent	06997538190
cobas omni MGP Reagent	06997546190
cobas omni Specimen Diluent	06997511190
cobas omni Wash Reagent	06997503190
Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer	07435967001 and 07094361001 or 08030073001 and 08387281001
cobas omni Secondary Tubes 13x75 (optional)	06438776001
cobas® PCR Media Tube Replacement Cap Kit	07958056190
cobas® PCR Media Disposable Tube Stand (Optional)	07958064190
MPA RACK 16 MM LIGHT GREEN 7001-7050***	03143449001
RD5 RACK – RD Standard rack 0001-0050 LR***	11902997001

* MPA 16 mm or RD5 racks are required to use **cobas®** SARS-CoV-2 Qualitative. Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

MPA 16 mm rack is the preferred rack for use with samples collected in **cobas® PCR Media tubes. If RD5 racks are used, make sure to fill in the sample tubes with not less than the recommended minimum sample input. The tubes sit higher in an RD5 rack because of the rubber gasket at the bottom of each tube position. Therefore, it is possible that when using RD5 racks, the system could accept tubes that are below the minimum sample input volume and cause pipetting errors later in the run.

Alternative collection kits for swab specimens

Table 8 Alternative specimen collection kits used with **cobas®** SARS-CoV-2 Qualitative for swab specimens

Collection Kit	P/N
cobas® PCR Media Uni Swab Sample Kit	07958030190
cobas® PCR Media Dual Swab Sample Kit	07958021190
cobas® PCR Media 100 tube kit	06466281190
cobas® Uni Swab 100 Kit	09205098190

Instrumentation and software required

The **cobas**® 6800/8800 Systems software and **cobas**® SARS-CoV-2 Qualitative analysis package for the **cobas**® 6800/8800 Systems must be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 9 Instrumentation

Equipment	P/N
cobas ® 6800 System (Moveable Platform)	05524245001 or 06379672001
cobas ® 6800 System (Fixed Platform)	05524245001 or 06379664001
cobas ® 8800 System	05412722001
Sample Supply Module	06301037001

Refer to the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide for additional information.

Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For in vitro diagnostic use.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.^{1,2} Only personnel proficient in handling infectious materials and the use of cobas® SARS-CoV-2 Qualitative and the cobas® 6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- The use of sterile disposable pipettes and nuclease-free pipette tips is recommended. Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
- **Some positive samples may not be detected when diluted and tested in pools.** SARS-CoV-2 RNA concentration is reduced when a positive sample is pooled with other samples, and the reduction corresponds inversely to the pool size. For example, if there is only one positive sample in a pool of 6, the concentration in the original sample would need to be 6 times the assay limit of detection in order for the concentration in the pool to be at the limit of detection.
- Inform your local competent authority about any serious incidents which may occur when using this assay.
- Reliable saliva results are dependent on proper specimen collection, handling, and storage. In case of visible particles or discoloration in the saliva sample, samples should not be further processed, but patient shall be asked to provide a new sample. Food particles and increased levels of mucin might cause failures in processing of the saliva specimen.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.

- **cobas® SARS-CoV-2 Qualitative, cobas® SARS-CoV-2 Qualitative Control Kit, cobas® Buffer Negative Control Kit, cobas omni MGP Reagent, and cobas omni Specimen Diluent** contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas® SARS-CoV-2 Qualitative kits, cobas® SARS-CoV-2 Qualitative Control Kit, cobas® Buffer Negative Control Kit and cobas omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas® 6800/8800** instrument, follow the instructions in the **cobas® 6800/8800 Systems – User Assistance and/or User Guide** to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Sample collection – swab specimen types

Ensure that the correct collection device is used with the appropriate sample type by referring to the table below:

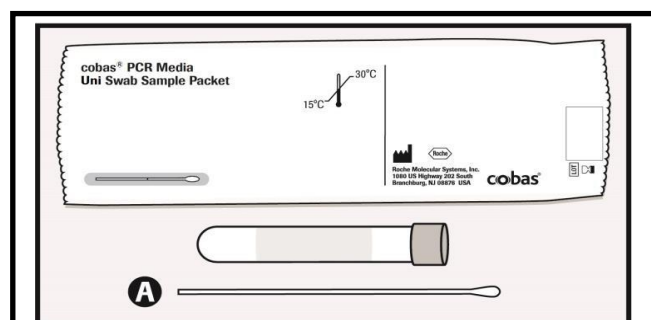
Table 10 Overview of collection devices and sample types

Collection Device	Sample Type		
	Nasopharyngeal	Oropharyngeal	Nasal
Copan Universal Transport Media (UTM-RT®)	✓	✓	✓
BD™ Universal Viral Transport (UVT)	✓	✓	✓
cobas® PCR Media Uni Swab Sample Kit			✓
cobas® PCR Media Dual Swab Sample Kit			✓
cobas® PCR Media Kit (and 100 tube PCR Media Kit)			✓
0.9% Physiological saline			✓

- Collect nasal, nasopharyngeal and oropharyngeal specimens according to standard collection technique using flocked or polyester-tipped swabs and immediately place in 3 mL of Copan Universal Transport Medium (UTM-RT) or BD™ Universal Viral Transport (UVT).
- Collect nasal specimens according to standard collection technique using flocked or polyester-tipped swabs and immediately place into cobas® PCR Media tube from cobas® PCR Media Kit (P/N 06466281190).
- Collect nasal specimens using the cobas® PCR Media Uni Swab Sample Kit (P/N 07958030190) or cobas® PCR Media Dual Swab Sample Kit (P/N 07958021190) according to instructions below.
- Refer to the Instructions for Use of the Collection Devices for hazard information.

Nasal (anterior nares) swab specimen collection - clinician or self-collected on site

WARNING: DO NOT PRE-WET SWAB IN cobas® PCR MEDIA BEFORE COLLECTION!

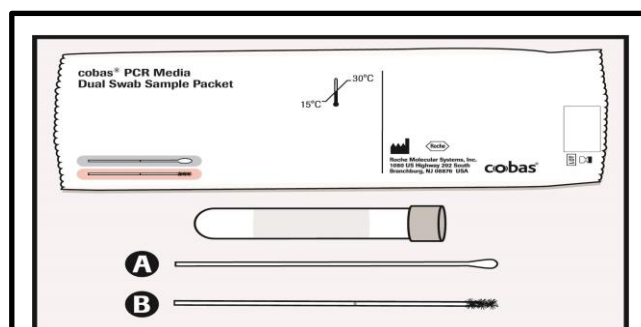


The cobas® PCR Media Uni Swab Sample kit contains:

cobas® PCR Media tube

Woven Swab: A

OR

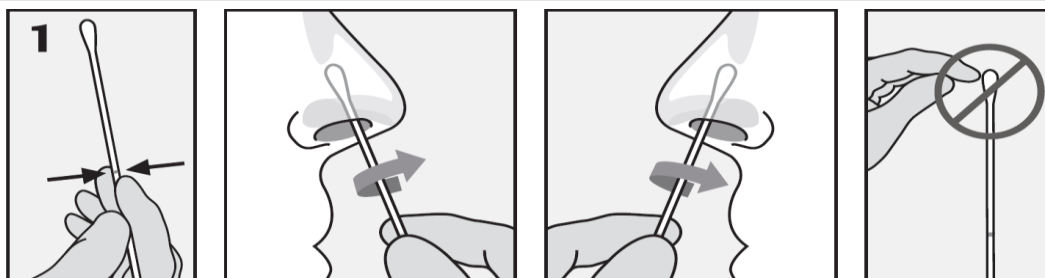


The cobas® PCR Media Dual Swab Sample kit contains:

cobas® PCR Media Tube

Woven Swab: A

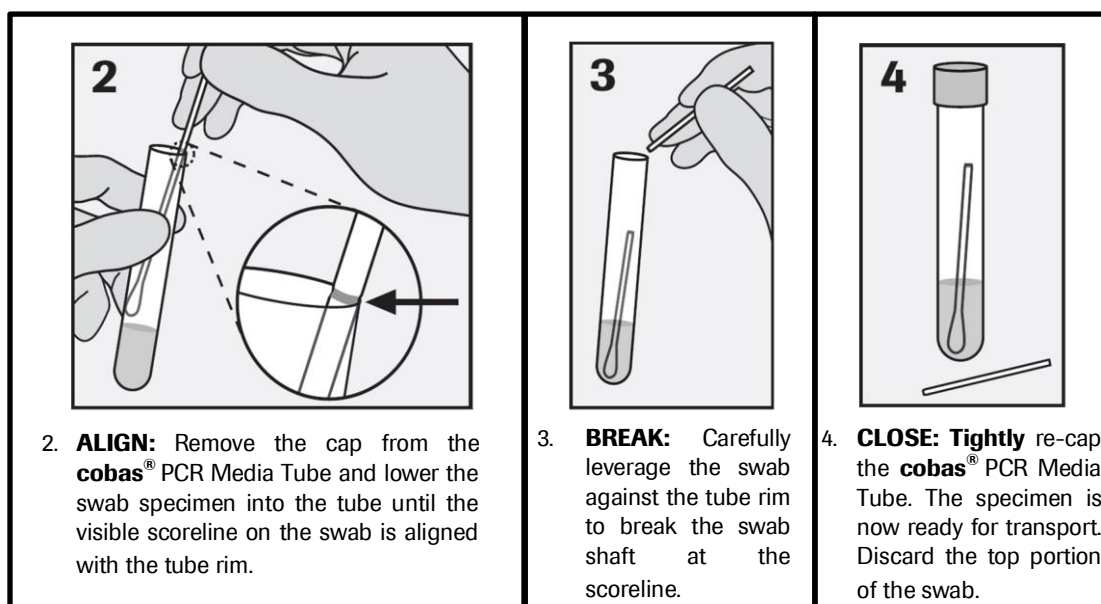
Flocked Swab: B



DO NOT PRE-WET SWAB IN cobas® PCR MEDIA BEFORE COLLECTION!

1. **COLLECT:** Hold the woven swab (Swab A) or the flocked swab (Swab B) with the scoreline above your hand. Insert the swab 1-2 cm into one of the anterior nares. Rotate the swab against the nasal mucosa for about 3 seconds and withdraw. Repeat with the other anterior nares using the same swab.

Do not let the swab touch any surface before placing it into the collection tube.



- Collect nasal specimens according to standard collection technique using flocked or polyester-tipped swabs and immediately place in 3 mL of 0.9% physiological saline.

Sample collection – saliva

- Do NOT eat or brush teeth 60 minutes before collecting the saliva sample.
- Collect saliva on the floor of your mouth and allow to pool without swallowing. Do not cough.
- Spit passively collected saliva into a sterile graduated collection container.
- Repeat the procedure above until at least 1 mL, but no more than 5 mL of saliva are collected in the container.
- Replace cap on the saliva collection container.
- Return the saliva collection container to the healthcare personnel.

Raw saliva needs to be liquified within 9 days of collection (48 hours at 2-25°C followed by 7 days below -18°C) by estimating the volume of saliva and adding double the amount of 0.9% physiological saline solution to the saliva collection container. After addition of saline, the sample needs to be mixed (e.g., vortex 10 – 20 seconds) prior to further storage or processing.

Transport and storage – swab specimen types

- Transportation of collected specimens must comply with all applicable regulations for the transport of etiologic agents.
- Samples collected in UTM-RT®,
 - After collection, specimens can be stored for up to 48 hours at 2-25°C followed by up to 3 days at 2-8°C and at ≤ -70°C for up to 30 days.
- Samples collected in **cobas**® PCR Media,
 - After collection, specimens can be stored for up to 24 hours at 2-25°C followed by up to 3 days at 2-8°C and at ≤ -70°C for up to 30 days.

- Samples collected in 0.9% physiological saline,
 - After collection, specimens can be stored for up to 48 hours at 2-25°C followed by up to 3 days at 2-8°C and at $\leq -70^{\circ}\text{C}$ for up to 30 days.
- Specimen are stable for up to two freeze/thaw cycles when frozen at $\leq -70^{\circ}\text{C}$.

Transport and storage – saliva

- Transportation of collected specimens must comply with all applicable regulations for the transport of etiologic agents.
- Raw saliva samples collected in a sterile polypropylene collection device,
 - After collection, specimens can be stored for up to 48 hours at 2-25°C followed by up to 7 days $\leq -18^{\circ}\text{C}$.
- Liquefied saliva,
 - After addition of 2 parts of 0.9% physiological saline solution and intensive mixing, the liquefied saliva specimens can be stored for up to 48 hours at 2-25°C followed by up to 7 days at 2-8°C.

Instructions for use

Procedural notes

- Do not use cobas® SARS-CoV-2 Qualitative, cobas® SARS-CoV-2 Qualitative Control Kit, cobas® Buffer Negative Control Kit, or cobas omni reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Ensure that specimen barcode labels on sample tubes are visible through the openings on the side of the sample racks. Refer to the cobas® 6800/8800 Systems User Guide for proper barcode specifications and additional information on loading sample tubes.
- Refer to the cobas® 6800/8800 Systems – User Assistance and/or User Guide for proper maintenance of instruments.

Running cobas® SARS-CoV-2 Qualitative utilizing swab specimens

For testing swab specimens, cobas® SARS-CoV-2 Qualitative can be run with a minimum required sample volume of 0.6 mL in the cobas omni Secondary Tube for specimens collected in Copan Universal Transport Medium (UTM-RT), BD™ Universal Viral Transport (UVT), cobas® PCR Media or 0.9% physiological saline. Specimens collected using cobas® PCR Media Uni Swab Sample Kit or cobas® PCR Media Dual Swab Sample Kit can be run in their primary collection tube with a minimum required sample volume of 1.0 mL.

Specimens collected in cobas® PCR Media, 0.9% physiological saline, UTM-RT or UVT

Specimens collected in tubes compatible with the cobas® 6800/8800 Systems may be loaded directly onto the cobas® 6800/8800 Systems. Specimens collected in Copan Universal Transport Medium (UTM-RT), BD™ Universal Viral Transport (UVT), cobas® PCR Media or 0.9% physiological saline tubes which are not compatible with the cobas® 6800/8800 Systems must be transferred into a secondary tube prior to processing on the cobas® 6800/8800 Systems. The cobas omni Secondary Tube is the preferred option. Samples should be processed using the sample type selection in the user interface (UI) as described in Table 11. Additional tubes for testing cobas® SARS-CoV-2 Qualitative are available. Contact your local Roche representative for detailed testing instructions and an order list of primary tubes and secondary tubes compatible with the instruments.

Always use caution when transferring specimens from a primary collection tube to a secondary tube.

Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.

Always use a new pipette tip for each specimen.

Ensure samples are equilibrated to room temperature prior to transfer into a cobas omni Secondary Tube.

Follow the steps below to transfer patient sample from a primary collection tube into a cobas omni Secondary Tube:

- Unscrew the primary sample tube cap.
- Lift the cap and any attached swab to allow a pipette to be inserted into the sample tube.
- Transfer 0.6 mL into the prepared barcoded secondary tube.
- Transfer secondary tube to a rack. Close the primary sample tube cap.

Specimens collected using cobas® PCR Media Uni or Dual Swab Sample Kit or cobas® PCR Media together with cobas® Uni Swab 100 Kit

Samples collected using cobas® PCR Media Uni Swab Sample Kit or cobas® PCR Media Dual Swab Sample Kit or cobas® PCR Media together with the cobas® Uni Swab 100 Kit must be uncapped and can be loaded directly onto racks for processing on the cobas® 6800/8800 Systems. Transfer into a secondary tube is not necessary. cobas® PCR Media tubes fit on to the MPA RACK 16 MM LIGHT GREEN 7001-7050 (P/N 03143449001) and can be processed with the swab remaining in the tube. Samples collected using cobas® PCR Media Uni Swab Sample Kit or cobas® PCR Media Dual Swab Sample Kits or cobas® PCR Media together with the cobas® Uni Swab 100 Kit should be processed using the ‘cobas® PCR media swab’ sample type selection in the user interface (UI) of the cobas® SARS-CoV-2 Qualitative as described in Table 11.

A properly collected swab specimen should have a single swab with the shaft broken at the scoreline. Swab shafts which are broken above the score line will appear longer than normal and may also be bent over to fit into the cobas® PCR Media tube. This may create an obstruction to the pipetting system which might cause the loss of sample, test results and/or mechanical damage to the instrument. In the event that a swab specimen has an improperly broken shaft, remove the swab prior to sample processing on the cobas® 6800/8800 Systems. Use caution when disposing of specimen swabs; avoid splashing or touching swabs to other surfaces during disposal to prevent contamination.

Incoming cobas® PCR Media primary swab specimen tubes with no swabs or with two swabs have not been collected according to the instructions in their respective collection kit instruction for use and should not be tested. If the sample containing two swabs in the cobas® PCR Media primary tubes must be tested, transfer 0.6 mL into the prepared barcoded secondary tube.

Occasionally, incoming swab specimens contain excessive mucus which may induce a pipetting error (e.g., clot or other obstruction) on the cobas® 6800/8800 Systems. Prior to retesting of specimens that exhibited clots during initial processing, remove and discard the swab, then re-cap and vortex these specimens for 30 seconds to disperse the excess mucus.

Swab specimens can be processed twice on the cobas® 6800/8800 Systems while the swab is in the collection tube. If additional testing is required, or if the first test fails due to specimen pipetting error (e.g., clot or other obstruction), the swab must be removed and the remaining fluid must have a minimum volume of 1.0 mL.

Running cobas® SARS-CoV-2 Qualitative utilizing saliva specimens

Raw saliva specimens collected in a sterile polypropylene container, need to be liquified prior to processing. For liquifaction the volume of raw saliva is estimated and the double amount of 0.9% physiological saline is added. The collection device must be recapped and the solution mixed (e.g., vortex 10 – 20 seconds) prior to the required transfer into a cobas omni Secondary Tube and processing on the cobas® 6800/8800 Systems. Liquified saliva samples transferred to cobas omni Secondary Tubes should be processed using the ‘Saliva’ sample type selection in the user interface (UI) of the cobas® SARS-CoV-2 Qualitative.

Always use caution when transferring specimens from a primary collection container to the cobas omni Secondary Tube.

Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.

Always use a new pipette tip for each specimen.

Ensure samples are equilibrated to room temperature prior to liquifaction and transfer into a cobas omni Secondary Tube.

Follow the steps below to transfer saliva samples from a primary collection container into a **cobas omni** Secondary Tube:

- Unscrew the primary sample tube cap and lift the cap.
- Estimate the volume of raw saliva and add the double amount of 0.9% physiological saline.
- Recap the tube and mix (e.g., vortex 10-20 seconds) until a homogenous solution results.
- Unscrew the primary sample container cap and lift the cap.
- Transfer 1.2 mL into the prepared barcoded **cobas omni** Secondary Tube.
- Close the primary sample container cap.
- Transfer **cobas omni** Secondary Tube to the rack.

Table 11 Sample type selection in the user interface of the cobas® SARS-CoV-2 Qualitative

Collection kit/Matrix type	Minimum volume (mL) Processing tube	Process as Sample Type
Copan Universal Transport Medium BD™ Universal Viral Transport 0.9% physiological saline cobas® PCR Media Kit	0.6 mL cobas omni Secondary Tube	VTM
cobas® PCR Media Uni or Dual Swab Sample Kit cobas® PCR Media Kit together with the cobas® Uni Swab 100 Kit	1.0 mL Primary tube	cobas® PCR media swab
Liquified Saliva in a sterile polypropylene container	1.2 mL cobas omni Secondary Tube	Saliva

Sample pooling for SARS-CoV-2 testing

Pools of up to and including 6 samples may be tested using cobas® SARS-CoV-2 Qualitative. The pool size implemented by the laboratory should be based on the required efficiency gains, the positivity rate of SARS-CoV-2 in the testing population, and the potential risks of testing in pools. Combination of multiple sample types in a pool has not been validated.

When resource availability is sufficient to meet testing demand, it is recommended that laboratories consider whether the risks of reduced test sensitivity with pooling outweigh the benefits of resource conservation.

- Use a process that ensures traceability between individual sample IDs and pool IDs.
- To reduce potential contamination of the cobas® 6800/8800 Systems, do not transfer samples into the secondary tubes while the samples are in the Roche 5 position racks (RD5 and/or MPA).
- Ensure appropriate sample handling techniques to reduce the risk of cross-contamination of pools and original patient samples.

Note: Sample pooling does not apply to saliva specimen.

Pooling methods

1. Identify a uniquely labeled secondary tube for pooling.
2. Associate the samples to be pooled with the pool tube identification using either a pooling worksheet or validated sample tracking system.
3. Roche suggests using Biological Safety Cabinet or other approved safety measures during sample handling (i.e., sample transfer to secondary tube).
4. For manual pooling, it is recommended to work with only the samples for one pool at a time.
5. Ensure each sample has sufficient volume for pool construction and any possible resolution testing (pool deconvolution) that may be required. Example: for pools of 6, a minimum volume of 100 µL (for pool) plus 600 µL (for resolution) are required for a minimum sample volume of 700 µL available prior to pooling (Table 12).

Table 12 Minimum sample volumes for pooling

Pool Size	Volume required for pool (mL)	Volume required for resolution testing (mL)	Minimum volume required prior to pooling (mL)
6	0.100	0.600	0.700
5	0.120	0.600	0.720
4	0.150	0.600	0.750
3	0.200	0.600	0.800
2	0.300	0.600	0.900

6. Using a calibrated micropipettor with a fresh pipette tip for each sample, carefully pipette each individual sample associated with that pool into the appropriate secondary tube to prepare the pool.
7. Ensure complete mixing after addition of all samples to the secondary tube (i.e., through pipetting up and down). Use caution to avoid creating bubbles, foam or aerosols while mixing.
8. For manual pooling, it is recommended to visually compare the pooled sample volume in the secondary tube to a secondary tube containing the target pool volume. If the pooling tube level is less or more than the standard pool volume, then the manually prepared pool should be discarded and prepared again.
9. Process pooled samples as described in Figure 1.

Pool result reporting and follow-up testing

Interpretation of pool results is the same as for individual results as described in the **Interpretation of results** section.

- If the result of the pool is negative, then each constituent sample can be reported as negative. The result report should include a comment that pooling was used during testing. Refer to the **Warnings and precautions** section for additional information regarding decreased sensitivity of pool testing.
- If the result of the pool is positive or presumptive positive, then each of the constituent samples must be re-tested as a separate individual sample. Use the laboratory defined tracking system to ensure the correct individual samples are tested. Individual test results supersede the pool result.

Running cobas® SARS-CoV-2 Qualitative

The test procedure is described in detail in the **cobas® 6800/8800 Systems – User Assistance and/or User Guide**. Figure 1 below summarizes the procedure.

Figure 1 cobas® SARS-CoV-2 Qualitative procedure on the cobas® 6800/8800 Systems

1	Log onto the system Press Start to prepare the system Order tests
2	Refill reagents and consumables as prompted by the system <ul style="list-style-type: none">• Load test specific reagent cassette• Load control cassettes• Load pipette tips• Load processing plates• Load MGP reagent• Load amplification plates• Refill specimen diluent• Refill lysis reagent• Refill wash reagent
3	Loading samples onto the system <ul style="list-style-type: none">• Load sample racks and clotted tip racks onto the sample supply module• Confirm samples have been accepted into the transfer module
4	Start the run by choosing the Start manually button on the user interface or have it start automatically after 120 minutes or if the batch is full
5	Review and export results
6	Remove and cap any sample tubes meeting the minimum volume requirements if needed for future use Clean up the instrument <ul style="list-style-type: none">• Unload empty control cassettes• Empty amplification plate drawer• Empty liquid waste• Empty solid waste

Results

The **cobas**® 6800/8800 Systems automatically detect the SARS-CoV-2, for each individually processed sample and control, displaying individual target results for samples as well as test validity and overall results for controls.

Quality control and validity of results

- One **cobas**® Buffer Negative Control [BUF (-) C] and one Positive Control [SARS-CoV-2 QL (+)C] are processed with each batch.
- In the **cobas**® 6800/8800 Systems software and/or report, check for flags and their associated results to ensure the batch validity.
- All flags are described in the **cobas**® 6800/8800 Systems User Guide.
- The batch is valid if no flags appear for any controls. If the batch is invalid, repeat testing of the entire batch.

Validation of results is performed automatically by the **cobas**® 6800/8800 Systems software based on negative and positive control performance.

Interpretation of results

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 Systems software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.
- Results display examples for **cobas**® SARS-CoV-2 Qualitative are shown in Table 13.
- The “Valid” and “Overall Result” columns are not applicable to sample results for the **cobas**® SARS-CoV-2 Qualitative.
- Invalid results for one or more target combinations are possible and are reported out specifically for each target. If any individual target result is invalid, the presence or absence of that individual target cannot be determined.
- Other initial valid target results can be interpreted as described in Table 14.

Display examples for **cobas**® SARS-CoV-2 Qualitative are shown in Table 13.

Table 13 Example of cobas® SARS-CoV-2 Qualitative results display

Test*	Sample ID	Valid*	Flags	Sample type	Overall result*	Target 1	Target 2
SCoV2- QL 400 µL	Sample _01	NA		VTM	NA	SCoV2 Negative	PanSarβ Negative
SCoV2- QL 400 µL	Sample _C1	NA	Y40T	VTM	NA	Invalid	Invalid
SCoV2- QL 400 µL	Sample _B1	NA		VTM	NA	SCoV2 Negative	PanSarβ Positive
SCoV2- QL 400 µL	Sample _B2	NA		VTM	NA	SCoV2 Positive	PanSarβ Positive
SCoV2- QL 400 µL	Sample _D1	NA		VTM	NA	SCoV2 Negative	PanSarβ Negative
SCoV2- QL 400 µL	Sample _A6	NA		VTM	NA	SCoV2 Positive	PanSarβ Negative
SCoV2- QL 400 µL	Sample _E1	NA	C01H2	VTM	NA	SCoV2 Positive	Invalid
SCoV2- QL 400 µL	Sample _A2	NA	C01H1	VTM	NA	Invalid	PanSarβ Positive
SCoV2- QL	C161420284090428828404	Yes		(-) Ctrl	Valid	Valid	Valid
SCoV2- QL	C161420284093009580264	Yes		SCoV2-QL (+) C	Valid	Valid	Valid

* Depends on sample type used.

**The “Valid” and “Overall Result” columns are not applicable to sample results for the cobas® SARS-CoV-2 Qualitative. Refer to Table 14, cobas® SARS-CoV-2 Qualitative results interpretation, for specific instructions on test results interpretation.

Table 14 cobas® SARS-CoV-2 Qualitative results interpretation

Target 1	Target 2	Interpretation
SCoV2 Positive	PanSarb Positive	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
SCoV2 Positive	PanSarb Negative	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected. A positive Target 1 result and a negative Target 2 result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target 2, target region, or 3) other factors.
SCoV2 Negative	PanSarb Positive	All Target Results were valid. Result for SARS-CoV-2 RNA is Presumptive Positive. A negative Target 1 result and a positive Target 2 result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target 1 target region in the oligo binding sites, or 3) infection with some other Sarbecovirus (e.g., SARS-CoV or some other Sarbecovirus previously unknown to infect humans), or 4) other factors. For samples with a Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
SCoV2 Negative	PanSarb Negative	All Target Results were valid. Result for SARS-CoV-2 RNA is Not Detected.
SCoV2	Invalid	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Invalid	PanSarb Positive	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Presumptive Positive. For samples with a Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
SCoV2 Negative	Invalid	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	PanSarb Negative	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	Invalid	All Target Results were invalid.* Sample should be retested. If the result is still invalid, a new specimen should be obtained.

*For further details also refer to section **Procedural limitations**

Procedural limitations

- **cobas® SARS-CoV-2 Qualitative** has been evaluated only for use in combination with the **cobas® SARS-CoV-2 Qualitative Control Kit**, **cobas® Buffer Negative Control Kit**, **cobas omni MGP Reagent**, **cobas omni Lysis Reagent**, **cobas omni Specimen Diluent**, and **cobas omni Wash Reagent** for use on the **cobas® 6800/8800 Systems**.
- Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection.
- Reliable results depend on proper sample collection, storage and handling procedures.
- Due to the nature of the saliva sample type and the individual patient sample variability, an increased invalid and clot rate may occur. Additionally, food particles and increased levels of mucin indicated by potentially discolored samples might cause failures in processing of the saliva specimen. Refer to the **Sample collection – saliva** section for the appropriate precautions to be taken during collection to assure optimal performance.
- Occasionally, incoming saliva specimens may induce a pipetting error (e.g. clot or other obstruction) on the **cobas® 6800/8800 Systems**. A potential additional processing step prior to retesting the specimens that exhibited clots during initial processing is to centrifuge the samples at 2000g for 1 minute and reload the samples on the instrument.
- This test is intended to be used for the detection of SARS-CoV-2 RNA in nasal, nasopharyngeal and oropharyngeal swab samples collected in a Copan UTM-RT System (UTM-RT) or BD™ Universal Viral Transport System (UVT) and nasal swab samples collected in **cobas® PCR Media** and 0.9% physiological saline. Additionally the test is intended to be used detection of SARS-CoV-2 RNA in saliva specimen liquified with 0.9% physiological saline. Testing of other sample types with **cobas® SARS-CoV-2 Qualitative** may result in inaccurate results.
- As with any molecular test, mutations within the target regions of **cobas® SARS-CoV-2 Qualitative** could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies. Users should follow their own specific policies/procedures.
- False negative or invalid results may occur due to interference. The Internal Control is included in **cobas® SARS-CoV-2 Qualitative** to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
- The addition of AmpErase enzyme into the **cobas® SARS-CoV-2 Qualitative Master Mix** reagent enables selective amplification of target RNA; however, good laboratory practices and careful adherence to the procedures specified in this Instructions For Use document are necessary to avoid contamination of reagents.

Use of pooling based on prevalence

Pooling may increase throughput in laboratories testing samples from populations with low prevalence of SARS-CoV-2. In populations with higher prevalence, smaller pool sizes or individual sample testing may be indicated.

When considering pooling strategies, laboratories should consider the appropriateness of the pooling strategy based on the positivity rate in the testing population and efficiency of the pooling workflow. Laboratories may also consider the sensitivity of pooled testing based on the assay's limit of detection.

Table 15 provides estimated maximal efficiency gained based on N-sample pooling and on the percent of SARS-CoV-2 positive samples in a population.

Table 15 Efficiency of pooling based on prevalence

P, percent of positive subjects in the tested population	$n_{\text{maxefficiency}}$ (n corresponding to the maximal efficiency)	Efficiency (F) of n-sample pooling (a maximum increase in the number of tested patients when Dorfman n-pooling strategy used)
1% - 4%	6	4.44 - 2.60
5% - 6%	6	2.32 - 2.10
7% - 12%	6	1.92 - 1.42
13% - 25%	6	1.36 - 1.01
1% - 4%	5	4.02 - 2.60
5% - 6%	5	2.35 - 2.15
7% - 12%	5	1.98 - 1.49
13% - 25%	5	1.43 - 1.04
1% - 4%	4	3.46 - 2.50
5% - 6%	4	2.30 - 2.13
7% - 12%	4	1.99 - 1.54
13% - 25%	4	1.48 - 1.07
1% - 4%	3	2.75 - 2.23
5% - 6%	3	2.10 - 1.99
7% - 12%	3	1.89 - 1.53
13% - 25%	3	1.48 - 1.10
1% - 4%	2	1.92 - 1.73
5% - 6%	2	1.67 - 1.62
7% - 12%	2	1.57 - 1.38
13% - 25%	2	1.35 - 1.07

Because a positive pool requires individual retesting of each sample in the pool, the efficiency of any pooling strategy depends on the positivity rate. The efficiency (F) of n-sample pooling for positivity rate (P) can be calculated using the following formula $F=1/(1+1/n-(1-P)n)$. The efficiency (F) indicates how many more samples can be tested with n-sample pools compared to individual testing. For example, a 6-sample pooling strategy increases the number of tested samples by 2.10 times for positivity rate P of 6% ($F = 2.10$). At $F = 2.10$, 1,000 tests can cover testing of 2,100 samples on average.

Non-clinical performance evaluation

Analytical sensitivity (Limit of Detection) – swab specimen types

Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates tested positive.

To determine the LoD, the WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146) was serially diluted in simulated clinical matrix. A total of 5 concentration levels, and 3 independent dilution series, were tested with a total of 24 replicates per concentration and lot, with an additional 60 replicates of a blank sample (i.e., clinical sample pools).

As shown in Table 16 and Table 17, the concentration level with observed hit rates greater than or equal to 95% were 250 and 125 IU/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively and the Probit predicted 95% hit rates were 200 and 102 IU/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively.

Table 16 Summary of LoD for SARS-CoV-2 using WHO International Standard (NIBSC code: 20/146)

Viral Strain	Kit lot	95% Probit [IU/mL]	95% CI of Probit [IU/mL]	Hit rate ≥ 95% [IU/mL]	Mean Ct at ≥ 95% Hit rate
WHO International Standard for SARS-CoV- 2 RNA (NIBSC code: 20/146)	Lot 1	202	157-319	250	33.2
	Lot 2	121	97-183	125	34.1
	Lot 3	259	196-413	250	33.2
	Lot 1-3	200	170-252	250	33.4

Table 17 Summary of LoD for pan-Sarbecovirus using WHO International Standard (NIBSC code: 20/146)

Viral Strain	Kit lot	95% Probit [IU/mL]	95% CI of Probit [IU/mL]	Hit rate ≥ 95% [IU/mL]	Mean Ct at ≥ 95% Hit rate
WHO International Standard for SARS-CoV- 2 RNA (NIBSC code: 20/146)	Lot 1	83	64-127	125	35.2
	Lot 2	67	46-454	125	36.0
	Lot 3	132	99-233	125	34.8
	Lot 1-3	102	83-140	125	35.3

Analytical sensitivity (Limit of Detection) – saliva specimen types

Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates tested positive.

To determine the LoD, the WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146) was serially diluted in pools of negative Saliva clinical specimens. A total of 8 concentration levels and 3 independent dilution series/saliva pools, were tested with a total of 24 replicates per concentration and lot, with an additional 72 replicates of a blank sample (i.e., clinical sample pools).

As shown in Table 18 and Table 19, the concentration levels with observed hit rates greater than or equal to 95% were 150 IU/mL and 75 IU/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively and the Probit predicted 95% hit rates were 96 and 69 IU/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively.

Table 18 Summary of LoD for SARS-CoV-2 using WHO International Standard (NIBSC code: 20/146)

Viral Strain	Kit lot	95% Probit [IU/mL]	95% CI of Probit [IU/mL]	Hit rate ≥ 95% [IU/mL]	Mean Ct at ≥ 95% Hit rate
WHO International Standard for SARS-CoV- 2 RNA (NIBSC code: 20/146)	Lot 1	118	83-206	150	34.0
	Lot 2	87	64-144	150	34.0
	Lot 3	83	62-131	150	33.8
	Lot 1-3	96	79-124	150	33.9

Table 19 Summary of LoD for pan-Sarbecovirus using WHO International Standard (NIBSC code: 20/146)

Viral Strain	Kit lot	95% Probit [IU/mL]	95% CI of Probit [IU/mL]	Hit rate ≥ 95% [IU/mL]	Mean Ct at ≥ 95% Hit rate
WHO International Standard for SARS-CoV- 2 RNA (NIBSC code: 20/146)	Lot 1	55	42-91	75	36.6
	Lot 2	75	52-140	75	36.3
	Lot 3	79	55-150	75	36.4
	Lot 1-3	69	56-93	75	36.4

Inclusivity

The inclusivity of cobas® SARS-CoV-2 for the detection of SARS-CoV-2 was confirmed by testing nine SARS-CoV-2 strains, including six variant strains. The lowest target analyte at which all four tested replicates were positive are reported in Table 20.

Table 20 Summary of inclusivity

Strain	Catalog Number	Lot Number	Test Concentration with 100% Positivity
Hong Kong/VM20001061/2020	0810590CFHI	325659	1.06E+02 cp/mL
Italy-INMI1	0810589CFHI	325658	1.00E+02 cp/mL
USA-WA1/2020	0810587CFHI	325656	5.03E+01 cp/mL
UK (B.1.1.7)	0810614CFHI	326230	2.4E+01 cp/mL
Japan / Brazil (P.1)	NR-54982	70042875	1.9E+02 cp/mL
South Africa (B.1.351)	0810613CFHI	326229	2.4E+01 cp/mL
US NY (B.1.526)	NR-55359	70043342	1.9E+02 cp/mL
India (B.1.617.1)	NR-55486	70044706	2.5E+02 cp/mL
India (B.1.617.2)	NR-55611	70045238	7.0E+01 cp/mL

Precision

Within-laboratory precision was examined using a panel of SARS-CoV-2 (USA-WA1/2020, heat-inactivated) cultures diluted in simulated clinical matrix in universal transport media. Sources of variability were examined with a panel consisting of three concentration levels, using three lots of cobas® SARS-CoV-2 Qualitative reagents and three instruments over a course of 15 instrument days (2 runs/day x 3 instruments x 5 days/instrument) for a total of 30 runs. A description of the precision panel and the observed positivity rates are shown in Table 21. All negative panel members tested negative throughout the study. Analysis of standard deviation and percent coefficient of variation (CV) of the Ct values from tests performed on positive panel members (see Table 22) yielded overall CV percentage ranging from 1.1% to 2.2% for cobas® SARS-CoV-2 Qualitative.

Table 21 Summary of within laboratory precision

Target	Panel Member	Level (x LoD)	Positive Results	Total Results	Positivity %	Two-sided 95% CI Lower Bound	Two-sided 95% CI Upper Bound
Target 1 (SARS-CoV-2)	Weak positive	~0.3x	9	90	10%	5%	18%
	Low positive	~1.0x	82	90	91%	83%	96%
	Moderate positive	~3.0x	90	90	100%	96%	100%
Target 2 (pan-Sarbecovirus)	Weak positive	~0.3x	31	90	34%	25%	45%
	Low positive	~1.0x	84	90	93%	86%	97%
	Moderate positive	~3.0x	90	90	100%	96%	100%
N/A	Negative	Blank	0	90	0%	0%	4%

Table 22 Overall mean, standard deviation, and percent coefficient of variation for Ct values by positive panel member

Target	Level (x LoD)	Hit rate	Mean Ct	Instrument-to-Instrument		Lot-to-Lot		Day-to-Day		Run-to-Run		Within Run		Total	
				SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Target 1 (SARS-CoV-2)	~0.3x	10.0%	32.51	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	1.4	0.5	1.4
	~1.0x	91.1%	32.1	0.0	0.0	0.2	0.6	0.1	0.3	0.0	0.0	0.6	1.8	0.6	1.9
	~3.0x	100.0%	31.18	0.0	0.0	0.2	0.7	0.0	0.0	0.0	0.0	0.3	0.9	0.4	1.1
Target 2 (pan-Sarbeco virus)	~0.3x	34.4%	35.36	0.0	0.0	0.5	1.3	0.3	0.8	0.1	0.2	0.5	1.5	0.8	2.2
	~1.0x	93.3%	34.21	0.0	0.0	0.1	0.3	0.2	0.6	0.0	0.0	0.7	2	0.7	2.2
	~3.0x	100.0%	32.9	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4	1.1	0.4	1.1

Analytical specificity / cross-reactivity

A panel of 48 viruses, bacteria, and fungi (including those commonly found in respiratory tract) were tested with cobas® SARS-CoV-2 Qualitative to assess analytical specificity. The organisms listed in Table 23 were spiked at concentrations of 1×10^5 units/mL for viruses and 1×10^6 units/mL for other organisms, unless otherwise noted.

Testing was performed with each potential interfering organism in the absence and presence of SARS-CoV-2 target (spiked at ~3x LoD). None of the organisms interfered with the test performance. Testing of SARS-CoV-1 generated an expected pan-Sarbecovirus positive result.

Table 23 Cross-reactivity test results

Microorganism	Concentration
Human coronavirus 229E	1.0×10^5 TCID ₅₀ /mL
Human coronavirus OC43	1.0×10^5 TCID ₅₀ /mL
Human coronavirus HKU1	1.0×10^5 TCID ₅₀ /mL
Human coronavirus NL63	1.0×10^5 TCID ₅₀ /mL
MERS coronavirus	1.0×10^5 genomic equivalent/mL
SARS coronavirus	1.0×10^5 PFU/mL
Adenovirus B (Type 34)	1.0×10^5 TCID ₅₀ /mL
Bocavirus	1.0×10^5 cp/mL
Cytomegalovirus	1.0×10^5 TCID ₅₀ /mL
Epstein Barr virus	1.0×10^5 cp/mL
Human Metapneumovirus (hMPV)	1.0×10^5 TCID ₅₀ /mL
Measles virus	1.0×10^5 TCID ₅₀ /mL
Mumps virus	1.0×10^5 TCID ₅₀ /mL
Parainfluenza virus Type 1	1.0×10^5 TCID ₅₀ /mL
Parainfluenza virus Type 2	1.0×10^5 TCID ₅₀ /mL
Parainfluenza virus Type 3	1.0×10^5 TCID ₅₀ /mL

Parainfluenza virus Type 4	1.0E+05 TCID ₅₀ /mL
Influenza A (H1N1)	1.0E+05 TCID ₅₀ /mL
Influenza A virus (H1N1-2009, H1N3, H3N2)	1.0E+05 TCID ₅₀ /mL
Influenza B	1.0E+05 TCID ₅₀ /mL
Enterovirus E (Type 1)	1.0E+05 TCID ₅₀ /mL
Parechovirus	1.0E+05 TCID ₅₀ /mL
Respiratory syncytial virus	1.0E+05 PFU/mL
Rhinovirus	1.0E+05 TCID ₅₀ /mL
<i>Candida albicans</i>	1.0E+06 CFU/mL
<i>Chlamydia pneumoniae</i>	1.0E+06 TCID ₅₀ /mL
<i>Corynebacterium diphtheriae</i>	1.0E+06 CFU/mL
<i>Escherichia coli</i>	1.0E+06 CFU/mL
<i>Haemophilus influenzae</i>	1.0E+06 CFU/mL
<i>Lactobacillus gasseri</i>	1.0E+06 CFU/mL
<i>Legionella pneumophila</i>	1.0E+06 CFU/mL
<i>Legionella jordanis (non-pneumophila)</i>	1.0E+06 CFU/mL
<i>Moraxella catarrhalis</i>	1.0E+06 CFU/mL
<i>Mycobacterium tuberculosis</i>	1.0E+06 cells/mL
<i>Neisseria elongata</i>	1.0E+06 CFU/mL
<i>Neisseria meningitidis</i>	1.0E+06 CFU/mL
<i>Pseudomonas aeruginosa</i>	1.0E+06 CFU/mL
<i>Pneumocystis jirovecii</i>	1:20 of Patient Sample
<i>Staphylococcus aureus</i>	1.0E+06 CFU/mL
<i>Staphylococcus epidermidis</i>	1.0E+06 CFU/mL
<i>Streptococcus pneumoniae</i>	1.0E+06 CFU/mL
<i>Streptococcus pyogenes</i>	1.0E+06 CFU/mL
<i>Streptococcus salivarius</i>	1.0E+06 CFU/mL
<i>Bordetella pertussis</i>	1.0E+06 CFU/mL
<i>Mycoplasma pneumoniae</i>	1.0E+06 CFU/mL
Pooled Nasal Wash	1:20 of Patient Sample

Interference

The effect of exogenous substances potentially secreted into respiratory specimens was evaluated (Table 24). Each potentially interfering substance was tested at or above clinically relevant levels in negative simulated clinical matrix stabilized in universal transport media in absence and presence of SARS-CoV-2 target (spiked at ~3x LoD).

None of the substances interfered with the test performance by generating false-negative, false-positive or invalid results.

Table 24 List of exogenous substances tested for interference

Substance	Concentration
Oxymetazoline	0.011 mg/mL
Galphimia glauca, Luffa operculata, Sabadilla	0.023 mg/mL
Lidocaine and Phenylephrine	2.68 mg/mL
Budesonide	0.039 mg/mL
Phenol	0.47 mg/mL
Fluticasone propionate	166.67 µg/mL
Mupirocin	0.20 mg/mL
Zanamivir	0.0015 mg/mL
Oseltamivir	0.0073 mg/mL
Benzocaine and Menthol	5.00 mg/mL
Tobramycin	0.018 mg/mL
Petroleum Jelly	1% (w/v)
Nicotine	1% (w/v)
Camphor-synthetic eucalyptus oil and menthol ointment	1% (w/v)
0.65% NaCl, Phenylcarbino, Benzalkonium chloride	1% (w/v)

Endogenous substances that may be present in respiratory specimens were tested for interference (Table 25). Each potentially interfering substance was tested at or above clinically relevant levels in negative simulated clinical matrix stabilized in universal transport media in absence and presence SARS-CoV-2 target (spiked at ~3x LoD).

None of the substances interfered with the test performance by generating false-negative, false-positive or invalid results.

Table 25 List of endogenous substances tested for interference

Substance	Concentration
Human Genomic DNA	20 ng/µL
Mucus	One sputum swab/mL
Human Peripheral Blood Mononuclear Cells (PBMC)	1.0E+03 cells/µL
Human Whole Blood	1% (v/v)
Human Whole Blood	2% (v/v)
Human Whole Blood	5% (v/v)

Matrix equivalency – UTM-RT/UVT, cobas® PCR Media and 0.9% physiological saline

Equivalence between different collection media (UTM-RT/UVT, cobas® PCR Media, and saline) was evaluated using the WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146). The WHO International Standard was used to formulate to a target concentration of approximately 2x LoD (low positive) and 4x LoD (moderate positive) into paired individual negative clinical samples, stabilized either in Universal Transport Media (UTM-RT/UVT), cobas® PCR Media (CPM), or 0.9% physiological saline (NaCl). At least 20 replicates per low positive sample and 10 replicates per moderate positive sample were tested for each collection media type. All replicates tested were positive for SARS-CoV-2 in all the three collection media types. UTM-RT/UVT, cobas® PCR Media, and 0.9% physiological saline are acceptable for use with cobas® SARS-CoV-2 Qualitative.

Whole system failure

The whole system failure rate was assessed by testing 100 specimens of simulated clinical matrix spiked with WHO International Standard for SARS-CoV-2 RNA (acid-heat inactivated England/02/2020 isolate, NIBSC code: 20/146) to a concentration of approximately 3x LoD. The results of this study determined that all replicates were valid and positive for SARS-CoV-2, resulting in a whole system failure rate of 0% with an upper one-sided 95% confidence interval of 2.95%.

Cross contamination

Studies were performed to evaluate potential cross contamination on the cobas® 6800/8800 Systems using cobas® SARS-CoV-2 Qualitative. Cross-contamination can cause false positive results. In this performance study, the sample-to-sample cross-contamination rate of cobas® SARS-CoV-2 Qualitative was 0.0% (0/239; with an upper one-sided 95% CI of 1.25%) in UTM samples and 0.6% (3/480; with a 95%CI from 0.1% to 1.8%) in Saliva samples when alternating high viral level positive and negative samples were tested over multiple runs. High viral level positive samples in the study were prepared to generate a Ct value that exceeds the 95th percentile of all positive samples observed via real world monitoring of cobas® SARS-CoV-2 Qualitative (>10 million results). The likelihood of encountering such specimens in the routine use of cobas® SARS-CoV-2 Qualitative is proportional to SARS-CoV-2 prevalence in the testing population. Therefore, the sample-to-sample cross-contamination rate for saliva samples in routine use of cobas® SARS-CoV-2 Qualitative will likely be less than 0.6% x 5% x SARS-CoV-2 prevalence (in percent) in the testing population. With an assumed prevalence of 10%, the estimated cross-contamination rate would be 0.6% x 5% x 10% = 0.003%.

Performance in sample pools

The performance of cobas® SARS-CoV-2 Qualitative when testing nasopharyngeal samples collected in UTM or UVT was evaluated using one cobas® 6800 System and one cobas® 8800 System. Thirty positive samples were tested individually and in pools of 6 containing 1 positive and 5 negative samples, and in pools of 3 containing 1 positive and 2 negative samples. Additionally, negative samples were tested individually, in 20 negative pools of 6, and in 20 negative pools of 2.

The 30 individual positive specimens had pan-Sarbecovirus Target 2 Ct values between 15.1 – 35.3, including a subset of 8 Low Positive samples (~27% of the samples) with Target 2 Ct values between 33.4 and 35.3. The Low Positive subset of samples targeted within 2-3 Ct (actual 1.1 – 3) of the mean Ct for Target 2 at the Limit of Detection.

The performance of testing sample pools of 6 and pools of 3 containing one positive sample each, compared to testing individual samples, is shown in Table 26 and Table 27, respectively. Positive and presumptive positive results (as defined in

Table 14) were used for the Positive Percent Agreement (pools vs. individual) calculations, as all the constituent samples would require re-testing as separate individual samples. Results are summarized for all samples, and separately summarized for the subset of Low Positive samples, for each tested pool size.

Table 26 Reactivity in positive sample pools of 6

Samples in Pools of 6	Negative Pool Results	Invalid Pool Results	Positive or Presumptive Positive Pool Results	Total N valid Pool Results	Positive Percent Agreement (pools vs, individual)
Positive (Including Low Positive)	0	0	30*	30	100% (30/30) (95% CI: 88.6 – 100%)
Low Positive	0	0	8*	8	100% (8/8) (95% CI: 67.6 – 100%)

*Note: One low positive sample was presumptive positive when tested in a pool of 6.

Table 27 Reactivity in positive sample pools of 3

Samples in Pools of 3	Negative Pool Results	Invalid Pool Results	Positive or Presumptive Positive Pool Results	Total N valid Pool Results	Positive Percent Agreement (pools vs, individual)
Positive (Including Low Positive)	0	0	30	30	100% (30/30) (95% CI: 88.6 – 100%)
Low Positive	0	0	8	8	100% (8/8) (95% CI: 67.6 – 100%)

The performance of testing sample pools of 6 and pools of 2 containing only negative samples compared to testing individual samples, is shown in Table 28.

Table 28 Specificity in negative sample pools of 6 and pools of 2

Pool Size	Negative Pool Results	Invalid Pool Results	Positive or Presumptive Positive Pool Results	Total N valid Pool Results	Observed Negative Rate
Pools of 6	20	0	0	20	100% (20/20) (95% CI: 83.9 – 100%)
Pools of 2	20	0	0	20	100% (20/20) (95% CI: 83.9 – 100%)

Note: Some positive samples may not be detected when diluted and tested in pools. Performance estimations above may underestimate the loss of detection from testing in pools. Laboratories should also consider the assay's limit of detection when evaluating testing in pools (see **Warnings and precautions**).

Clinical performance evaluation

Performance with clinical specimens – swab specimen types

The performance of cobas® SARS-CoV-2 Qualitative was evaluated across three studies with archived or fresh, prospectively collected specimens. Combined, all three studies compared the performance of cobas® SARS-CoV-2 Qualitative across four external testing sites (one site in the EU, 3 in the US) using a common highly sensitive CE-IVD SARS-CoV-2 assay as the comparator method. The specimens in all studies were collected into VTM.

The first study consisted of archived nasopharyngeal swab (NPS) specimens from individuals with signs and symptoms of a respiratory infection evaluated at one external site. The second study consisted of one external site evaluating archived specimens from individuals without symptoms or other reasons to suspect COVID-19. The final study was a large multi-center study with three external testing sites evaluating prospectively collected fresh clinical specimens from individuals with signs and symptoms of a respiratory infection. Participants from 12 geographically distributed enrollment centers provided NPS and NS (nasal swab) specimens as part of a dual collection procedure where (a) the collection order was varied such that the first specimen collected will be ~50% NPS and ~50% NS, and (b) the collection method for NS specimens was also varied to yield ~50% self-collected and ~50% healthcare worker-collected.

Across the three studies, a total of 1,500 SARS-CoV-2 nasopharyngeal swab specimen results were evaluable and included in the data analysis. The accuracy (method correlation) of the cobas® SARS-CoV-2 Qualitative in comparison to a highly sensitive CE-IVD SARS-CoV-2 assay is shown in Table 29. Overall, the cobas® SARS-CoV-2 Qualitative sensitivity (PPA) was 97.2% (140/144) and specificity (NPA) was 99.9% (1,354/1,356).

Table 29 Summary of NPS clinical performance of cobas® SARS-CoV-2 Qualitative

Specimen Type	Target	Total (N)	PPA	PPA LCL 95% Score CI	PPA UCL 95% Score CI	NPA	NPA LCL 95% Score CI	NPA UCL 95% Score CI
Nasopharyngeal	SARS-CoV-2	1500	97.2% (140/144)	93.1%	98.9%	99.9% (1,354/1,356)	99.5%	100%

CI= confidence interval, LCL= lower confidence limit, NPA= negative percent agreement, PPA= positive percent agreement, UCL= upper confidence limit.

Additionally, the aforementioned prospective multi-center evaluation study was designed to evaluate the performance of the cobas® SARS-CoV-2 Qualitative test using NPS and NS specimens from subjects suspected of respiratory infection. This study used a composite comparator method wherein laboratory sites used up to 3 highly-sensitive CE-IVD SARS-CoV-2 assays to determine the infective status by majority rule. The composite comparator result was defined as the concordant results from 2 comparator assays (test A and test B). In case of discordance between the initial 2 comparator assays, the sample was tested by a third assay (test C) and the result of that test determined the composite comparator status.

When compared with the composite comparator result, cobas® SARS-CoV-2 Qualitative yielded a sensitivity (PPA) of 98.7% for NPS and 96.2% for NS specimens. The specificity (NPA) was 99.7% for NPS and 100% for NS specimens. cobas® SARS-CoV-2 Qualitative also demonstrated similar performance when using self-collected and healthcare worker collected nasal swab specimens as shown in Table 30.

Table 30 Summary of NPS/NS clinical performance of cobas® SARS-CoV-2 Qualitative – prospective evaluation

Specimen Type	Target	Total (N)	PPA	PPA LCL 95% Score CI	PPA UCL 95% Score CI	NPA	NPA LCL 95% Score CI	NPA UCL 95% Score CI
Nasopharyngeal*	SARS-CoV-2	938	98.7% (77/78)	93.1 %	99.8 %	99.7% (857/860)	99.0 %	99.9 %
Nasal Swab	SARS-CoV-2	941	96.2% (76/79)	89.4 %	98.7 %	100.0% (862/862)	99.6 %	100.0 %
Nasal Swab - Self Collected	SARS-CoV-2	481	100.0% (40/40)	91.2 %	100.0 %	100.0% (441/441)	99.1 %	100.0 %
Nasal Swab - HCW Collected	SARS-CoV-2	460	92.3% (36/39)	79.7 %	97.3 %	100.0% (421/421)	99.1 %	100.0 %

CI= confidence interval, LCL= lower confidence limit, NPA= negative percent agreement, PPA= positive percent agreement, UCL= upper confidence limit, HCW= healthcare worker.

* Nasopharyngeal specimen data from the prospective study are included in both Table 29 and Table 30. Test A of the SARS-CoV-2 composite comparator was the same method used as the single comparator in the summary analysis of all three studies.

Performance with clinical specimens – saliva specimen

The performance of cobas® SARS-CoV-2 Qualitative was evaluated with prospectively collected specimens. The study compared the performance of cobas® SARS-CoV-2 Qualitative at one external testing site within the EU against the paired nasopharyngeal swab result of cobas® SARS-CoV-2 Qualitative as the comparator. Nasopharyngeal specimens were collected into VTM and saliva specimens were collected as raw saliva into a sterile device.

The study was evaluating prospectively collected clinical specimens from individuals with signs and symptoms of a respiratory infection, as well from individuals without signs and symptoms of a respiratory infection. Participants provided nasopharyngeal swab and saliva specimens as part of a dual collection procedure.

In total, 130 paired patient specimens were evaluable and included in the data analysis, which included 82 (63.1%) who were symptomatic and 48 (36.9%) who were asymptomatic at the time of testing. The accuracy (method correlation) of the cobas® SARS-CoV-2 Qualitative using saliva in comparison to the cobas® SARS-CoV-2 Qualitative using nasopharyngeal swab specimen is shown in Table 31. Overall, the cobas® SARS-CoV-2 Qualitative Positive Percent Agreement (PPA) between the specimen types saliva and nasopharyngeal swab was 87.1% (54/62) and Negative Percent Agreement (NPA) was 95.6% (65/68).

Table 31 Summary of clinical performance of cobas® SARS-CoV-2 Qualitative using saliva in comparison to NPS

Specimen Type	Target	Total (N)	PPA	PPA LCL 95% Score CI	PPA UCL 95% Score CI	NPA	NPA LCL 95% Score CI	NPA UCL 95% Score CI
Saliva	SARS-CoV-2	130	87.1% (54/62)	76.6%	93.3%	95.6% (65/68)	87.8%	98.5%

CI= confidence interval, LCL= lower confidence limit, NPA= negative percent agreement, PPA= positive percent agreement, UCL= upper confidence limit.

The Positive Percent Agreement of the cobas® SARS-CoV-2 Qualitative using saliva in comparison to the cobas® SARS-CoV-2 Qualitative using nasopharyngeal swab specimen split into viral load groups is shown in Table 32. The cobas® SARS-CoV-2 Qualitative Positive Percent Agreement (PPA) between the specimen types saliva and nasopharyngeal swab was 95.0% (19/20) for NPS samples with a high viral level (Ct of target 1 (SARS-CoV-2) \leq 23), 100.0% (20/20) for NPS samples with a moderate viral level (Ct of target 1 (SARS-CoV-2) $>$ 23 to 30), and 68.2% (15/22) for NPS samples with a low viral level at and below the Limit of Detection of the NPS sample type (Ct of target 1 (SARS-CoV-2) $>$ 30).

Table 32 Positive Percent Agreement cobas® SARS-CoV-2 Qualitative using saliva in comparison to the viral level detected in the paired NPS sample

Viral level based on Ct* of paired NPS specimen	Target	Total (N)	PPA	PPA LCL 95% Score CI	PPA UCL 95% Score CI
High (NPS Ct \leq 23)	SARS-CoV-2	20	95.0% (19/20)	76.4 %	99.1 %
Moderate (NPS $>$ Ct 23 to 30)	SARS-CoV-2	20	100.0% (20/20)	83.9 %	100.0 %
Low (NPS Ct $>$ 30, at and below LoD of NPS sample type)	SARS-CoV-2	22	68.2% (15/22)	47.3 %	83.6 %

CI= confidence interval, LCL= lower confidence limit, NPA= negative percent agreement, PPA= positive percent agreement, UCL= upper confidence limit.

* Ct of target 1 (SARS-CoV-2)

Testing of the 11 saliva specimens where results were discrepant between the paired nasopharyngeal swab and saliva specimen by an alternative highly sensitive CE-IVD PCR test resulted in a 100% agreement with the cobas® SARS-CoV-2 Qualitative saliva result. All 3 cobas® SARS-CoV-2 Qualitative NPS negative, but saliva positive results were confirmed as saliva positive by the alternative test, and all 8 cobas® SARS-CoV-2 Qualitative NPS positive, but saliva negative results were confirmed as saliva negative by the alternative test. This indicates that discrepant results when using saliva as a sample type are dependent on the differences between the two specimen types rather than on the assay performance.

Reproducibility

The reproducibility of cobas® SARS-CoV-2 Qualitative was evaluated across multiple factors that theoretically could affect reported results, including: reagent lot, testing site/instrument, day, and run. The evaluation was conducted at 3 testing sites, using 3 reagent lots, with a 4-member panel of positive and negative samples resulting in a total number of 216 tests per concentration (not including controls). The positive panel members contained SARS-CoV-2 viral culture material [WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146)] at 3 different concentrations in universal transport medium (UTM) based simulated clinical matrix. Each site tested two reagent lots for 6 days. Two runs were performed each day and 3 replicates of each panel member were performed for each run. An overall SARS-CoV-2 positive result was determined by a positive detection in either or both of the SARS-CoV-2 or/and pan-Sarbecovirus channels. The evaluation results are summarized in Table 33.

The test results showed good lot-to-lot, instrument-to-instrument (site), day-to-day, and between batch variation for the \sim 0.3x LoD, \sim 1x LoD, and \sim 3x LoD panel members (Table 33). Regardless of viral targets and viral concentrations, most of the variability was within batches, ranging from 79.5% to 100%. Site-to-site variability ranged from 0.0% to 10.1%, and between-batch variability ranged from 0.0% to 16.0%.

Table 33 Overall mean estimate, standard deviations, and coefficients of variation (%) for cycle threshold values by viral target and expected viral concentration (positive panel members)

Viral Target	Panel Member Concentration	n ^a /N	Mean Ct ^b	Site SD	Site CV(%)	Lot SD	Lot CV(%)	Day SD	Day CV(%)	Batch SD	Batch CV(%)	Within Batch SD	Within Batch CV(%)	Total SD ^b	Total CV(%) ^c
SARS-CoV-2	~0.3x LoD	45/216	33.6	0.00	0.0	0.00	0.0	0.11	0.3	0.00	0.0	0.35	1.1	0.37	1.1
SARS-CoV-2	~1x LoD	196/216	33.2	0.00	0.0	0.09	0.3	0.00	0.0	0.17	0.5	0.37	1.1	0.42	1.3
SARS-CoV-2	~3x LoD	216/216	32.2	0.05	0.2	0.02	0.1	0.00	0.0	0.03	0.1	0.24	0.8	0.25	0.8
pan-Sarbecovirus	~0.3x LoD	158/216	36.5	0.18	0.5	0.00	0.0	0.00	0.0	0.00	0.0	0.71	2.0	0.74	2.0
pan-Sarbecovirus	~1x LoD	214/216	35.4	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.67	1.9	0.67	1.9
pan-Sarbecovirus	~3x LoD	216/216	34.1	0.11	0.3	0.05	0.2	0.00	0.0	0.00	0.0	0.32	0.9	0.34	1.0

Ct = cycle threshold; LoD = limit of detection; SD = standard deviation; CV(%) = percent coefficient of variation; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Note: SARS-CoV-2 is a dual target assay. Inactivated viral culture material was diluted to ~0.3/1/3x LoD based on the target 2 (SARS-CoV-2) LoD.

^a n is the number of positive tests which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.

^b The mean and total standard deviation (SD) estimates were calculated from the PROC MIXED procedure.

^c Total CV(%) = (SD/Mean)*100.

The system showed a 99.1% negative percent agreement with a 95% CI of 96.7%-99.9%. Of the 216 valid tests, 2 tests were positive (1 each for SARS-CoV-2 and pan-Sarbecovirus). Post-amplification DNA sequencing confirmed the presence of an amplification product in 1 sample (pan-Sarbecovirus positive, Ct 36.7) and did not detect amplification product for either target in the other (SARS-CoV-2 positive, Ct 34.4). The Ct values and the curve analysis of the reactive negative panel member may suggest a low level of contamination during specimen handling.

Additional information

Key test features

Sample type	Nasopharyngeal and oropharyngeal swab samples collected in the Copan UTM-RT System or the BD™ UVT System Nasal swab samples collected in the Copan UTM-RT System, the BD™ UVT System, the cobas ® PCR Media, and 0.9% physiological saline Saliva samples
Minimum amount of sample required	Swab specimen types: 0.6 or 1.0 mL ^{*,**} Liquified Saliva: 1.2 mL
Sample processing volume	Swab specimen types: 0.4 mL Liquified Saliva: 0.85 mL














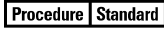






































*Dead volume of 0.2 mL is identified for the **cobas** **omni** Secondary Tubes. Dead volume of 0.6 mL is identified for the **cobas**® PCR Media primary tubes. Other tubes compatible with **cobas**® 6800/8800 Systems (consult User Assistance Guide) may have different dead volume and require more or less minimum volume.

**Additional volume is required if pooling.

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 34 Symbols used in labeling for Roche PCR diagnostics products

 Age/DOB	 Device not for near-patient testing	 QS IU/PCR	QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
 Ancillary Software	 Device not for self-testing		
 Assigned Range [copies/mL]	 Distributor (Note: The applicable country/region may be designated beneath the symbol)	 SN	Serial number
 Assigned Range [IU/mL]	 Do not re-use	 Site	Site
 Authorized representative in the European Community	 Female	 Procedure Standard	Standard Procedure
 Barcode Data Sheet	 For IVD performance evaluation only	 STERILE EO	Sterilized using ethylene oxide
 Batch code	 Global Trade Item Number	 Store in dark	
 Biological risks	 Importer	 Temperature limit	
 Catalogue number	 In vitro diagnostic medical device	 Test Definition File	
 CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	 Lower Limit of Assigned Range	 This way up	
 Collect date	 Male	 Procedure UltraSensitive	Ultrasensitive Procedure
 Consult instructions for use	 Manufacturer	 UDI	Unique Device Identifier
 Contains sufficient for <n> tests	 Negative control	 ULR	Upper Limit of Assigned Range
 Content of kit	 Non-sterile	 Urine Fill Line	Urine Fill Line
 Control	 Patient Name	 Rx Only	US Only: Federal law restricts this device to sale by or on the order of a physician.
 Date of manufacture	 Patient number	 Use-by date	
 Device for near-patient testing	 Peel here		
 Device for self-testing	 Positive control		
	 QS copies / PCR		QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.

Technical support

For technical support (assistance) please reach out to your local affiliate:

https://www.roche.com/about/business/roche_worldwide.htm

Manufacturer and importer

Table 35 Manufacturer and importer



Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876 USA
www.roche.com

Made in USA



Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Trademarks and patents

This product is covered by one or more of US Patent Nos. 8962293, 9102924, 8609340, 9234250, 8097717, 8192958, 10059993, 10358675, 8129118, and 6727067, and foreign equivalent patents of each.

COBAS, COBAS OMNI, and AMPERASE are trademarks of Roche.

The trademark “Armored RNA®” is owned by Asuragen, Inc. and Cenetron Diagnostics, Ltd.

All other product names and trademarks are the property of their respective owners.

Carryover prevention technology in the AmpErase® enzyme is covered by U.S. Patent 7,687,247 owned by Life Technologies and licensed to Roche Molecular Systems, Inc.

See <http://www.roche-diagnostics.us/patents>

Copyright

©2021 Roche Molecular Systems, Inc.



Roche Diagnostics GmbH
Sandhofer Str. 116
68305 Mannheim
Germany



References

1. Center for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.
2. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.

Document revision

Document Revision Information	
Doc Rev. 1.0 12/2021	First Publishing.