Rx Only

cobas® SARS-CoV-2

Qualitative assay for use on the cobas® 6800/8800 Systems

For use under the Emergency Use Authorization (EUA) only

For in vitro diagnostic use

cobas® SARS-CoV-2 - 192T	P/N: 09175431190
cobas® SARS-CoV-2 - 480T	P/N: 09343733190
cobas® SARS-CoV-2 Control Kit	P/N: 09175440190
cobas® 6800/8800 Buffer Negative Control Kit	P/N: 07002238190

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Intended use

cobas® SARS-CoV-2 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 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. **cobas**® SARS-CoV-2 is for use only under Emergency Use Authorization (EUA) in laboratories certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high or moderate complexity tests.

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. Testing of pooled samples is limited to laboratories certified under CLIA, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

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; 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, recent exposures and epidemiological information. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities. **cobas**® SARS-CoV-2 is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and on the use of the **cobas**® 6800/8800 Systems. **cobas**® SARS-CoV-2 is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and explanation of the test

Explanation of the test

cobas® SARS-CoV-2 is a qualitative test for use on the **cobas**® 6800 System and **cobas**® 8800 System for the detection of the 2019 novel coronavirus (SARS-CoV-2) RNA in 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 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 software, 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 with each **cobas**® SARS-CoV-2 run.

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 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 deoxythimidine 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 can be found in Table 1. Materials required, but not provided can be found in Table 2, Table 3, Table 4, Table 7, Table 8, 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 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

cobas® SARS-CoV-2
Store at 2-8°C
192 test cassette (P/N 09175431190)
480 test cassette (P/N 09343733190)

Kit components	Reagent ingredients	Quantity per kit	
		192 tests	**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. 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 Master Mix Reagent 2 (SARS-CoV-2 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 Control Kit

cobas® SARS-CoV-2 Control Kit Store at 2–8°C (P/N 09175440190)		
Kit components	**Reagent ingredients**	**Quantity per kit**
SARS-CoV-2 Positive Control (SARS-CoV-2 (+)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 07002238190)		
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	  **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H314: Causes severe burns and eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/fume/gas/mist/vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face 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. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
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 test kits. See listing of additional materials required (Table 7).

** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance

Reagent storage and handling 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 -192T	2–8°C
cobas® SARS-CoV-2 -480T	2–8°C
cobas® SARS-CoV-2 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

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 – 192T	Date not passed†	90 days from first usage†	Max 40 runs†	Max 40 hours†
cobas® SARS-CoV-2 – 480T	Date not passed†	90 days from first usage†	Max 20 runs†	Max 20 hours†
cobas® SARS-CoV-2 Control Kit	Date not passed†	Not applicable[a]	Not applicable	Max 8 hours†
cobas® Buffer Negative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 10 hours
cobas omni Lysis Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable

[a] Single use reagents

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

† The performance has not been established for suggested use cycles and time, but is based on similar reagents used on the same system.

Additional materials required

Table 7 Materials and consumables for use on **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 16mm and RD5 racks are required to use **cobas**® SARS-COV-2. 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 16mm 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.

Table 8 Alternative Specimen collection kits used with **cobas**® SARS-CoV-2

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

Instrumentation and software required

The **cobas**® 6800/8800 software and **cobas**® SARS-CoV-2 analysis package 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 (Option Moveable)	05524245001 and 06379672001
cobas® 6800 System (Fix)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001
Instrument Gateway	06349595001

For additional information, please refer to the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide.
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 under Emergency Use Authorization only.
- The **cobas**® SARS-CoV-2 for use on the **cobas**® 6800/8800 Systems is a real-time RT-PCR test that has not been FDA cleared or approved but has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform moderate or high complexity tests. Testing of pooled samples is limited to laboratories certified under CLIA, 42 U.S.C. §263a, that meet requirements to perform high complexity tests only.
- The **cobas**® SARS-CoV-2 for use on the **cobas**® 6800/8800 Systems is a real-time RT-PCR test that has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of the **cobas**® SARS-CoV-2 for use on the **cobas**® 6800/8800 Systems is a real-time RT-PCR test that is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or the authorization is revoked sooner.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- 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 and **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.

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 test kits, **cobas**® SARS-CoV-2 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 kits, **cobas**® SARS-CoV-2 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

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

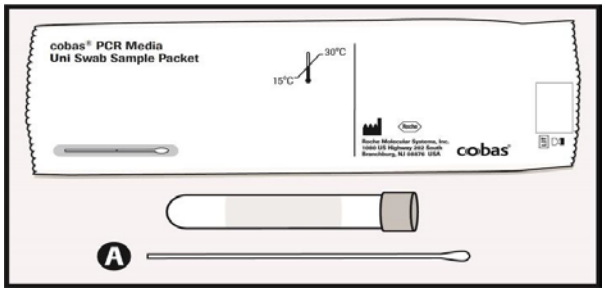
Table 10 Overview of collection devices and sample types

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.

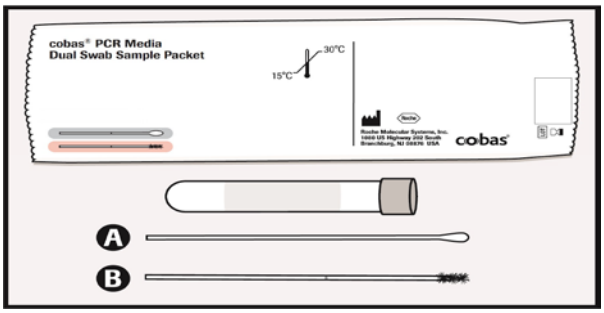
Nasal (anterior nares) swab specimen collection – healthcare provider or self-collected on site

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

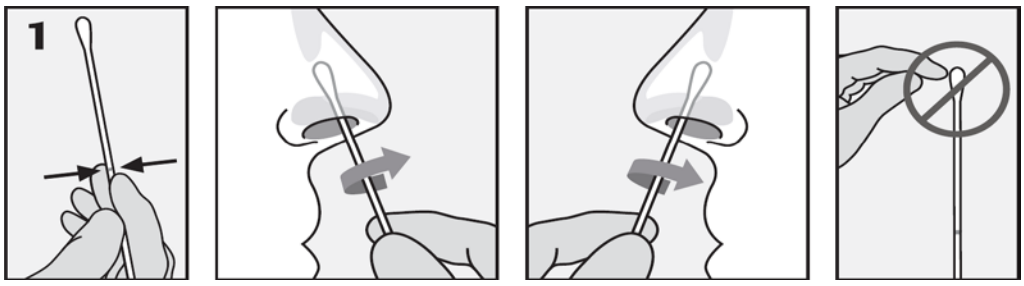


The **cobas**® PCR Media Uni Swab Sample kit contains:
cobas® PCR Media tube, Woven Swab A

OR

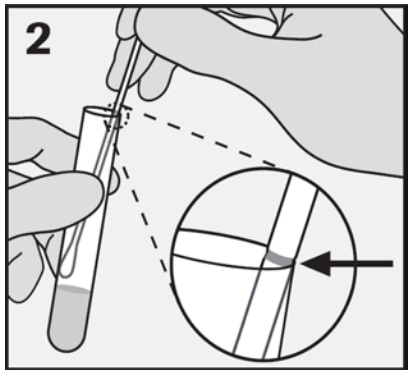


The **cobas**® PCR Media Dual Swab Sample kit contains:
cobas® PCR Media Tube, Woven Swab A, Flocked Swab B

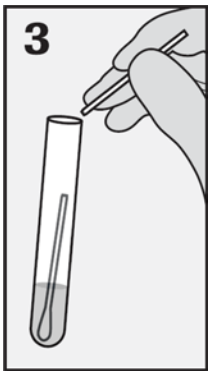


DO NOT PRE-WET SWAB IN cobas® PCR MEDIA BEFORE COLLECTION!

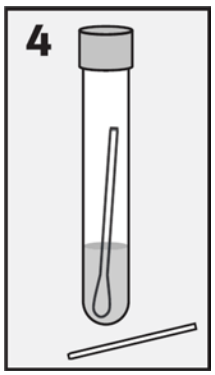
1. **COLLECT**: Hold the woven swab (Swab A) or the flocked swab (Swab B) with the scoreline above your hand. Insert the swab 1–2 cm into one of the anterior nares. Rotate the swab against the nasal mucosa for about 3 seconds and withdraw. Repeat with the other anterior nare using the same swab.



2. **ALIGN**: Remove the cap from the **cobas**® PCR Media Tube and lower the swab specimen into the tube until the visible scoreline on the swab is aligned with the tube rim.



3. **BREAK**: Carefully leverage the swab against the tube rim to break the swab shaft at the scoreline.



4. **CLOSE**: **Tightly** re-cap the **cobas**® PCR Media Tube. The specimen is now ready for transport. Discard the top portion of the swab.

- 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.

Transport and storage

- Transportation of collected specimens must comply with all applicable regulations for the transport of etiologic agents.
- Transport and store samples collected in **cobas**® PCR Media or 0.9% physiological saline as follows:
  - After collection, specimens in **cobas**® PCR Media or 0.9% physiological saline should be stored at 2-8°C and processed within 48 hours.
- Sample stability when using **cobas**® SARS-CoV-2 has not been established for suggested temperatures and time, but is based on viability data from testing similar viruses in the UTM-RT or UVT Systems as stated in Copan UTM-RT System Instructions For Use and shown below:
  - After collection, the specimen should be stored at 2-25°C and processed within 48 hours.
  - If delivery and processing exceed 48 hours, specimens should be transported in dry ice and once in laboratory frozen at -70°C or colder.

Instructions for use

Procedural notes

- Do not use **cobas**® SARS-CoV-2 reagents, **cobas**® SARS-CoV-2 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.
- Refer to the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide for proper maintenance of instruments.

Running cobas® SARS-CoV-2

cobas® SARS-CoV-2 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 Copan Universal Transport Medium (UTM-RT), BD™ Universal Viral Transport (UVT), **cobas**® PCR Media or 0.9% physiological saline must be transferred into a cobas omni Secondary tube prior to processing on the **cobas**® 6800/8800 Systems. Samples transferred to **cobas omni** Secondary tubes should be processed using the 'Swab' sample type selection in the user interface (UI) of the **cobas**® SARS-CoV-2 as described in Table 11.

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

Samples collected using **cobas**® PCR Media Uni Swab Sample Kit or **cobas**® PCR Media Dual Swab Sample 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 should be processed using the '**cobas**® PCR Media swab' sample type selection in the user interface (UI) of the **cobas**® SARS-CoV-2 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 IFU 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.

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

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	**Swab**
cobas® PCR Media Uni or Dual Swab Sample Kit	1.0 mL Primary tube	**cobas® PCR Media swab**

Sample pooling for SARS-CoV-2 testing

Pools of up to and including 6 samples may be tested using **cobas**® SARS-CoV-2. 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.

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

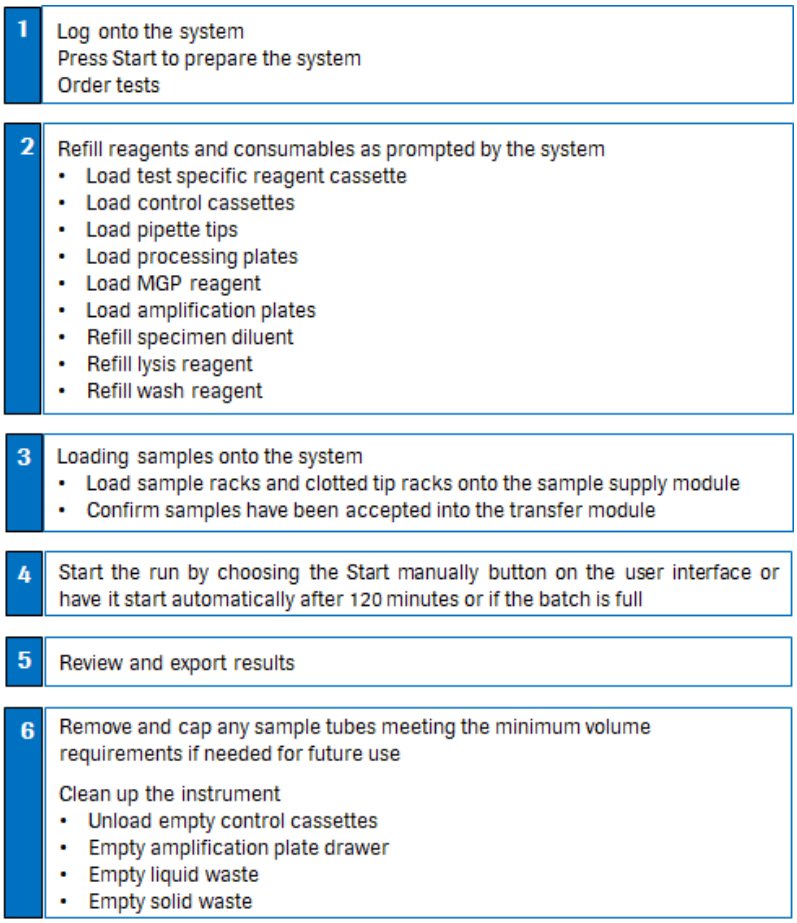
Interpretation of pool results is the same as for individual results as described in the **Interpretation of results** section.

- If the result of the pool is negative, then each constituent sample is 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. If the result of the pool is invalid, each constituent sample should be re-tested as a separate individual sample in order to avoid potential delay in reporting valid patient results. However, if the invalid is due to entire run failure or other instrument malfunctions, the pool may be re-tested if sufficient volume is available.

cobas® SARS-CoV-2 procedure

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 procedure



Results

The **cobas**® 6800/8800 Systems automatically detect the SARS-CoV-2, for each individually processed or pooled 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 [(-) Ctrl] and one [SARS-CoV-2 (+)C] are processed with each batch.
- In the **cobas**® 6800/8800 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 software based on negative and positive control performance.

Interpretation of results

cobas® SARS-CoV-2 for System Software v1.2

Display examples for **cobas**® SARS-CoV-2 for System Software v1.2 are shown in Figure 2.

Figure 2 Example of **cobas**® SARS-CoV-2 results display for System Software v1.2

Test	Sample ID	Valid*	Flags	Sample type	Overall result*	Target 1	Target 2
SARS-CoV-2 400 µL	Swab_01	Yes		Swab	**Negative**	Negative	Negative
SARS-CoV-2 400 µL	Swab _C1	No	Y40T	Swab	**Invalid**	Invalid	Invalid
SARS-CoV-2 400 µL	Swab _B1	Yes		Swab	**Reactive**	Negative	Positive
SARS-CoV-2 400 µL	Swab _B2	Yes		Swab	**Positive**	Positive	Positive
SARS-CoV-2 400 µL	Swab _D1	Yes		Swab	**Negative**	Negative	Negative
SARS-CoV-2 400 µL	Swab _A6	Yes		Swab	**Reactive**	Positive	Negative
SARS-CoV-2 400 µL	Swab _E1	No	C01H2	Swab	**Invalid**	Positive	Invalid
SARS-CoV-2 400 µL	Swab _A2	No	C01H1	Swab	**Invalid**	Invalid	Positive
SARS-CoV-2	C161420284090428828404	Yes		(-) Ctrl	**Valid**	Valid	Valid
SARS-CoV-2	C16142028409300958026 4	Yes		SARS-CoV-2 (+) C	**Valid**	Valid	Valid

* The “Valid” and “Overall Result” columns are not applicable to sample results for the **cobas**® SARS-CoV-2. Values reported in these columns are not applicable and do not impact the validity of results reported within individual Target Result columns. Refer to Table 13, **cobas**® SARS-CoV-2 results interpretation, for specific instructions on test results interpretation.

cobas® SARS-CoV-2 for System Software v1.3 or higher

Display examples for **cobas**® SARS-CoV-2 for System Software v1.3 or higher are shown in Figure 3.

Figure 3 Example of **cobas**® SARS-CoV-2 results display for System Software v1.3 or higher

Test	Sample ID	Valid*	Flags	Sample type	Overall result*	Target 1	Target 2
SARS-CoV-2 400 µL	Swab_01	NA		Swab	**NA**	Negative	Negative
SARS-CoV-2 400 µL	Swab _C1	NA	Y40T	Swab	**NA**	Invalid	Invalid
SARS-CoV-2 400 µL	Swab _B1	NA		Swab	**NA**	Negative	Positive
SARS-CoV-2 400 µL	Swab _B2	NA		Swab	**NA**	Positive	Positive
SARS-CoV-2 400 µL	Swab _D1	NA		Swab	**NA**	Negative	Negative
SARS-CoV-2 400 µL	Swab _A6	NA		Swab	**NA**	Positive	Negative
SARS-CoV-2 400 µL	Swab _E1	NA	C01H2	Swab	**NA**	Positive	Invalid
SARS-CoV-2 400 µL	Swab _A2	NA	C01H1	Swab	**NA**	Invalid	Positive
SARS-CoV-2	C161420284090428828404	Yes		(-) Ctrl	**Valid**	Valid	Valid
SARS-CoV-2	C161420284093009580264	Yes		SARS-CoV-2 (+) C	**Valid**	Valid	Valid

* The "Valid" and "Overall Result" columns are not applicable to sample results for the **cobas**® SARS-CoV-2. Values reported in these columns are not applicable and do not impact the validity of results reported within individual Target Result columns. Refer to Table 13, **cobas**® SARS-CoV-2 results interpretation, for specific instructions on test results interpretation.

Interpretation of results

The following result interpretation applies to both **cobas**® 6800/8800 software version 1.2 and **cobas**® 6800/8800 software version 1.3 and higher.

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

- A valid batch may include both valid and invalid sample results.
- **The "Valid" and "Overall Result" columns are not applicable to sample results for the cobas® SARS-CoV-2. Values reported in these columns are not applicable and do not impact the validity of results reported within individual Target Result columns.**
- Invalid results for one or more target combinations are possible and are reported out specifically for each channel.
- Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.

Results and their corresponding interpretation for detecting SARS-CoV-2 are shown below (Table 13).

Table 13 cobas® SARS-CoV-2 results interpretation

Target 1	Target 2	Interpretation
Positive	**Positive**	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Positive	**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.
Negative	**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.
Negative	**Negative**	All Target Results were valid. Result for SARS-CoV-2 RNA is Not Detected.
Positive	**Invalid**	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Invalid	**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.
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	**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.

Procedural limitations

- **cobas**® SARS-CoV-2 has been evaluated only for use in combination with the **cobas**® SARS-CoV-2 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.
- Reliable results depend on proper sample collection, storage and handling procedures.
- 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. Testing of other sample types with **cobas**® SARS-CoV-2 may result in inaccurate results.
- 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.
- As with any molecular test, mutations within the target regions of **cobas**® SARS-CoV-2 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 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 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.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Conditions of Authorization for the Laboratory

The **cobas**® SARS-CoV-2 test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas

To assist clinical laboratories running the **cobas**® SARS-CoV-2 test, the relevant Conditions of Authorization are listed verbatim below, and are required to be met by laboratories performing the EUA test.

A. Authorized laboratories[1] using the **cobas**® SARS-CoV-2 test must include with result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
B. Authorized laboratories using the **cobas**® SARS-CoV-2 test must perform the **cobas**® SARS-CoV-2 test as outlined in the **cobas**® SARS-CoV-2 Instructions for Use. Deviations from the authorized procedures, including

the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the **cobas**® SARS-CoV-2 test are not permitted.

C. Authorized laboratories that receive the **cobas**® SARS-CoV-2 test must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

D. Authorized laboratories using the **cobas**® SARS-CoV-2 test must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories using specimen pooling strategies when testing patient specimens with the **cobas**® SARS-CoV-2 test must include with negative test result reports for specific patients whose specimen(s) were the subject of pooling, a notice that pooling was used during testing and that "Patient specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing."

F. Authorized laboratories implementing pooling strategies for testing patient specimens must use the "Use of pooling based on prevalence" and "Monitoring plan for use of pooling" recommendations available in the authorized labeling to evaluate the appropriateness of continuing to use such strategies based on the recommendations in the protocol.

G. Authorized laboratories must keep records of specimen pooling strategies implemented including type of strategy, date implemented, and quantities tested, and test result data generated as part of the Protocol for Monitoring of Specimen Pooling Testing Strategies. For the first 12 months from the date of their creation, such records will be made available to FDA within 48 business hours for inspection upon request, and will be made available within a reasonable time after 12 months from the date of their creation.

H. Authorized laboratories must collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Roche Diagnostics US Customer Technical Support 1-800-526-1247 any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.

I. All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.

J. RMS, its authorized distributor(s) and authorized laboratories using the **cobas**® SARS-CoV-2 test must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] Authorized laboratories are laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high or moderate complexity tests, except testing of pooled samples is limited to laboratories certified under CLIA, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

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, efficiency of the pooling workflow, and Positive Percent Agreement (PPA) for the desired pool size (where applicable). Laboratories should retain the generated test results data for inspection by the FDA upon request.

If the laboratory has historical data generated in the laboratory testing individual samples:

To maximize pooling efficiency, if the laboratory has historical data from the previous 7-10* days generated in the laboratory testing individual samples, we recommend estimating the positivity rate ($P_{individual}$) based on individual testing results. For this analysis, calculate the number of patients tested during those days. Also calculate the number of patients with positive results (K). The positivity rate for individual testing $P_{individual}$ is the number of positive results (K) divided by the total number of tested patients during these 7-10* days. The laboratory should then identify the n number of samples to pool based on the current positivity rate $P_{individual}$ using Table 14 below.

If the laboratory has historical data for individual samples from the previous 7-10 days, consider the following:

- If $P_{individual}$ is less than 5%, n=6 should be selected
- For calculation of efficiency of 6-sample pooling, use formula: $F=1/(1+1/6-(1-P)^6)$. For example, for $P_{individual}$=1%, the efficiency F is 4.44 for n=6 and it means that 1,000 tests can cover testing of 4,440 patients on average.
- If $P_{individual}$ is greater than 25%, then Dorfman pooling of patient specimens is not efficient and should not be applied.

Table 14 provides estimated maximal efficiency gained based on N-sample pooling and on the percent of SARS-CoV-2 positive samples in a population.

Table 14 Efficiency of pooling based on prevalence

P, percent of positive subjects in the tested population	*$n_{maxefficiency}$ (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 patients can be tested with n-sample

pools compared to individual testing. For example, a 5-sample pooling strategy increases the number of tested patients by 2.15 times for positivity rate P of 6% (F=2.15). At F=2.15, 1,000 tests can cover testing of 2,150 patients on average.

Prior to implementation of pooling, it is recommended that laboratories apply the Ct interval ranges for Zone 0, Zone 1, and Zone 2 and PPA equation to their historical individual testing data (if available) to estimate the PPA for their testing population described in the **In silico estimated performance in pools of 6, 5, 4 and 3** section. A PPA of at least 85% is acceptable for implementation of testing using pools of 6 samples or less (i.e., pools of 5, 4, 3 or 2). Furthermore, a PPA of at least 85% for a pool size of n is acceptable for implementation of testing using pools of n samples or less (i.e., a PPA of at least 85% in a pool of 5, 4, 3 or 2 is acceptable for implementation of testing using pools of that size or smaller).

If the laboratory does not have historical data generated in the laboratory testing individual samples:
If the laboratory does not have historical data from the previous 7-10 days, it may implement 6, 5, 4, 3 or 2 specimen pooling because the **cobas**® SARS-CoV-2 EUA assay has been validated for 6-specimen pooling. However, note that without $P_{individual}$, the laboratory may choose a pooling size that does not maximize pooling efficiency.

Monitoring plan for use of pooling

Laboratories should evaluate the appropriateness of the pooling and pool size using the FDA recommended monitoring procedure described below. Laboratories may also consider the sensitivity of pooled testing based on the assay's Limit of Detection.

Ongoing assessment of positivity rate during application of the initial selected n-sample pooling strategy:

a. If historical data on testing individual samples from the laboratory is <u>available</u>:
   - The percent positivity rate, P_{pools}, should be updated daily using a moving average of the data from pooled samples from the previous 7-10* days. If P_{pools} is less than 85% of $P_{individual}$ ($P_{pools} < 0.85 \cdot P_{individual}$), then it is recommended that the pool size be adjusted to maximize pooling efficiency, according to the criteria in Table 14.
   - It is recommended that $n_{maxefficiency}$, using P_{pools} and Table 14, be re-assessed periodically while sample pooling is implemented by the laboratory to ensure maximum pooling efficiency.

b. If historical data on testing individual samples from the laboratory is <u>unavailable</u>:
   - After initiating the pooling strategy, calculate the initial pooling positivity rate ($P_{pools\text{-}initial}$) for the first 7-10* days using a moving average of the data from n pool testing results.
   - If $P_{pools\text{-}initial}$ is greater than 25%, then Dorfman pooling of patient specimens is not efficient and should cease.
   - Following the first 7-10* day period of sample pooling, calculate the pooling positivity rate ($P_{pools\text{-}x}$) for the next 7-10* day period based on n pool testing results.
     If $P_{pools\text{-}x}$ is less than 90% of $P_{pool\text{-}initial}$ ($P_{pools\text{-}x} < 0.90 \cdot P_{pools\text{-}initial}$), it is recommended that the pool size be adjusted to maximize pooling efficiency, according to the criteria in Table 14.
   - It is recommended that that $n_{maxefficiency}$, using $P_{pools\text{-}x}$ and Table 14, be re-assessed periodically while sample pooling is implemented by the laboratory to ensure maximum pooling efficiency.

* It is recommended that $P_{individual}$ be calculated from the previous 7-10 days, while P_{pools} and $P_{pools\text{-}x}$ are calculated from data collected during a 7-10 day time frame. However, when determining if 7-10 days is appropriate, take into consideration the laboratory testing volume and percent positivity, among other factors. Note that if the number of individual or pooled positive results collected during a given time frame is less than 10, $P_{individual}$, P_{pools} and $P_{pools\text{-}x}$ may not be representative of the percent positivity in the testing population and the laboratory may want to consider extending the testing time period to increase the chance of capturing positives.

Non-clinical performance evaluation

Key performance characteristics

Analytical sensitivity

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 test positive.

To determine the LoD, a cultured virus of an isolate from a US patient (USA-WA1/2020, catalog number NR-52281, lot number 70033175, 2.8E+05 $TCID_{50}$/mL[1]) was serially diluted in simulated clinical matrix. A total of 7 concentration levels, with 3-fold serial dilutions between the levels, were tested with a total of 21 replicates per concentration, with an additional 10 replicates of a blank sample (i.e., simulated clinical matrix).

As shown in Table 15, the concentration level with observed hit rates greater than or equal to 95% were 0.009 and 0.003 $TCID_{50}$/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively. As shown in Table 16, the Probit predicted 95% hit rates were 0.007 and 0.004 $TCID_{50}$/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively.

Table 15 LoD determination using USA-WA1/2020 strain

Strain	Concentration [$TCID_{50}$/mL]	Total valid results	Hit rate [%]^		Mean Ct*	
			Target 1	Target 2	Target 1	Target 2
USA-WA1/2020 (stock concentration 2.8E+05 $TCID_{50}$/mL)	0.084	21	100	100	31.0	33.0
	0.028	21	100	100	31.8	34.1
	0.009	21	100	100	32.7	35.2
	0.003	21	38.1	100	33.5	36.4
	0.001	21	0	52.4	n/a	37.9
	0.0003	21	0	14.3	n/a	37.2
	0.0001	21	0	9.5	n/a	38.5
	0 (blank)	10	0	0	n/a	n/a

^ All replicates where Target 1 was positive were also positive for Target 2.

* Calculations only include positive results.

Table 16 Probit predicted 95% hit rates using USA-WA1/2020 strain

Strain	Probit Predicted 95% Hit Rate [$TCID_{50}$/mL]	
	Target 1	Target 2
USA-WA1/2020 (stock concentration 2.8E+05 $TCID_{50}$/mL)	0.007 (95% CI: 0.005 – 0.036)	0.004 (95% CI: 0.002 – 0.009)

[1] The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-WA1/2020, NR-52281.

The analytical sensitivity of the assay was tested with AccuPlex SARS-CoV-2 (Lot #105324), a quantitated reference material – recombinant Sindbis virus particle containing target sequences from the SARS-CoV-2 genome. The concentration level in a dilution series with observed hit rates greater than or equal to 95% was 46 copies/mL for both Target 1 and Target 2. Probit model 95% LoD estimates based on these data were 25 copies/mL (95% CI: 17 – 58 copies/mL) for Target 1 and 32 copies/ml (95% CI: 21 – 73 copies/mL) for Target 2.

Reactivity/inclusivity

In silico analysis concluded that **cobas**® SARS-CoV-2 will detect all analyzed SARS-CoV-2 sequences in NCBI and in GISAID databases.

cobas® SARS-CoV-2 had 99.99% match to all but one sequence in NCBI and nine sequences in GISAID for Target 1 (NCBI (n = 87,333); GISAID (n = 657,736)).

cobas® SARS-CoV-2 had 99.99% match to all but twenty-seven sequences in GISAID for Target 2 (NCBI (n = 87,333); GISAID (n = 657,736)).

None of the sequences in NCBI or GISAID had mismatches in both target regions, therefore there is no predicted impact on the **cobas**® SARS-CoV-2 test performance.

Cross-reactivity

In silico analysis

The in silico analysis for possible cross-reactions with all the organisms listed in Table 17 was conducted by mapping primers in **cobas**® SARS-CoV-2 individually to the sequences downloaded from NCBI and GISAID databases. If any two of the primers were mapped to a sequence on opposite strands with short distance apart, potential amplifications were flagged. No potential unintended cross reactivity is expected based on this in silico analysis.

Table 17 In silico analysis for SARS-CoV-2

Strain	In Silico Analysis for % Identity to Target 1 (nCoV)	In Silico Analysis for % Identity to Target 2 (Pan-Sarbecovirus 1)
CoV 229E	74.47	No alignment was found*
CoV OC43	72.26	No alignment was found*
CoV HKU1	76.52	No alignment was found*
CoV NL63	71.32	No alignment was found*
SARS-CoV	95.04	100
MERS	No alignment was found*	No alignment was found*
AdV	No alignment was found*	No alignment was found*
HMPV	No alignment was found*	No alignment was found*
HPIV1	No alignment was found*	No alignment was found*
HPIV2	No alignment was found*	No alignment was found*
HPIV3	No alignment was found*	No alignment was found*
HPIV4	No alignment was found*	No alignment was found*
Flu A	No alignment was found*	No alignment was found*
Flu B	No alignment was found*	No alignment was found*

Strain	In Silico Analysis for % Identity to Target 1 (nCoV)	In Silico Analysis for % Identity to Target 2 (Pan-Sarbecovirus 1)
EV	No alignment was found*	No alignment was found*
RSV	No alignment was found*	No alignment was found*
RV	No alignment was found*	No alignment was found*
Chlamydia pneumoniae	No alignment was found*	No alignment was found*
Haemophilus influenzae	No alignment was found*	No alignment was found*
Legionella pneumophila	No alignment was found*	No alignment was found*
MTB Mycobacterium bovis subsp. Bovis	No alignment was found*	No alignment was found*
Streptococcus pneumoniae	No alignment was found*	No alignment was found*
Streptococcus pyrogenes	No alignment was found*	No alignment was found*
Bordetella pertussis	No alignment was found*	No alignment was found*
Mycoplasma pneumoniae	No alignment was found*	No alignment was found*
Pneumocystis jirovecii	No alignment was found*	No alignment was found*
Influenza C	No alignment was found*	No alignment was found*
Parechovirus	No alignment was found*	No alignment was found*
Candida albicans	No alignment was found*	No alignment was found*
Corynebacterium diphtheriae	No alignment was found*	No alignment was found*
Legionella non-pneumophila	No alignment was found*	No alignment was found*
Bacillus anthracis (Anthrax)	No alignment was found*	No alignment was found*
Moraxella catarrhalis	No alignment was found*	No alignment was found*
Neisseria elongate and meningitides	No alignment was found*	No alignment was found*
Pseudomonas aeruginosa	No alignment was found*	No alignment was found*
Staphylococcus epidermidis	No alignment was found*	No alignment was found*
Staphylococcus salivarius	No alignment was found*	No alignment was found*
Leptospira	No alignment was found*	No alignment was found*
Chlamydia psittaci	No alignment was found*	No alignment was found*
Coxiella burnetii (Q-Fever)	No alignment was found*	No alignment was found*
Staphylococcus aureus	No alignment was found*	No alignment was found*

Note: * The amplicon sequences were blasted against all the exclusive sequences with very low stringency cutoff (50% and 100bp). No alignment were found passing the cutoff and no concerns for cross-reactivity were observed.

Cross reactivity testing

Cross-reactivity of **cobas**® SARS-CoV-2 was evaluated by testing whole organisms. As listed in Table 18, a panel of multiple unique sub-species of microorganisms were tested. High titer stocks of the potentially cross-reacting microorganisms were spiked into negative simulated clinical matrix to a concentration level of 1.0E+05 units/mL for viruses and 1.0E+06 units/mL for other microorganisms, unless otherwise noted.

None of the organisms tested interfered with **cobas**® SARS-CoV-2 performance by generating false positive results.

Table 18 Cross-reactivity test results

Microorganism	Concentration	Target 1 Result	Target 2 Result
Human coronavirus 229E	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Human coronavirus OC43	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Human coronavirus HKU1	1.0E+05 cp/mL	Negative	Negative
Human coronavirus NL63	1.0E+05 $TCID_{50}$/mL	Negative	Negative
MERS coronavirus	1.0E+05 genomic equivalent/mL	Negative	Negative
SARS coronavirus	1.0E+05 PFU/mL	Negative	Positive
Adenovirus B (Type 34)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Human Metapneumovirus (hMPV)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 1	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 2	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 3	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Parainfluenza virus Type 4	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Influenza A (H1N1)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Influenza B	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Enterovirus E (Type 1)	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Respiratory syncytial virus	1.0E+05 PFU/mL	Negative	Negative
Rhinovirus	1.0E+05 $TCID_{50}$/mL	Negative	Negative
Chlamydia pneumonia	1.0E+06 $TCID_{50}$/mL	Negative	Negative
Haemophilus influenzae	1.0E+06 CFU/mL	Negative	Negative
Legionella pneumophila	1.0E+06 CFU/mL	Negative	Negative
Mycobacterium tuberculosis	1.0E+06 cells/mL	Negative	Negative
Streptococcus pneumonia	1.0E+06 CFU/mL	Negative	Negative
Streptococcus pyrogenes	1.0E+06 CFU/mL	Negative	Negative
Bordetella pertussis	1.0E+06 CFU/mL	Negative	Negative
Mycoplasma pneumoniae	1.0E+06 CFU/mL	Negative	Negative
Pooled human nasal wash	5 - 50%	Negative	Negative

Sample type equivalency

Equivalence between nasopharyngeal swab (NPS) and oropharyngeal swab (OPS) sample types was evaluated using cultured virus (USA-WA1/2020 strain) spiked into paired negative samples (individual samples, not pooled) to prepare contrived low positive (approximately 1.5x Target 1 LoD) and moderate positive (approximately 4x Target 1 LoD) samples for each sample type. A total of 21 low positive paired samples, 11 moderate positive paired samples, and 11 negative paired samples were tested.

As shown in Table 19, all low positive and moderate positive paired samples were positive in both sample matrices. All negative paired samples were negative in both sample types. The observed Ct values for contrived positive samples were comparable in both sample types.

Table 19 Result comparison of nasopharyngeal to oropharyngeal sample types

Sample Type	Sample Concentration	N	Target 1		Target 2	
			% Positive	Mean Ct (95% CI)	% Positive	Mean Ct (95% CI)
NPS	~1.5x LoD (Target 1)	21	100	31.9 (31.7 – 32.0)	100	33.6 (33.5 – 33.7)
OPS			100	32.2 (31.8 – 32.6)	100	33.7 (33.4 – 34.1)
NPS	~4x LoD (Target 1)	11	100	30.9 (30.3 – 31.5)	100	32.2 (31.6 – 32.9)
OPS			100	31.5 (31.2 – 31.9)	100	32.7 (32.4 – 33.0)
NPS	Negative	11	0	n/a	0	n/a
OPS			0	n/a	0	n/a

Matrix equivalency – UTM-RT and cobas® PCR Media

Equivalence between samples collected in UTM-RT and **cobas**® PCR Media (CPM) was evaluated using cultured virus (USA-WA1/2020 strain) spiked into paired negative nasopharyngeal samples from patients with signs and symptoms of an upper respiratory infection (individual samples, not pooled) to prepare contrived low positive (approximately 1.5x LoD) and moderate positive (approximately 4x LoD) samples for each collection media. A total of 21 low positive paired samples, 11 moderate positive paired samples, and 11 negative paired samples were tested.

As shown in Table 20, all low positive and moderate positive paired samples were positive in both sample matrices. All negative paired samples were negative in both sample matrices. The observed Ct values for contrived positive samples were comparable in both sample matrices.

Table 20 Result comparison of UTM-RT to **cobas**® PCR Media

Collection Media	Sample Concentration	N	Target 1		Target 2	
			% Positive	Mean Ct (95% CI)	% Positive	Mean Ct (95% CI)
UTM	~1.5x LoD	21	100	31.8 (31.6 - 32.0)	100	34.0 (33.8 - 34.2)
CPM			100	32.2 (31.9 - 32.4)	100	34.7 (34.4 – 35.0)
UTM	~4x LoD	11	100	30.7 (30.1 - 31.2)	100	32.4 (31.7 - 33.1)
CPM			100	31.6 (31.0 - 32.1)	100	33.7 (32.9 - 34.5)
UTM	Negative	11	0	n/a	0	n/a
CPM			0	n/a	0	n/a

Matrix equivalency –UTM-RT and 0.9% physiological saline

Equivalence between samples collected in UTM-RT and 0.9% physiological saline was evaluated using cultured virus (USA-WA1/2020 strain) spiked into paired negative samples (individual samples, not pooled) to prepare contrived low positive (approximately 1.5x LoD) and moderate positive (approximately 4x LoD) samples for each collection media. Three samples were collected from each of 45 healthy donors using swabs from **cobas**® PCR Media Dual Swab Sample Kit; two nasal samples (NS) collected using dual flocked/woven polyester swabs stored in UTM and one nasal sample (other nostril) collected using a woven polyester swab stored in 0.9% physiological saline. A total of 17 low positive paired samples, 11 moderate positive paired samples, and 45 negative paired samples were tested.

As shown in Table 21, all low positive and moderate positive paired samples were positive in both sample matrices. All negative paired samples were negative in both sample matrices. The observed Ct values for contrived positive samples were comparable in both sample matrices.

Table 21 Result comparison of UTM-RT to 0.9% physiological saline

Collection Device	Sample Concentration	N	Target 1		Target 2	
			% Positive	Mean Ct (95% CI)	% Positive	Mean Ct (95% CI)
Flocked Swab in UTM-RT	~1.5x LoD	17	100	32.2 (32.0 - 32.4)	100	33.6 (33.6 - 33.7)
Woven Swab in UTM-RT		16	100	31.6 (31.1 - 32.1)	100	33.2 (32.7 - 33.8)
Woven Swab in Saline		17	100	31.7 (31.4 - 32.0)	100	33.5 (33.2 - 33.8)
Flocked Swab in UTM-RT	~4x LoD	11	100	31.2 (31.1 - 31.4)	100	32.6 (32.4 - 32.7)
Woven Swab in UTM-RT			100	30.9 (30.4 - 31.4)	100	32.4 (31.9 - 33.0)
Woven Swab in Saline			100	31.0 (30.8 - 31.3)	100	32.6 (32.5 - 32.7)
Flocked Swab in UTM-RT	Negative	45	0	n/a	0	n/a
Woven Swab in UTM-RT			0	n/a	0	n/a
Woven Swab in Saline			0	n/a	0	n/a

FDA SARS-CoV-2 reference panel testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (concentrated stock T1 from FDA panel), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The results are summarized in Table 22.

Table 22 Summary of LoD confirmation result using the FDA SARS-CoV-2 reference panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Clinical Sample	1.8 x10^3 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

Performance in sample pools

Clinical performance of **cobas**® SARS-CoV-2 when testing nasopharyngeal samples collected in UTM or UVT was evaluated using one **cobas**® 6800 System and one **cobas**® 8800 System. 30 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 23 and Table 24, respectively. Positive and presumptive positive results (as defined in Table 13) were used for the Positive Percent Agreement (pools vs. individual) calculations, as all the constituent samples would require re-testing as a 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 23 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 24 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 25.

Table 25 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%)

In silico estimated performance in pools of 6, 5, 4 and 3

A model based on observed data in the sample pooling validation study above estimates detection in pooled samples testing using historical Ct values from individual testing. As indicated in Table 26 below, for n=6 and n=3, the model using Passing-Bablok regression analysis estimates Ct shifts for Target T1 and Target T2, and identifies three Ct intervals where a pool of n samples containing a positive sample in one of these intervals is expected to be detected 100% of the time (Zone 0, not shown, includes any sample with Ct earlier than the Zone 1 range), detected <100% (e.g., 85% of the time for n=6) (Zone 1), or detected 0% of the time probability (Zone 2). For n=5 and n=4, in addition, a linear model between Ct shift and number of n samples in pools (log2n) was also considered.

In silico performance estimation rules:

1. The Ct shifts evaluated between individual and pools of n=6, 5, 4, and 3 are shown in Table 26.
2. Samples with T1 Ct values in Zone 2 AND T2 Ct values in Zone 2 will be detected 0% of the time.
3. Samples with T1 Ct values in Zone 1 AND with T2 Ct values in Zone 1 will be detected with probability less than 100% depending on n (column "Percent of Detection" in Table 26).

Table 26 In silico performance estimation rules

Pool Size	Target	Ct Shift	Zone 1*	Percent of Detection	Zone 2**	Percent of Detection
N = 6	T1	1.0	33.3 to < 44.0	85.0%	44.0 to 45	0%
	T2	1.3	35.3 to < 43.7		43.7 to 45	
N = 5	T1	0.8	33.3 to < 44.2	86.6%	44.2 to 45	0%
	T2	1.2	35.3 to < 43.8		43.8 to 45	
N = 4	T1	0.7	33.3 to < 44.3	88.5%	44.3 to 45	0%
	T2	1.0	35.3 to < 44.0		44.0 to 45	
N = 3	T1	0.5	33.3 to < 44.5	90.0%	44.5 to 45	0%
	T2	0.9	35.3 to < 44.1		44.1 to 45	

* Zone 1 is a Ct range [Highest observed individual Ct in the pooling validation study to (Ct of 45 minus Ct shift)]

** Zone 2 is a Ct range (Ct of 45 minus Ct shift)

Application of the in silico performance estimation rules to historical data from individual positive and presumptive positive results over the same time period from 3 geographically diverse sites in the US is presented in Table 27 below,

Table 27 Results of in silico performance estimation from 3 geographically diverse sites in the US

Pool Size	Site 1 (N=31,916)			Site 2 (N=29,806)			Site 3 (N=3,243)		
	Percent in Zone 1	Percent in Zone 2	PPA	Percent in Zone 1	Percent in Zone 2	PPA	Percent in Zone 1	Percent in Zone 2	PPA
N = 6	8.8%	0%	98.7%	8.0%	0%	98.8%	11.3%	0%	98.3%
N = 5	8.8%	0%	98.8%	8.0%	0%	98.9%	11.3%	0%	98.5%
N = 4	8.8%	0%	99.0%	8.0%	0%	99.1%	11.3%	0%	98.7%
N = 3	8.8%	0%	99.1%	8.0%	0%	99.2%	11.3%	0%	98.9%

Example of an application of the in silico performance estimation rules for n=6 to historical data testing individual specimens from 3 geographically diverse sites in the US:

- Using historical data from Site 1, the percent of samples is 8.8% in zone 1 and 0% in zone 2. The estimated PPA is 100 – (8.8 x 0.15) – (0 x 1) = 100 – 1.3 – 0 = 98.7%.
- Using historical data from Site 2, the percent of samples is 8.0% in zone 1 and 0% in zone 2. The estimated PPA is 100 – (8 x 0.15) – (0 x 1) = 100 – 1.2 – 0 = 98.8%.
- Using historical data from Site 3, the percent of samples is 11.3% in zone 1 and 0% in zone 2. The estimated PPA is 100 – (11.3 x 0.15) – (0 x 1) = 100 – 1.7 – 0 = 98.3%.

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

Clinical performance with contrived samples

The performance of **cobas**® SARS-CoV-2 with prospectively collected nasopharyngeal swab clinical samples was evaluated using 100 individual negative clinical samples and 50 contrived positive clinical samples collected from patients with signs and symptoms of an upper respiratory infection.

Clinical samples were collected by qualified personnel according to the package insert of the collection device. Samples were handled as described in the package insert of the collection device and stored frozen until use. Samples were tested to be negative by a commercially available nucleic acid test for the qualitative detection of microorganisms associated with common upper respiratory tract infections.

Low positive and moderate positive contrived positive clinical samples were prepared by spiking cultured virus (USA-WA1/2020 strain) into individual negative clinical samples to approximately ~1.5x LoD (Target 1) (25 samples) and ~4x LoD (Target 1) (25 samples), respectively.

As shown in Table 28, all low positive and moderate positive samples were positive and all negative samples were negative in the background of individual clinical sample matrix.

Table 28 Clinical evaluation with nasopharyngeal swab samples

Sample Concentration	N	Target 1		Target 2	
		% positive (two-sided 95% CI)	Mean Ct	% positive (two-sided 95% CI)	Mean Ct
~1.5x LoD	25	100 (86.7 – 100)	31.6	100 (86.7 – 100)	33.2
~4x LoD	25	100 (86.7 – 100)	31.1	100 (86.7 – 100)	32.4
Negative	100	0 (n/a)	n/a	0 (n/a)	n/a

Performance against the expected results are:
Positive Percent Agreement 50/50 = 100% (95% CI: 92.9% - 100%)
Negative Percent Agreement 100/100 = 100% (95% CI: 96.3% - 100%)

Clinical performance with samples from individuals suspected of COVID-19

The performance of **cobas**® SARS-CoV-2 was compared to a highly sensitive RT-PCR EUA SARS-CoV-2 test (comparator) using nasopharyngeal samples collected in UTM from individuals suspected of COVID-19.

A total of 162 archived, de-identified, and prospectively collected fresh individual nasopharyngeal samples from individuals suspected of COVID-19 were included in the study. Thirty of these nasopharyngeal swab samples stored in Viral Transport Media (Copan UTM, BD™ UVT) were positive and 132 were negative for SARS-CoV-2 by the comparator. The study included 33.3% low positive samples (LP) with comparator Ct values that are within 3 Cts of the mean Ct value at the established comparator LoDs according to the respective instruction for use. The Cts of other positive samples were distributed over a broad range. Results are shown in Table 29.

Table 29 Clinical evaluation with nasopharyngeal swab samples from individuals suspected of COVID-19

		Highly Sensitive Comparator RT-PCR EUA Test		
		Positive	Negative	Total
cobas® SARS-CoV-2	**Positive**	30	6*	36
	Negative	0	126	126
	Total	30	132	162

*confirmed as true positives by post-amplification analysis of the amplicon

Percent Agreement	Result (%)	95% Score Confidence Interval (%)
PPA	100.0 (30/30)	88.6 – 100.0
NPA	95.5 (126/132)	90.4 – 97.9

Performance against the comparator results are:
Positive Percent Agreement 30/30 = 100% (95% CI: 88.6% - 100%)
Negative Percent Agreement 126/132 = 95.5% (95% CI: 90.4%-97.9%)

Discordant results between the **cobas**® SARS-CoV-2 assay and the comparator method were observed for 6 samples. These samples showed late Ct values, which are indicative of samples from individuals with viral loads close to or below the limit of detection of both the **cobas**® SARS-CoV-2 assay and the comparator test. Post-PCR analysis of the amplicon from all discordant samples confirmed the presence of SARS-CoV-2.

Clinical performance with samples from individuals without symptoms or other reasons to suspect COVID-19

The performance of **cobas**® SARS-CoV-2 was compared to a highly sensitive RT-PCR EUA SARS-CoV-2 test (comparator) using nasopharyngeal samples collected in UTM from individuals without symptoms or other reasons to suspect COVID-19.

A total of 143 consecutively collected, archived, de-identified individual nasopharyngeal samples from individuals without symptoms or other reasons to suspect COVID-19 were included in the study. Twenty-two of these nasopharyngeal swab samples were positive and 121 were negative for SARS-CoV-2 by the comparator. Results are shown in Table 30.

Table 30 Clinical evaluation with nasopharyngeal swab samples from individuals without symptoms or other reasons to suspect COVID-19

		Highly Sensitive Comparator RT-PCR EUA Test		
		Positive	Negative	Total
cobas® SARS-CoV-2	**Positive**	21	0	21
	Negative	1	121	122
	Total	22	121	143

Percent Agreement	Result (%)	95% Score Confidence Interval (%)
PPA	95.5 (21/22)	78.2 –99.2
NPA	100 (121/121)	96.9 – 100

Performance against the comparator results are:
Positive Percent Agreement 21/22 = 95.5% (95% CI: 78.2% - 99.2%)
Negative Percent Agreement 121/121 = 100% (95% CI: 96.9%-100%)

Discordant results between the **cobas**® SARS-CoV-2 assay and the comparator method were observed for 1 sample. This sample showed late Ct values, which are indicative of samples from individuals with viral loads close to or below the limit of detection of both the **cobas**® SARS-CoV-2 assay and the comparator test.

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
Minimum amount of sample required	0.6 or 1.0 mL*‡
Sample processing volume	0.4 mL
Test duration	Results are available within less than 3.5 hours after loading the sample on the system.

*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 31 Symbols used in labeling for Roche PCR diagnostics products

Age/DOB	Age or Date of Birth
SW	Ancillary Software
Assigned Range [copies/mL]	Assigned Range (copies/mL)
Assigned Range [IU/mL]	Assigned Range (IU/mL)
EC REP	Authorized representative in the European Community
BARCODE	Barcode Data Sheet
LOT	Batch code
	Biological risks
REF	Catalogue number
Collect Date	Collect date
	Consult instructions for use
Σ	Contains sufficient for <*n*> tests
CONTENT	Contents of kit
CONTROL	Control
	Date of manufacture
D	Distributed by
	Do not re-use
	Female
	For IVD performance evaluation only
GTIN	Global Trade Item Number
IVD	*In vitro* diagnostic medical device
LLR	Lower Limit of Assigned Range
	Male
	Manufacturer
CONTROL –	Negative control
NON STERILE	Non sterile
#	Patient number
?	Patient Name


Peel here

CONTROL + Positive control

QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.

QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.

SN Serial number

Site Site

Procedure Standard Standard Procedure

STERILE EO Sterilized using ethylene oxide


Store in the dark


Temperature limit

Test Definition File

CE CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an *in vitro* diagnostic medical device


This way up

UDI Unique Device Identification

Procedure UltraSensitive Ultrasensitive Procedure

ULR Upper Limit of Assigned Range

Urine Fill Line Urine Fill Line

Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.


Use-by date


Device for near-patient testing


Device Not for Near Patient Testing


Device for self-testing


Device not for self-testing

Technical support

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

Manufacturer and distributors

Table 32 Manufacturer and distributors

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

Made in USA

Distributed by	Roche Diagnostics GmbH Sandhofer Strasse 116 68305 Mannheim, Germany	Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457 USA (For Technical Assistance call the Roche Response Center toll-free: 1-800-526-1247)

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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.

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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.

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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. 3.0 05/2020	Workflow descriptions of the new sample type "**cobas**® PCR Media swab". Workflow to prepare the **cobas**® PCR Media tubes for processing. Data for analytical sensitivity for AccuPlex added. Removal of duplicate ingredients in formulation for positive control. Removal of repeat testing for samples with presumptive positive results. Update of figures in **Sample collection, transport, and storage** section with gloved hands. Removal of the limitation regarding nasal and mid-turbinate nasal collection in **Procedural limitations** section. Moved **Conditions of Authorization for the laboratory** section to the **Procedural limitations** section. Please contact your local Roche Representative if you have any questions.
Doc Rev. 4.0 10/2020	Added table with Overview of collection devices and sample types in **Sample handling, transport and storage** section. Data for analytical sensitivity using FDA SARS-CoV-2 reference panel added to **Non-Clinical performance evaluation** section. Data for sample pooling added to **Non-Clinical performance evaluation** section. Updated **Intended use**, **Summary and explanation of test**, and **Warnings and precautions** sections to include pooling. Added 3 sections for pooling: **Sample pooling**, **Use of pooling based on prevalence**, **Monitoring**. Please contact your local Roche Representative if you have any questions.
Doc Rev. 5.0 12/2020	Added 480T kits Updated harmonized symbol page. Please contact your local Roche Representative if you have any questions.
Doc Rev. 6.0 05/2021	Changed Intended Use statement to include Asymptomatic Screening Added text pointing to the sample collection kits' IFU for hazard information. "Refer to the Instructions for Use of the Collection Devices for hazard information." Added clinical data to the **Clinical performance evaluation** section Updated **Table 31**. Updated harmonized symbol page. Updated **Trademarks and patents** section. Please contact your local Roche Representative if you have any questions.
Doc Rev. 7.0 05/2021	Added clinical data to the **Clinical performance evaluation** section. Please contact your local Roche Representative if you have any questions.

Document Revision Information	
Doc Rev. 8.0 09/2021	Added the following statement to the **Procedural limitations** section: The performance of this test was established based on the evaluation of a limited number of clinical specimens.

cobas® SARS-CoV-2

IVD

KIT LOT

Rx Only

For USA: Emergency Use Authorization only

SW

cobas® SARS-CoV-2 ASAP Version 10.1.0 or higher

cobas® 6800/8800 System Software Version 1.2 or higher

This product has not been FDA cleared or approved, but has been authorized by FDA under an EUA for use by authorized laboratories.

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

The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

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09188185001-10

cobas® SARS-CoV-2

KIT LOT

IVD

Rx Only

For USA: Emergency Use Authorization only

SW

cobas® SARS-CoV-2 ASAP Version 10.3.0 or higher

cobas® 6800/8800 System Software Version 1.2 or higher

This product has not been FDA cleared or approved, but has been authorized by FDA under an EUA for use by authorized laboratories.

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