Rx Only

cobas® SARS-CoV-2 Duo

Qualitative and quantitative assay for use on the cobas® 5800/6800/8800 Systems

For in vitro diagnostic use

cobas® SARS-CoV-2 Duo	P/N: 09500111190
cobas® SARS-CoV-2 Duo Control Kit	P/N: 09500120190
cobas® Buffer Negative Control Kit	P/N: 09051953190

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Intended use

cobas® SARS-CoV-2 Duo for use on the **cobas**® 5800/6800/8800 Systems (**cobas**® SARS-CoV-2 Duo) is an automated real-time RT-PCR assay for the in vitro qualitative and quantitative detection of SARS-CoV-2 RNA in healthcare provider-instructed self-collected nasal (anterior nares and mid-turbinate) swab specimens (collected on site), and healthcare provider-collected nasal and nasopharyngeal swab specimens collected from individuals suspected of COVID-19 by their healthcare provider. **cobas**® SARS-CoV-2 Duo is intended for use as an aid in the diagnosis of patients suspected of COVID-19 by their healthcare provider.

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.

Summary and explanation of the test

Explanation of the test

cobas® SARS-CoV-2 Duo is an automated real-time RT-PCR assay for the in vitro qualitative and quantitative detection of SARS-CoV-2 RNA in collected nasal (anterior nares and mid-turbinate) and nasopharyngeal swab specimens collected in Copan Universal Transport Medium System (UTM-RT®) or BD™ Universal Viral Transport System (UVT) from individuals suspected of COVID-19. The viral load is quantified against a non-SARS-CoV-2 armored RNA quantitation standard (RNA-QS), which is introduced into each specimen during sample preparation. The RNA-QS also functions as an internal control to monitor the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control. The high positive and low positive external controls are manufactured by dilution from stock material with a titer traceable to the First WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146). Each **cobas**® SARS-CoV-2 Duo kit lot is calibrated traceable to the First WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146).

Principles of the procedure

cobas® SARS-CoV-2 Duo is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 5800 System is designed as one integrated instrument. 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**® 5800 System or the **cobas**® 6800/8800 software, which assigns 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 RNA-QS 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 are processed in the same way with each **cobas**® SARS-CoV-2 Duo run. The test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.

Selective amplification of SARS-CoV-2 target nucleic acid from the sample is achieved by the use of a dual target virus specific approach from highly-conserved regions of SARS-CoV-2 located in the ORF 1a and ORF 1a/b non-structural regions. Selective amplification of RNA QS is achieved by the use of non-competitive sequence specific forward and reverse primers which have no homology with the SARS-CoV-2 genome.

A thermostable DNA polymerase enzyme is used for amplification. The target and RNA QS sequences are amplified simultaneously utilizing a universal PCR amplification profile with pre-defined temperature steps and number of cycles.

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.

Amplified target is detected by cleavage of fluorescently labeled oligonucleotide probes. The **cobas**® SARS-CoV-2 Duo master mix contains two detection probes specific for SARS-CoV-2 target sequences and one for the RNA QS. The probes are labeled with target-specific fluorescent reporter dyes allowing simultaneous detection of SARS-CoV-2 target and RNA QS in two different target channels. The fluorescent signal of the intact probe is suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage by the 5' to 3' nuclease 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. Real-time detection and discrimination of PCR products are accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and RNA QS.

Reagents and materials

The materials provided for **cobas**® SARS-CoV-2 Duo can be found in Table 1. Materials required, but not provided can be found in Table 2, Table 3, Table 4, Table 8, Table 9, and Table 10.

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

cobas® SARS-CoV-2 Duo reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 5

Table 1: cobas® SARS-CoV-2 Duo

(SARS-CoV-2 Duo)

Store at 2-8°C
192 test cassette (P/N 09500111190)

Kit components	Reagent ingredients	Quantity per kit 192 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase, glycerol EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin from Bacillus subtilis. May produce an allergic reaction.	22.3 mL
RNA Quantitation Standard (RNA-QS)	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
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 mL
SARS-CoV-2 Duo Master Mix Reagent 2 (SARS-CoV-2 Duo 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 primers, < 0.01% Internal Control forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for SARS-CoV-2 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

Table 2: cobas® SARS-CoV-2 Duo Control Kit

(SARS-CoV-2 Duo CTL)

Store at 2–8°C
(P/N 09500120190)

Kit components	Reagent ingredients	Quantity per kit
SARS-CoV-2 Low Positive Control (SARS-CoV-2 L(+)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	5.2 mL (8 x 0.65 mL)
SARS-CoV-2 High Positive Control (SARS-CoV-2 H(+)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	5.2 mL (8 x 0.65 mL)

Table 3: cobas® Buffer Negative Control Kit

Store at 2-8°C

(P/N 09051953190)

Kit components	Reagent ingredients	Quantity per kit
cobas® Buffer Negative Control (BUF (–) C)	Tris buffer, < 0.1% sodium azide, EDTA, < 0.002% Poly rA RNA (synthetic)	16 mL (16 x 1 mL)

cobas omni reagents for sample preparation

Table 4: cobas omni reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	43% (w/w) guanidine thiocyanate***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol***, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H411: Toxic to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. EUH071: Corrosive to the respiratory tract. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/hearing protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. P391: Collect spillage. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
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 Duo test kits. See listing of additional materials required (Table 8 and Table 9).

** 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, Table 6 and Table 7.

When reagents are not loaded on the **cobas**® 5800 or **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 Duo	2–8°C
cobas® SARS-CoV-2 Duo Control Kit	2–8°C
cobas® Buffer Negative Control Kit	2–8°C
cobas omni Lysis Reagent	2–8°C
cobas omni MGP Reagent	2–8°C
cobas omni Specimen Diluent	2–8°C
cobas omni Wash Reagent	15–30°C

Reagent handling requirements for cobas® 5800 System

Reagents loaded onto the **cobas**® 5800 System are stored at appropriate temperatures and their expiration is monitored by the system. The system allows 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**® 5800 System.

Table 6: Reagent expiry conditions enforced by the **cobas**® 5800 System

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability
cobas® SARS-CoV-2 Duo	Date not passed	90 days from first usage	Max 40 runs	Max 36 days[b]
cobas® SARS-CoV-2 Duo Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 36 days[b]
cobas® Buffer Negative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 36 days[b]
cobas omni Lysis Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable

[a]Single use reagents

[b]Time is measured from the first time that reagent is loaded onto the **cobas**® 5800 System.

Reagent handling requirements for cobas® 6800/8800 Systems

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 7 are met. The system automatically prevents use of expired reagents. Table 7 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 7: 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 Duo	Date not passed	90 days from first usage	Max 40 runs	Max 40 hours
cobas® SARS-CoV-2 Duo 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[b]	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable

[a] Single use reagents

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

Additional materials required for cobas® 5800 System

Table 8: Materials and consumables for use on **cobas**® 5800 System

Material	P/N
cobas omni Processing Plate 24	08413975001
cobas omni Amplification Plate 24	08499853001
cobas omni Liquid Waste Plate 24	08413983001
Tip CORE TIPS with Filter, 1 mL	04639642001
Tip CORE TIPS with Filter, 300 µL	07345607001
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 or Solid Waste Bag With Insert	07435967001 or 08030073001
cobas omni Secondary Tubes 13x75 (optional)	06438776001
MPA RACK 13 or 16 MM[a]	N/A
RD5 RACK – RD Standard rack[a]	N/A
16-position tube carrier[a]	09224319001
5-position rack carrier[a, b]	09224475001

[a] Please contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack carriers accepted on the instruments and compatible with the assay.

[b] RD5 or MPA racks are required in combination with the 5-position Rack Carrier on the **cobas**® 5800 System.

Additional materials required for cobas® 6800/8800 Systems

Table 9: 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
MPA RACK 13 or 16 MM[a]	N/A
RD5 RACK – RD Standard rack[a]	N/A

[a] Please contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack carriers accepted on the instruments and compatible with the assay.

Instrumentation and software required

The **cobas**® 5800 software and **cobas**® SARS-CoV-2 Duo analysis package for the **cobas**® 5800 System must be installed on the **cobas**® 5800 instrument. The Data Manager software and computer unit for the **cobas**® 5800 System will be provided with the system.

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

Table 10: Instrumentation

Equipment	P/N
cobas® 5800 System	08707464001
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

Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems – User Assistance and/or User Guides for additional information.

Precautions and handling requirements

Warnings and precautions

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

- For in vitro diagnostic use.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[1,2] Only personnel proficient in handling infectious materials and the use of **cobas**® SARS-CoV-2 Duo and the **cobas**® 5800/6800/8800 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 0.6% 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.
- Inform your local competent authority and manufacturer about any serious incidents which may occur when using this assay.

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 Duo, **cobas**® SARS-CoV-2 Duo 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 Duo kits, **cobas**® SARS-CoV-2 Duo 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 at least 0.6% 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**® 5800 or **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 5800 or **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.

Store all samples at specified temperatures.

Sample stability is affected by elevated temperatures.

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.

Sample collection

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

Table 11: Overview of collection devices and sample types

Collection Device	**Sample Type** Nasopharyngeal	**Sample Type** Nasal (Anterior Nares and Mid-Turbinate)
Copan Universal Transport Media (UTM-RT®)	√	√
BD™ Universal Viral Transport (UVT)	√	√

- Collect nasal (anterior nares and mid-turbinate) and nasopharyngeal 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).
- Refer to the Instructions for Use of the Collection Devices for hazard information.

Transport and storage

- Transportation of collected specimens must comply with all applicable regulations for the transport of etiologic agents.
- Samples collected in UTM-RT® or BD™ Universal Viral Transport (UVT):
    - After collection, specimens can be stored for up to 24 hours at 2-25°C followed by up to 3 days at 2-8°C and at ≤ -70°C for up to 30 days.
    - Specimens are stable for up to two freeze/thaw cycles when frozen at ≤ -70°C.

Instructions for use

Procedural notes

- Do not use **cobas**® SARS-CoV-2 Duo reagents, **cobas**® SARS-CoV-2 Duo Control Kit, **cobas**® Buffer Negative Control Kit, or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Ensure that specimen barcode labels on sample tubes are visible through the openings on the side of the sample racks. Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems - User Assistance and/or User Guide for proper barcode specifications and additional information on loading sample tubes.
- Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems – User Assistance and/or User Guides for proper maintenance of instruments.

Running cobas® SARS-CoV-2 Duo

cobas® SARS-CoV-2 Duo 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®) and BD™ Universal Viral Transport (UVT).

Specimens collected in tubes compatible with **cobas**® 5800 and **cobas**® 6800/8800 Systems may be loaded directly onto the **cobas**® 5800 and **cobas**® 6800/8800 Systems. The swab must be removed from the sample tube prior to direct loading onto the system. Specimens collected in tubes which are not compatible with the **cobas**® 5800 and **cobas**® 6800/8800 Systems must be transferred into a secondary tube prior to processing on the **cobas**® 5800 or **cobas**® 6800/8800 Systems. The **cobas omni** Secondary Tube is the preferred option.

Additional tubes for testing with **cobas**® SARS-CoV-2 Duo are available. Contact your local Roche representative for detailed testing instructions and an order list of primary tubes and secondary tubes compatible with the instruments.

If using frozen samples in secondary tubes, place the samples at room temperature (15-30°C) until completely thawed and then briefly mix (e.g. vortex for 3-5 seconds) and centrifuge to collect all sample volume at the bottom of the 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.

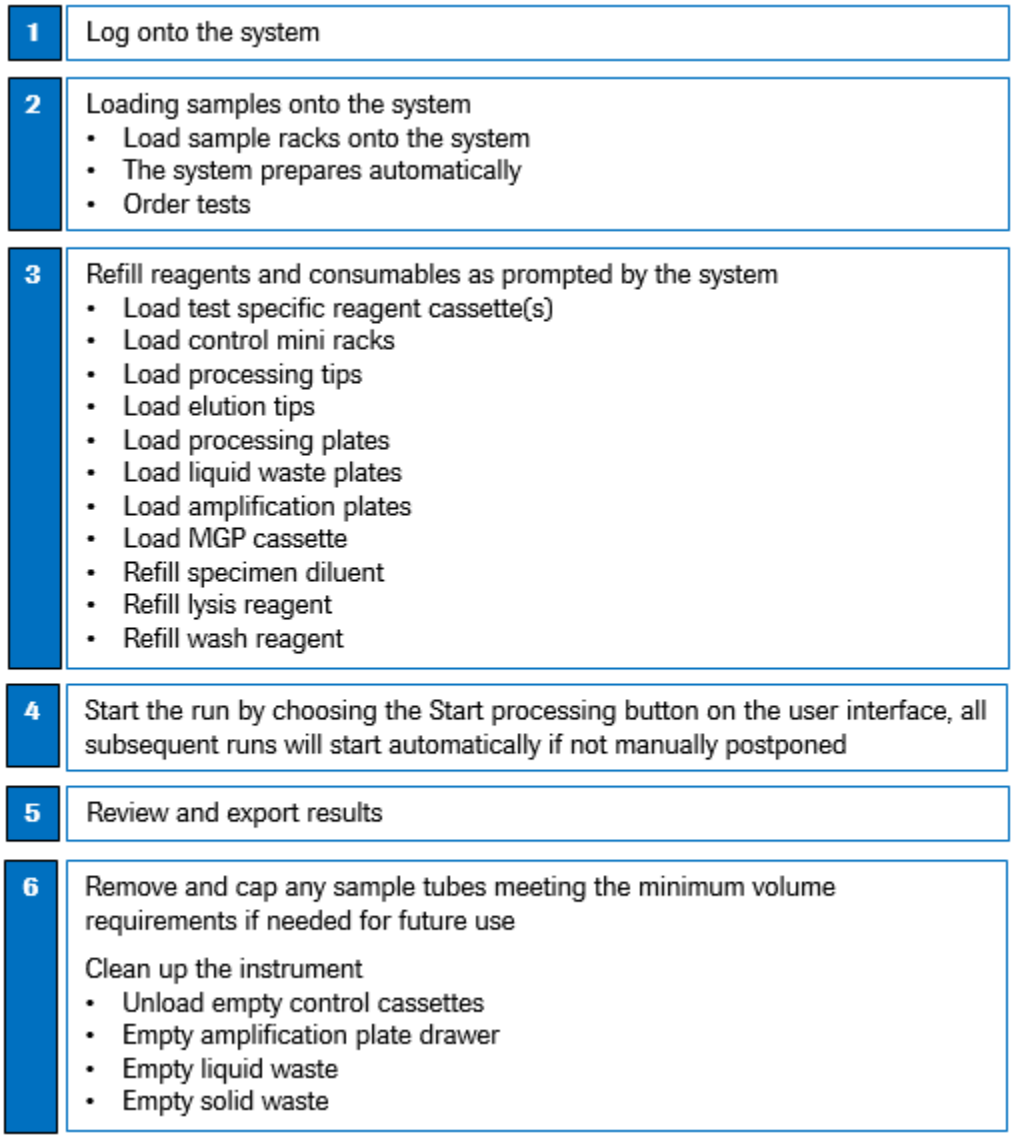
Table 12: Sample type selection in the user interface of the **cobas**® SARS-CoV-2 Duo

Collection kit/Matrix type	**Minimum volume (mL)** **Processing tube**	**Process as Sample Type**
Copan Universal Transport Medium® BD™ Universal Viral Transport	0.6 mL **cobas omni** Secondary tube	VTM (on **cobas**® 6800/8800) Viral transport medium (on **cobas**® 5800)

Running cobas® SARS-CoV-2 Duo on cobas® 5800 System

Figure 1 below summarizes the system workflow.

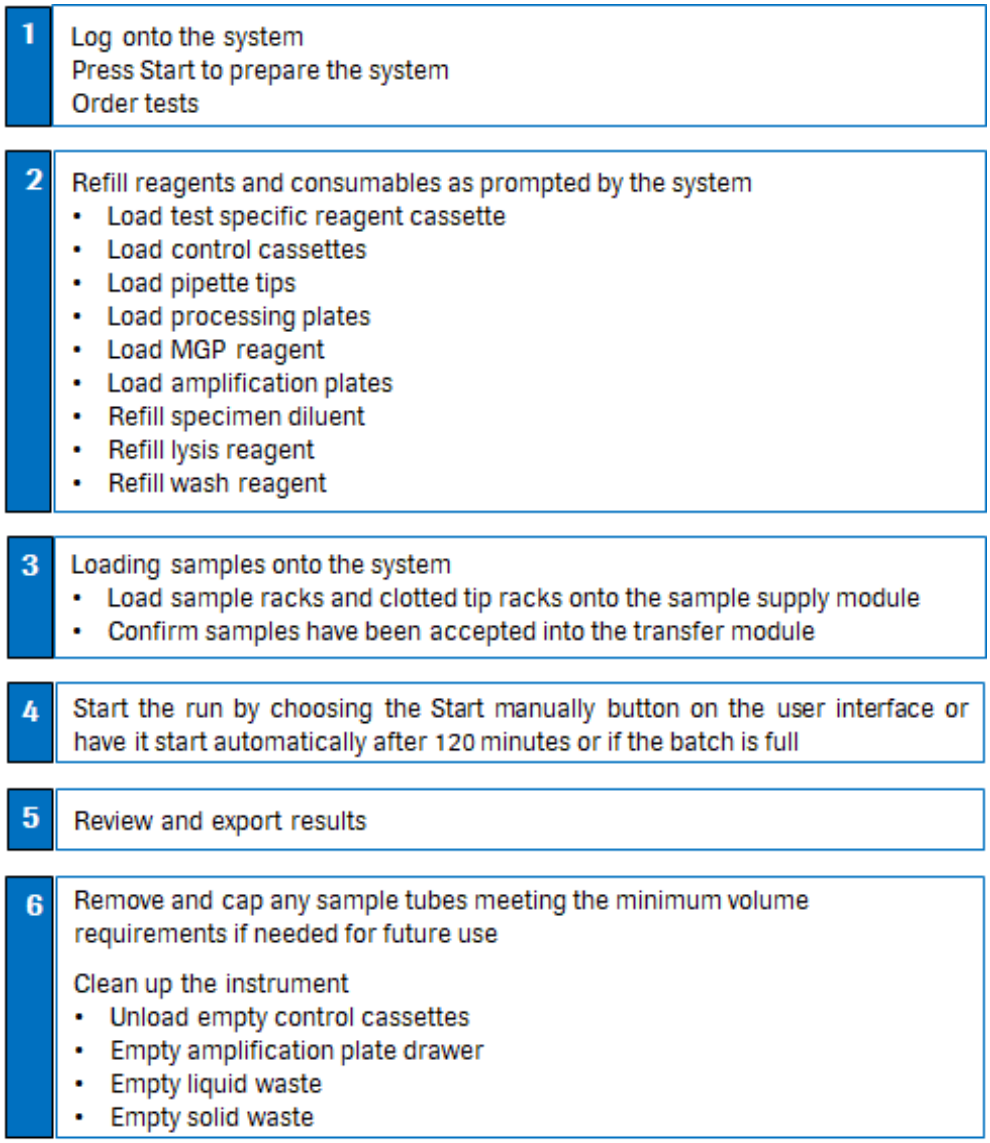
Figure 1: cobas® SARS-CoV-2 Duo workflow on the **cobas**® 5800 System



Running cobas® SARS-CoV-2 Duo on cobas® 6800/8800 Systems

Figure 2 below summarizes the system workflow.

Figure 2: cobas® SARS-CoV-2 Duo workflow on the **cobas**® 6800/8800 Systems



Results

The **cobas**® 5800 System and **cobas**® 6800/8800 Systems automatically detect SARS-CoV-2 RNA and determine the RNA concentration, for each sample and control. Individual target results for samples as well as test validity and overall results for controls are displayed on the user interface. The SARS-CoV-2 RNA concentration is expressed in International Units per milliliter (IU/mL).

Quality control and validity of results on the cobas® 5800 System

- One **cobas**® Buffer Negative Control [(-) Ctrl] and two positive controls, a low positive control [SARS-CoV-2 L(+)C] and a high positive control [SARS-CoV-2 H(+)C] are processed at least every 72 hours or with every new kit lot. Positive and/or negative controls can be scheduled more frequently based on laboratory procedures and/or local regulations.
- In the **cobas**® 5800 software and/or report, check for flags and their associated results to ensure the batch validity.
- 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**® 5800 software based on negative and positive control performance.

NOTE: The **cobas**® 5800 System will be delivered with the standard setting of running a set of controls (positive and negative) with every run, but can be configured to a less frequent scheduling up to every 72 hours based on laboratory procedures and/or local regulations. Please contact your Roche service engineer and/or Roche customer technical support for more information.

Control results on cobas® 5800 System

The results of the controls are shown in the **cobas**® 5800 software in the "Controls" app.

- Controls are marked with "Valid" in the column "Control result" if all Targets of the control are reported valid. Controls are marked with "Invalid" in the column "Control result" if all or one Target of the control are reported invalid.
- Controls marked with "Invalid" show a flag in the "Flags" column. More information on why the control is reported invalid including flag information is shown in the detail view.
- If one of the positive controls is invalid, repeat testing of the all positive controls and all associated samples. If the negative control is invalid, repeat testing of all controls and all associated samples.

Quality control and validity of results on the cobas® 6800/8800 Systems

- One **cobas**® Buffer Negative Control [(-) Ctrl] and two positive controls, a low positive control [SARS-CoV-2 L(+)C] and a high positive control [SARS-CoV-2 H(+)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

For a valid run/control batch, check each individual sample for flags in the **cobas**® 5800 System and **cobas**® 6800/8800 Systems softwares and/or reports. A valid batch may include both valid and invalid sample results.

Table 13: Target results for individual target result interpretation

Results (quantitative)	Results (qualitative)	Interpretation
Target Not Detected	Negative	SARS-CoV-2 RNA not detected. Report results as "SARS-CoV-2 not detected."
< Titer min	Positive	SARS-CoV-2 RNA is detected. Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as "SARS-CoV-2 detected, less than (Titer min)" Titer min = 100 IU/mL
Titer (IU/mL)	Positive	SARS-CoV-2 RNA is detected. Calculated titer is within the Linear Range of the assay – greater than or equal to Titer min and less than or equal to Titer max. Report results as "(Titer) of SARS-CoV-2 detected."
> Titer max[a]	Positive	SARS-CoV-2 RNA is detected. Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as "SARS-CoV-2 detected, greater than (Titer max)." Titer max = 1.00E+09 IU/mL
Invalid	Invalid	Results are invalid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.

[a]A sample result "> Titer max" indicates a positive sample that has a very high RNA level above 1,000,000,000 IU/mL and can therefore not be quantified. If a quantitative result is desired, the original sample should be diluted with SARS-CoV-2-negative transport media depending on the type of the original sample, and the test should be repeated. Multiply the reported result by the dilution factor.

Interpretation of results on the cobas® 5800 System

The results of the samples are shown in the **cobas**® 5800 software in the "Results" app.

For a valid control batch, check each individual sample for flags in the **cobas**® 5800 System software and/or report. The result interpretation should be as follows:

- Samples associated with valid controls are shown as "Valid" in the "Control result" column if all Control Target Results reported valid. Samples associated with a failed control batch are shown as "Invalid" in the "Control result" column if Control Results are reported invalid.
- If the associated controls of a sample result are invalid, a specific flag will be added to the sample result as follows:
  - Q05D: Result validation failure because of an invalid positive control.
  - Q06D: Result validation failure because of an invalid negative control.
- The values in "Result" column for individual sample target result should be interpreted as shown in Table 13. If one or more sample targets are marked with "Invalid" the **cobas**® 5800 software shows a flag in the "Flag-" column. More information on why the sample target(s) is reported invalid including flag information is shown in the detail view.

Table 14: Example of **cobas**® SARS-CoV-2 Duo results display for samples on the **cobas**® 5800 System

Sample ID	Test	Control result	Flag**	Status	Result		Creation date
Sample 1	SARS-CoV-2-Duo	Valid	-	Released	SCoV2 Quant Target Not Detected	SCoV2 Qual Negative	*7/3/2022* 4:23:33 PM
Sample 2	SARS-CoV-2-Duo	Valid	-	Released	SCoV2 Quant 7.38E+02 IU/mL (Ct 32.82)*	SCoV2 Qual Positive (Ct 32.82)*	*7/3/2022* 4:23:33 PM
Sample 3	SARS-CoV-2-Duo	Valid	-	Released	SCoV2 Quant > Titer max	SCoV2 Qual Positive (Ct 7.53)*	*7/3/2022* 4:23:33 PM
Sample 4	SARS-CoV-2-Duo	Valid	-	Released	SCoV2 Quant < Titer min	SCoV2 Qual Positive (Ct 39.18)*	*7/3/2022* 4:23:33 PM
Sample 5	SARS-CoV-2-Duo	Invalid		Released	SCoV2 Quant Invalid	SCoV2 Qual Invalid	*7/3/2022* 4:23:33 PM

* Illustrative Ct values

**The result overview shows a flag symbol in case of invalid results. Detailed flag descriptions are available in the result details.

Table 15: Example of **cobas**® SARS-CoV-2 Duo results display for controls on the **cobas**® 5800 System

Control ID*	Control result	Flags**	Test	Control name	Control mini rack lot No.	Run ID
C82110790015554527015	Valid	-	SARS-CoV-2-Duo	SARS-CoV-2 L(+)C	HD2494	5-524-20220715-0803
C26013093663850840557	Valid	-	SARS-CoV-2-Duo	SARS-CoV-2 H(+)C	HD2494	5-524-20220715-0803
C99483044721079909638	Valid	-	SARS-CoV-2-Duo	(-) Ctrl	G23281	5-524-20220715-0803
C83068286146351656371	Invalid		SARS-CoV-2-Duo	SARS-CoV-2 L(+)C	HD2493	5-524-20220706-0940
C28118401885958013514	Invalid		SARS-CoV-2-Duo	SARS-CoV-2 H(+)C	HD2493	5-524-20220706-0940

* Table applies for all Control types used.

**The result overview shows a flag symbol in case of invalid results. Detailed flag descriptions are available in the result details.

Interpretation of results on the cobas® 6800/8800 Systems

Display examples for **cobas**® SARS-CoV-2 Duo for System Software are shown Figure 3.

Figure 3: Example of **cobas**® SARS-CoV-2 Duo results display on the **cobas**® 6800/8800 Systems

Test	Sample ID	Valid	Flags	Sample Type	Overall Result	Target 1 (Quantitative)**	Target 2 (Qualitative)**
SARS-CoV-2-Duo	Sample_01	Yes		VTM	Target Not Detected	Target Not Detected	Negative
SARS-CoV-2-Duo	Sample_02	No	Y40T	VTM	Invalid	Invalid	Invalid
SARS-CoV-2-Duo	Sample_03	Yes		VTM	Titer	4.87e+007 IU/ml*	Positive
SARS-CoV-2-Duo	Sample_04	Yes		VTM	> Titer max	> Titer max	Positive
SARS-CoV-2-Duo	Sample_05	Yes		VTM	< Titer min	< Titer min	Positive
SARS-CoV-2-Duo	C1614202840904288 28404	Yes		SARS-CoV-2 H(+)C	Titer	1.16e+007 IU/ml*	Valid
SARS-CoV-2-Duo	C1614202840930095 80264	Yes		SARS-CoV-2 L(+)C	Titer	1.31e+003 IU/ml*	Valid
SARS-CoV-2-Duo	C1614202840930095 54953	Yes		(-) Ctrl	Target Not Detected	Target Not Detected	Valid

* Illustrative titer values

** The Target 1 and Target 2 labels reflect the user interface on the system and do not relate to the analyte targets. Target 1 represents the quantitative viral load values and Target 2 represents the qualitative SARS-CoV-2 result.

For a valid control batch, check each individual sample for flags in the **cobas**® 6800/8800 software and/or report. A valid control batch may include both valid and invalid sample results.

Procedural limitations

- **cobas**® SARS-CoV-2 Duo has been evaluated only for use in combination with the **cobas**® SARS-CoV-2 Duo 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**® 5800/6800/8800 Systems.
- Patient management decisions should not be made solely on the **cobas**® SARS-CoV-2 Duo test results, but rather with the consideration of clinical observations, patient history, recent exposures, epidemiological information and other diagnostic information.
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test can be used for the detection of SARS-CoV-2 RNA in nasal (anterior nares and mid-turbinate), and nasopharyngeal swab samples collected in a Copan UTM-RT System (UTM-RT®) or BD™ Universal Viral Transport System (UVT). Testing of other sample types with **cobas**® SARS-CoV-2 Duo 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 Duo 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 RNA-QS also functions as an internal control and is included in **cobas**® SARS-CoV-2 Duo 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 Duo 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.

Non-clinical performance evaluation

Key performance characteristics

Analytical sensitivity (Limit of Detection)

The Limit of Detection (LoD) study determines the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates test positive. The achieved LoD was 25.0 IU/mL based on hit rate analysis (24.1 IU/mL per Probit).

To determine the LoD, the 1st WHO International Standard for SARS-CoV-2 (NIBSC code 20/146) was serially diluted in negative simulated clinical matrix stabilized in UTM-RT®. Six concentration levels, with two-fold serial dilutions between the levels, were prepared on three days and tested with a total of 81 replicates per concentration across three reagent lots randomized with an additional 81 replicates of a blank sample (negative simulated clinical matrix stabilized in UTM-RT®).

The results are shown in Table 16.

Table 16: Summary LoD for all kit lots combined and individually

Viral Strain	Kit Lot	Number of Positives/ Number of Valid Replicates	Hit rate ≥ 95% [IU/mL]	Hit rate ≥ 95% Mean Ct	95% LoD PROBIT [IU/mL]	95% confidence interval [IU/mL]
WHO International Standard for SARS-CoV-2 (NIBSC code 20/146)	combined	77/81	25.0	38.2	24.1	19.3 - 32.8
WHO International Standard for SARS-CoV-2 (NIBSC code 20/146)	Kit Lot 1	27/27	25.0	38.1	20.3	14.1 - 39.6
WHO International Standard for SARS-CoV-2 (NIBSC code 20/146)	Kit Lot 2	27/27	50.0	37.2	25.8	18.0 - 48.0
WHO International Standard for SARS-CoV-2 (NIBSC code 20/146)	Kit Lot 3	27/27	50.0	37.1	25.6	18.1 - 46.0

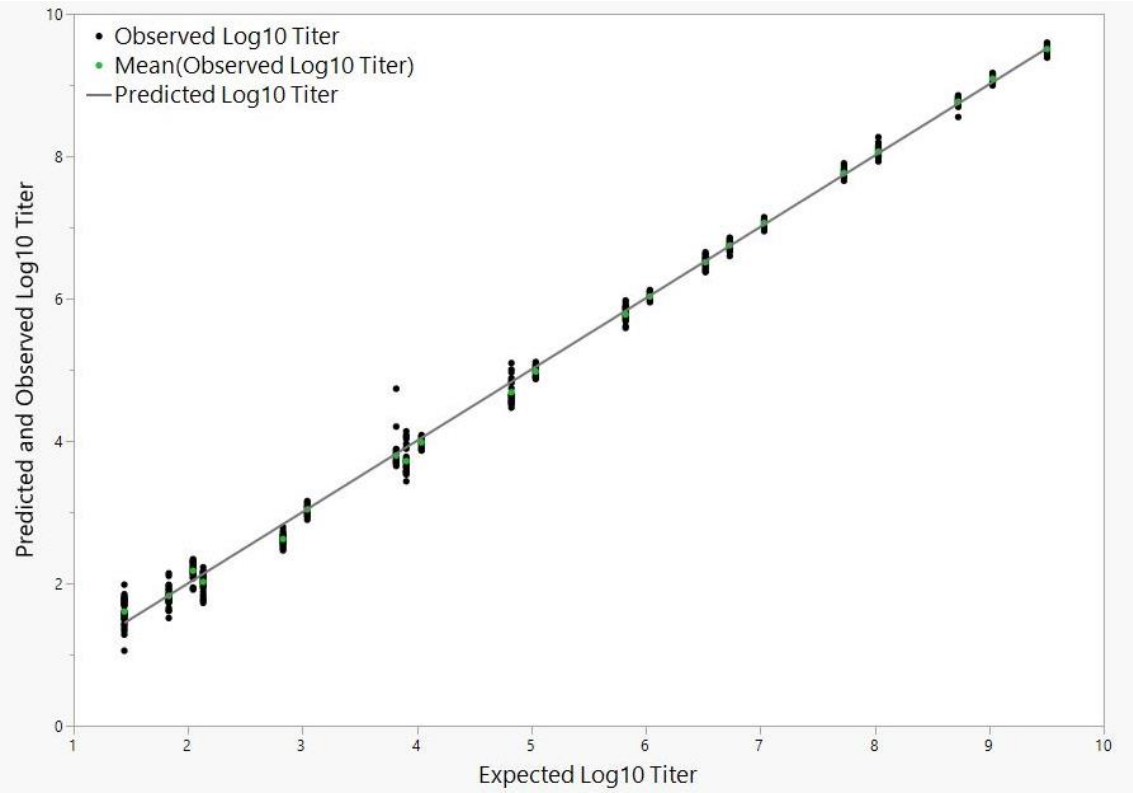
Linear range

Linearity of **cobas**® SARS-CoV-2 Duo was evaluated using a dilution series consisting of 19 concentration levels with SARS-CoV-2 RNA spanning the assay linear range. A high titer aRNA stock was used to prepare 14 panel members spanning the entire linear range. A clinical specimen was used to prepare seven panel members covering the intermediate - and lower levels of the linear range. The expected linear range of **cobas**® SARS-CoV-2 Duo is from lower limit of quantitation (LLoQ) of 1.00E+02 IU/mL to upper limit of quantitation (ULoQ) of 1.00E+09 IU/mL.The linearity panel was designed to range from concentrations below LLoQ to concentrations above ULoQ.

Each panel member was tested in 36 replicates (12 replicates for each of three lots of **cobas**® SARS-CoV-2 Duo reagents), and the results of the study are presented in Figure 4.

cobas® SARS-CoV-2 Duo was demonstrated to be linear from 1.0E+02 IU/mL to 1.0E+09 IU/mL and shows a maximum mean deviation from linearity of less or equal than ± 0.3 log_{10}. Across the linear range, the accuracy of the test was within ± 0.5 log_{10}. The lower limit of quantitation (LLoQ) was set and verified at 100 IU/mL.

Figure 4: Linear range determination



Precision – within laboratory

The quantitative precision of **cobas**® SARS-CoV-2 Duo was determined by analysis of serial dilutions of co-formulated SARS-CoV-2 aRNA Orf 1a and Orf 1a/b in negative simulated clinical matrix stabilized in UTM-RT®. For the assessment of the qualitative precision, the 1st WHO International Standard for SARS-CoV-2 (NIBSC code 20/146) was serially diluted in negative simulated clinical matrix stabilized in UTM-RT®. Each panel member was tested in 108 replicates across three (3) different test-specific reagent lots (kit lots) which results in 36 replicates per kit lot, using four (4) instruments and four (4) operators over six (6) days of testing.

The results are shown in Table 17 and Table 18. **cobas**® SARS-CoV-2 Duo showed high quantitative precision for three lots of reagents tested across a concentration range of 2.00E+02 IU/mL to 5.00E+08 IU/mL and a high qualitative precision at concentrations around the LoD.

Table 17: Summary of Total Precision as SD of log_{10} Titer Results from Quantitative Panels

Nominal Concentration [IU/mL]	Total precision as SD [log10]			Pooled SD [log10]
	Kit Lot 1	Kit Lot 2	Kit Lot 3	
5.0E+08	0.05	0.06	0.06	0.06
5.0E+06	0.04	0.06	0.15	0.10
5.0E+04	0.06	0.06	0.06	0.06
6.0E+03	0.05	0.06	0.05	0.05
2.0E+02	0.06	0.07	0.09	0.08

Table 18: Summary of Qualitative Precision for the Qualitative Panel

Level	Positive Results	Number of valid results	Positivity	Two-sided 95% CI Lower Bound	Two-sided 95% CI Upper Bound
~3.0 x LoD	108	108	100%	96.6%	100%
~1.0 x LoD	108	108	100%	96.6%	100%
~0.3 x LoD	89	108	82.4%	73.9%	89.1%
Blank	0	108	0.0%	0.0%	3.4%

SARS-CoV-2 variants verification

The performance of **cobas**® SARS-CoV-2 Duo on SARS-CoV-2 was evaluated by:

- Inclusivity - Verification of the limit of detection
- Verification of the linear range

Table 19 shows the tested SARS-CoV-2 variants.

Table 19: Overview of tested SARS-CoV-2 variants

SARS-CoV-2 variants	Lineage number
US-WA 1/2020	USA-WA1/2020
Alpha	Lineage B.1.1.7
Beta	Lineage B.1.351
Gamma	Lineage P.1
Delta	Lineage B.1.617.2
Omicron BA.1	Lineage B.1.1.529.1
Omicron BA.2	Lineage B.1.1.529.2

Inclusivity

Cultured isolates of SARS-CoV-2 variants (shown in Table 19) were diluted to two different concentration levels (1 x LoD and 0.5 x LoD). Testing was performed with 63 replicates for each level (21 replicates per each of three lots of **cobas**® SARS-CoV-2 Duo reagents). These results (shown in Table 20) verify that **cobas**® SARS-CoV-2 Duo detects seven tested different SARS-CoV-2 variants at a concentration of 24.1 IU/mL with a hit rate of ≥ 95%.

Table 20: Summary of Hit Rates for SARS-CoV-2 Strains (Variants)

Strain (Variant)	Concentration*	Number of Positives/ Number of Valid Replicates	Hit Rate [%]	Mean Target Ct
SARS-CoV-2 US-WA1/2020	~ 1 x LoD	63/63	100	38.2
	~ 0.5 x LoD	57/63	90.5	38.8
SARS-CoV-2 Alpha Variant (Lineage B.1.1.7)	~ 1 x LoD	63/63	100	38.5
	~ 0.5 x LoD	53/63	84.1	39.3
SARS-CoV-2 Beta Variant (Lineage B.1.351)	~ 1 x LoD	61/63	96.8	38.4
	~ 0.5 x LoD	44/63	69.8	39.3
SARS-CoV-2 Gamma Variant (Lineage P.1)	~ 1 x LoD	63/63	100	38.3
	~ 0.5 x LoD	55/63	87.3	39.3
SARS-CoV-2 Delta Variant (Lineage B.1.617.2)	~ 1 x LoD	60/63	95.2	38.5
	~ 0.5 x LoD	47/63	74.6	39.2
SARS-CoV-2 Omicron Variant BA.1 (Lineage B.1.1.529.1)	~ 1 x LoD	63/63	100	37.6
	~ 0.5 x LoD	59/63	93.7	38.5
SARS-CoV-2 Omicron Variant BA.2 (Lineage B.1.1.529.2)	~ 1 x LoD	61/63	96.8	37.8
	~ 0.5 x LoD	61/63	96.8	38.8

*Based on 95% Probit LoD all lots combined testing WHO International Standard for SARS-CoV-2 (NIBSC code 20/146)

Verification of linearity for SARS-CoV-2 variants

For the linearity verification of **cobas**® SARS-CoV-2 Duo, cell-free culture fluid (heat-inactivated) of SARS-CoV-2 USA-WA1/2020 strain, Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) variants and isolates of Omicron BA.1 (B.1.1.529.1) and Omicron BA.2 (B.1.1.529.2) variant were respectively used in serial dilution spanning the linear range of the assay. Testing was conducted with three lots of **cobas**® SARS-CoV-2 Duo reagent; 12 replicates per level (4 replicates per lot) were tested.

The maximum mean deviation from linearity was less than ± 0.3 $\log_{10}$ for the tested SARS-CoV-2 variants, as shown in Table 21. Therefore, the linear range of **cobas**® SARS-CoV-2 Duo was verified with all tested SARS-CoV-2 variants (shown in Table 19).

Table 21: Maximum Mean Deviation from Linearity for SARS-CoV-2 Strains (Variants)

Strain (Variant)	Predicted* value at Maximum Mean Deviation [log10]	Maximum Mean Deviation (observed – predicted*) [log10]	Deviation (Maximum Mean Deviation/predicted*) [%]
SARS-CoV-2 US-WA1/2020	2.10	0.20	9.39
SARS-CoV-2 Alpha Variant (Lineage B.1.1.7)	2.06	0.17	8.40
SARS-CoV-2 Beta Variant (Lineage B.1.351)	2.02	0.16	7.78
SARS-CoV-2 Gamma Variant (Lineage P.1)	2.04	0.21	10.44
SARS-CoV-2 Delta Variant (Lineage B.1.617.2)	2.02	0.26	12.75
SARS-CoV-2 Omicron Variant BA.1 (Lineage B.1.1.529.1)	5.95	0.11	1.85
SARS-CoV-2 Omicron Variant BA.2 (Lineage B.1.1.529.2)	3.30	-0.26	-7.75

*Weighted least square regression (1st order)

Matrix equivalency

Equivalency between nasopharyngeal swabs, nasal swabs and simulated clinical matrix stabilized in UTM-RT® was evaluated. The WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146) was used to formulate panels to a target concentration of ~2 x LoD and ~5 x LLoQ into pooled negative clinical samples of each sample type and negative simulated clinical matrix stabilized in UTM-RT®. Twenty-one replicates per concentration were tested for each sample type. All replicates tested with 2 x LoD panel were positive for each matrices with 100% hit rate. The difference of the mean $\log_{10}$ titer for each matrix for 5 x LLoQ panel did not exceed 0.20 log compared to the nominal log titer.

Analytical specificity (cross-reactivity and microbial interference)

The analytical specificity of **cobas**® SARS-CoV-2 Duo was evaluated by testing a panel of microorganisms, including those commonly found in respiratory tract at a concentration between 5.00E+03 units/mL and 1.00E+06 units/mL for bacteria and fungi and at 1.00E+05 units/mL for viruses.

The organisms are listed in Table 22. Testing was performed with each potential interfering organism in the absence and presence of SARS-CoV-2 target spiked at ~3 x LoD and ~5 x LLoQ. Negative results were obtained with **cobas**® SARS-CoV-2 Duo for all microorganism samples without SARS-CoV-2 target and positive results were obtained on all of the microorganism samples with SARS-CoV-2 target spiked at ~3 x LoD.

Furthermore, the accuracy of **cobas**® SARS-CoV-2 Duo was not affected when samples spiked with the selected potential cross reactants were tested at ~5 x LLoQ spiked SARS-CoV-2 as the mean $\log_{10}$ titer of each sample was within ± 0.20 $\log_{10}$ of the mean $\log_{10}$ titer of the respective spike control.

Table 22: Microorganisms tested for analytical specificity/cross reactivity

Viruses	**Bacteria**	**Fungi**
Adenovirus (AdV-1)	*Bordetella pertussis*	*Candida albicans*
Cytomegalovirus	*Chlamydia pneumoniae*	*Pneumocystis jirovecii*
Enterovirus (EV68)	*Corynebacterium diphtheriae*	-
Epstein Barr Virus	*Escherichia coli*	-
Human coronavirus 229E	*Haemophilus influenzae*	-
Human coronavirus HKU1	*Lactobacillus acidophilus (for Lactobacillus sp.)*	-
Human coronavirus NL63	*Legionella longbeachae (for Legionella non-pneumophila)*	-
Human coronavirus OC43	*Legionella pneumophila*	-
Human Metapneumovirus	*Moraxella catarrhalis*	-
Human Rhinovirus	*Mycobacterium bovis (for Mycobacterium tuberculosis* complex*)*	-
Influenza A (H3N2)	*Mycoplasma pneumoniae*	-
Influenza B	*Neisseria elongata*	-
Measles virus	*Neisseria meningitidis*	-
MERS-coronavirus	*Pseudomonas aeruginosa*	-
Mumps Virus	*Staphylococcus aureus*	-
Parainfluenza virus 1	*Staphylococcus epidermidis*	-
Parainfluenza virus 2	*Streptococcus pneumoniae*	-
Parainfluenza virus 3	*Streptococcus pyogenes*	-
Parainfluenza virus 4	*Streptococcus salivarius*	-
Parechovirus	-	-
Respiratory Syncytial Virus	-	-
SARS-coronavirus (SARS-CoV-1)	-	-

Analytical specificity – interfering substances

Elevated levels of mucin (0.1 – 0.5 % w/v) and whole blood (1.0 – 1.5 % v/v) in simulated clinical matrix stabilized in UTM-RT® were tested in absence and in the presence of SARS-CoV-2 target spiked at ~3 x LoD and ~ 5 x LLoQ. The tested whole blood levels were shown not to interfere with the performance of **cobas**® SARS-CoV-2 Duo test. Mucin was shown not to interfere with the performance of **cobas**® SARS-CoV-2 Duo test up to 0.15 %.

In addition, drug compounds listed in Table 23 were tested in presence and absence of SARS-CoV-2 target.

All potentially interfering substances have been shown to not interfere with the test performance. Negative results were obtained with **cobas**® SARS-CoV-2 Duo for all samples without SARS-CoV-2 target and positive results were obtained on all of the samples with SARS-CoV-2 target. Furthermore, the mean $\log_{10}$ titer of each of the positive SARS-CoV-2 samples containing potentially interfering substances was within ± 0.40 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 23: Drug compounds tested for interference with **cobas**® SARS-CoV-2 Duo test

Generic drug name	Active Ingredient	Concentration
NASIVIN Pur Spray 0.05%	Oxymetazoline	0.011 mg/mL
BUDESONID Sandoz Nasal Spray 64 mcg	Budesonide	0.039 mg/mL
AXOTIDE Diskus Multidose 250 mcg	Fluticasone propionate	0.167 mg/mL
Heel Luffeel Nasal Spray	Luffa operculata Thryallis glauca Histaminum Sulfur	2.99 mg/mL 2.99 mg/mL 1.50 mg/mL 1.50 mg/mL
Dolo-Dobendan	Benzocaine	5 mg/mL
Chloraseptic max	Glycerin Phenol	10.31 mg/mL 0.47 mg/mL
XYLOCAIN Spray 10%	Lidocaine	2.68 mg/mL
BACTROBAN Nasal Ointment	Mupirocin	0.20 mg/mL
RELENZA Disk 5 mg	Zanamivir	0.0015 mg/mL
TAMIFLU Kaps 75 mg	Oseltamivir	0.0073 mg/mL
OBRACIN Inj Solution 40 mg/mL	Tobramycin	0.018 mg/mL
DEXERYL Creme	Petroleum Jelly	1% w/v
Snuff Tobacco	Nicotine	1% w/v
VICKS VapoRub	Eucalyptus Oil and Menthol	1% w/v

Reproducibility

The reproducibility of **cobas**® SARS-CoV-2 Duo on the **cobas**® 5800/6800/8800 Systems was evaluated for the qualitative and quantitative detection of SARS-CoV-2 RNA across reagent lot, testing site/instrument, day, and run. **cobas**® SARS-CoV-2 Duo on the **cobas**® 6800/8800 Systems showed a good qualitative and quantitative reproducibility across 3 reagent lots, 4 sites/instruments, and 6 days, within runs, as did **cobas**® SARS-CoV-2 Duo on the **cobas**® 5800 System (Table 24 and Table 25). All systems detected 100% of the 3 x LoD samples of the qualitative panel. In addition, the quantitative component of the assay showed a very good total precision SD, between 0.12 and 0.08, across the linear range (represented by the quantitative panel members) on the three systems. There was one negative panel member at one of the three sites that showed the presence of SARS-CoV-2 RNA by the assay. Post-amplification amplicon analysis by heminested PCR confirmed the presence of SARS-CoV-2. Negative water runs excluded instrument contamination. Since the observation was limited to a single site, the conclusion of the discrepant analysis was that carryover contamination may have occurred during specimen handling during the pre-analytical phase of the assay.

Table 24: Overall percentage agreement, mean estimate, standard deviation, and coefficient of variation (%) for cycle threshold values by Qualitative Panel Members on the **cobas**® 5800/6800/8800 Systems

Panel Member Concentration	n[a]/N	Percent Agree-ment (%)[b]	Mean Ct	Site SD	Site CV%	Lot SD	Lot CV%	Day SD	Day CV%	Run SD	Run CV%	Within Run SD	Within Run CV%	Total SD	Total CV%
Negative	467/468	99.8	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
~0.3 × LOD	332/468	70.9	40.0	0.10	0.26	0	0	0	0	0	0	1.26	3.15	1.26	3.16
~1.0 × LOD	463/468	98.9	38.6	0.41	1.07	0.18	0.46	0.14	0.38	0	0	1.02	2.64	1.12	2.91
~3.0 × LOD	468/468	100.0	36.9	0.39	1.07	0.06	0.18	0.14	0.38	0.11	0.32	0.61	1.65	0.75	2.04

Ct = cycle threshold, LoD = limit of detection, SD = standard deviation, CV(%) = percent coefficient of variation, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, nc = not calculable.

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

[b] Percent agreement with expected results

Table 25: Overall percentage agreement, mean estimate, standard deviation, and coefficient of variation (%) for SARS-CoV-2 RNA Concentration (log10 IU/mL) by Quantitative Panel Members on the **cobas**® 5800/6800/8800 Systems

Expected SARS-CoV-2 RNA Concentration (IU/mL)	Panel Member Concentration (log_{10} IU/mL)	n[a]/N	Percent Agreement (%)[b]	Mean Ct	Site SD	Site CV%	Lot SD	Lot CV%	Day SD	Day CV%	Run SD	Run CV%	Within Run SD	Within Run CV%	Total SD	Total CV%
2.00E+02 (Near LLoQ)	2.301	467/467	100.0	2.4	0.02	0.91	0.08	3.20	0.01	0.55	0.02	0.64	0.09	3.73	0.12	5.07
6.00E+03	3.778	468/468	100.0	3.8	0.01	0.29	0.05	1.34	0.01	0.17	0.00	0.08	0.06	1.58	0.08	2.10
5.00E+04	4.699	468/468	100.0	4.7	0.01	0.18	0.05	1.01	0.01	0.30	0.00	0.00	0.06	1.38	0.08	1.75
5.00E+06	6.699	468/468	100.0	6.7	0.00	0.00	0.03	0.51	0.01	0.16	0.00	0.00	0.08	1.13	0.08	1.25
5.00E+08	8.699	468/468	100.0	8.7	0.01	0.06	0.05	0.53	0.01	0.12	0.00	0.00	0.06	0.75	0.08	0.93

SD = standard deviation, CV(%) = percent coefficient of variation, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, .

[a]n is the number of positive tests which contribute to the analysis. N is the total number of valid tests for the panel member.

[b]Percent agreement with expected results.

Clinical performance evaluation

The performance of **cobas**® SARS-CoV-2 Duo was evaluated for the qualitative detection of SARS-CoV-2 at three sites using prospectively collected clinical and contrived nasopharyngeal (NPS) and nasal swab (NS) samples from patients with signs and symptoms of a respiratory infection, collected in Copan UTM-RT or BD™ UVT. Clinical samples were collected by qualified personnel according to the package insert of the collection device. Results of **cobas**® SARS-CoV-2 Duo were compared to **cobas**® SARS-CoV-2 & Influenza A/B. A total of 1245 NPS and NS specimens were tested in the study of which 1109 provided valid results (n=1064 clinical specimns and n=45 contrived specimens). The respective PPA and NPA point estimates between **cobas**® SARS-CoV-2 Duo and the comparator were 98.3% (95% Score CI 94.0-99.5%) and 99.6% (95% Score CI 98.5-99.9%) for all (clinical and contrived) NPS samples and 98.5% (95% Score CI 91.9-99.7%) and 98.6% (95% Score 97.0-99.4%) for all (clinical and contrived) NS samples (Table 26).

Table 26: Performance of the **cobas**® SARS-CoV-2 Duo against an EUA authorized highly sensitive comparator test for NPS and NS specimens

Specimen Type	Total (N)	PPA	PPA LCL 95% Score CI	PPA UCL 95% Score CI	NPA	NPA LCL 95% Score CI	NPA UCL 95% Score CI
Overall	1109	98.4 % (181/184)	95.3 %	99.4 %	99.1 % (917/925)	98.3 %	99.6 %
NPS	612	98.3 % (116/118)	94.0 %	99.5 %	99.6 % (492/494)	98.5 %	99.9 %
NS	497	98.5 % (65/66)	91.9 %	99.7 %	98.6 % (425/431)	97.0 %	99.4 %

Note: CI = confidence interval, PPA = positive percent agreement, NPA = negative percent agreement,

LCL = lower confidence limit, UCL = upper confidence limit.

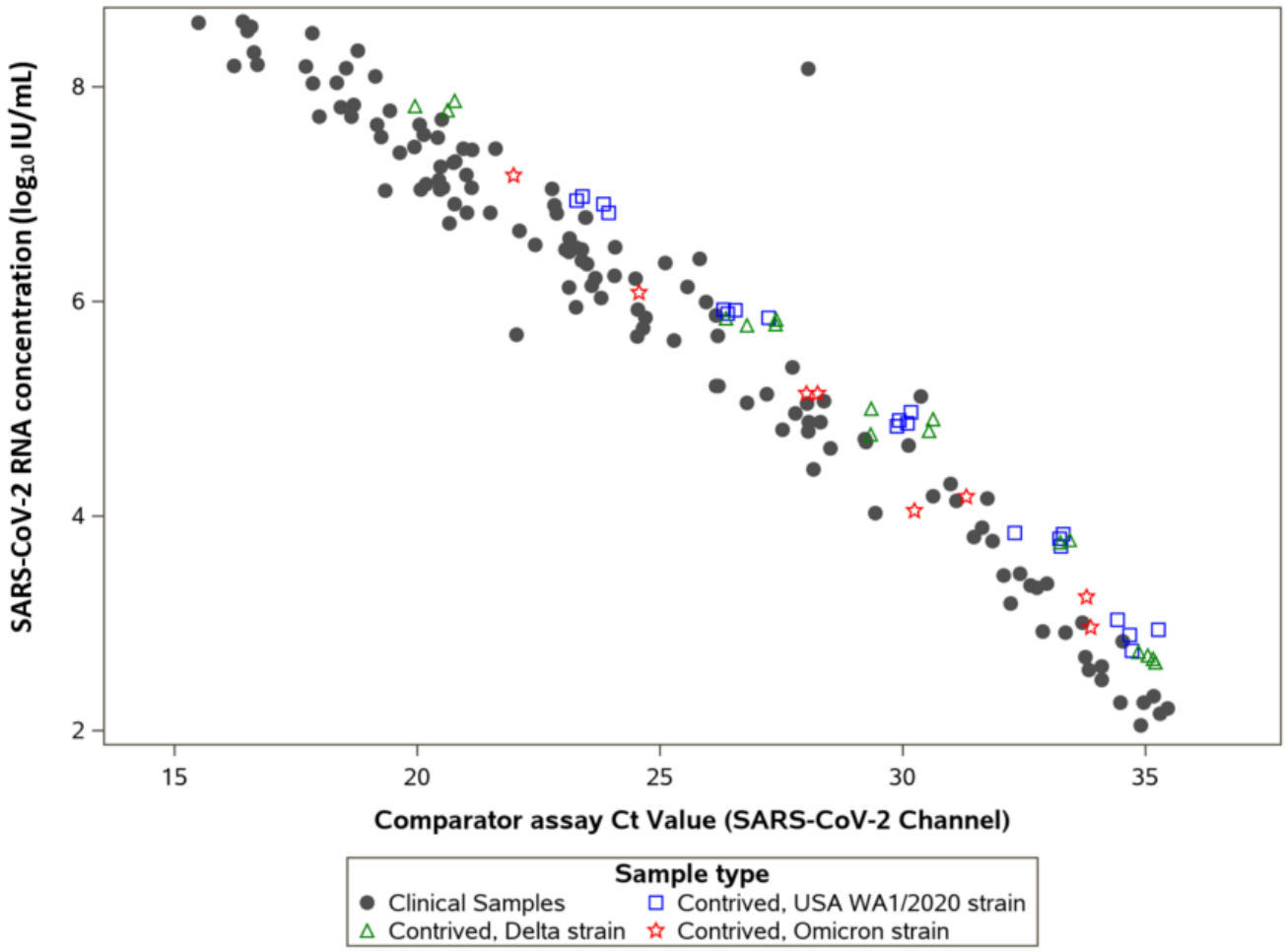
NPS = nasopharyngeal swab; NS = nasal swab.

SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Discrepant results were observed in a total of 11 clinical specimens (4 NPS and 7 NS). The Ct distributions of the two assays and the viral load results by **cobas**® SARS-CoV-2 Duo did not reveal a difference between the two clinical specimen types. **cobas**® SARS-CoV-2 Duo identified (confirmed by post-amplification amplicon analysis) the presence of SARS-CoV-2 in 2 of the discrepant NPS and 6 of the discrepant NS samples with late Ct values and low viral loads (Ct 38.4-42.2 and viral load of <1.00E+02 IU/mL, with exception of 1 sample with a Ct of 30 and viral load of 5.35E+03 IU/mL), suggesting a higher sensitivity by the assay versus the comparator. The comparator identified the presence of SARS-CoV-2 in three **cobas**® SARS-CoV-2 Duo negative NPS clinical specimens with Ct values close to the LoD of the assay.

Additional analysis was conducted versus the qualitative comparator regarding the quantitative component of the assay. Despite the qualitative comparator not being standardized to a quantitation standard, the analysis showed a strong linear correlation (r=-0.97) of **cobas**® SARS-CoV-2 Duo with the comparator Ct values in clinical and contrived samples that were positive by both assays with different viral RNA levels (Figure 5).

Figure 5: Correlation of SARS-CoV-2 RNA concentration (log_{10} IU/mL) vs. Ct Values for the comparator assay (SARS-CoV-2 channel) among clinical and contrived samples



System equivalency / system comparison

System equivalency of the **cobas**® 5800, **cobas**® 6800 and **cobas**® 8800 Systems was demonstrated via performance studies. The results presented in the Instructions for Use support equivalent performance for all systems.

Additional information

Key test features

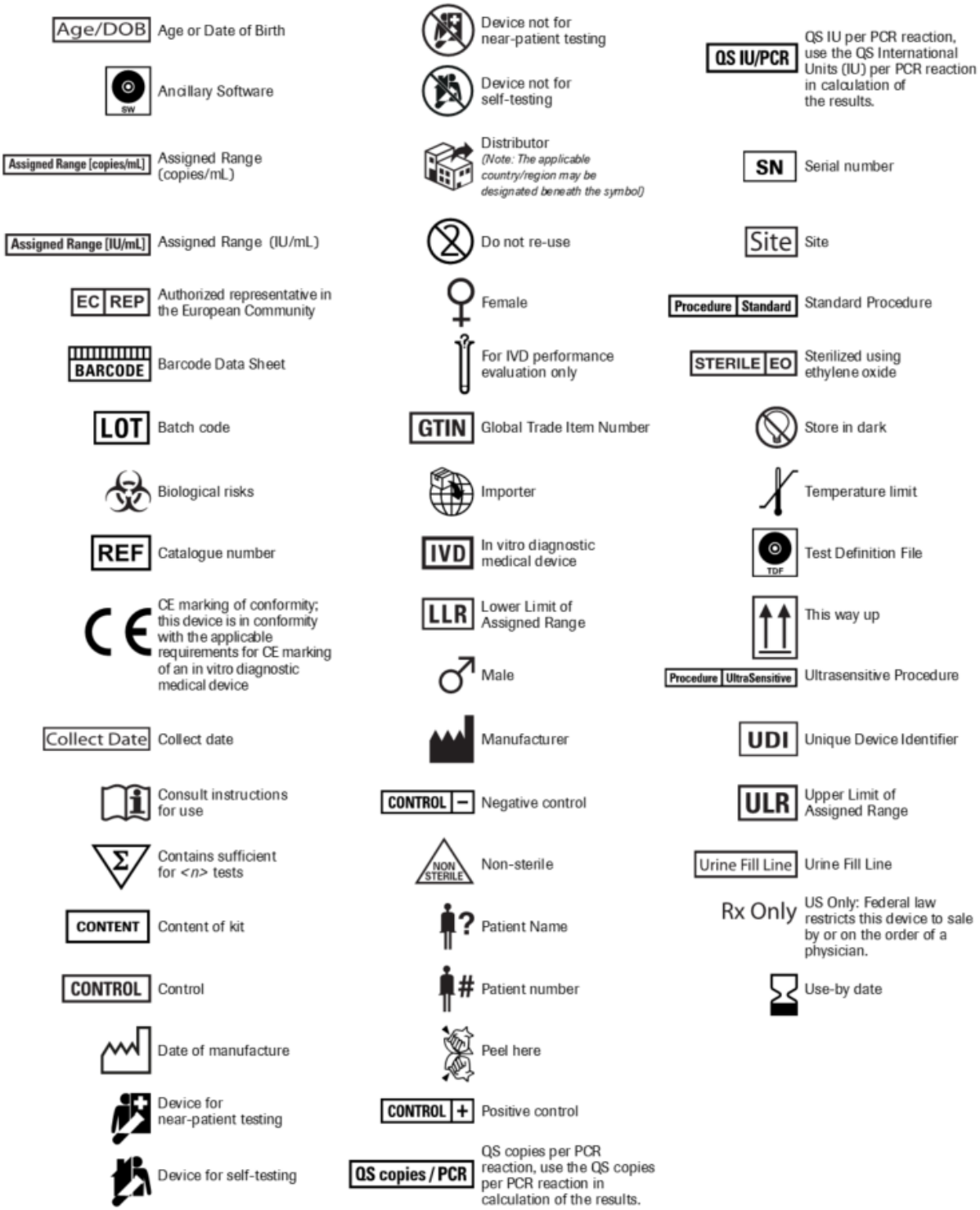
Sample type	Nasopharyngeal swab samples collected in the Copan UTM-RT® System or the BD™ UVT System Nasal swab samples collected in the Copan UTM-RT® System or the BD™ UVT System
Minimum amount of sample required	0.6 mL*
Sample processing volume	0.4 mL

*Dead volume of 0.2 mL should be considered for the **cobas omni** Secondary tubes. Other tubes compatible with the **cobas**® 5800 and **cobas**® 6800/8800 Systems (consult User Assistance Documents) may have different dead volume and require more or less minimum volume.

Symbols

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

Table 27: Symbols used in labeling for Roche PCR diagnostics products



Technical support

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

Manufacturer and importer

Table 28: Manufacturer and importer

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

Made in USA

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Trademarks and patents

See https://diagnostics.roche.com/us/en/about-us/patents

Copyright

CE 2797

EC REP

Roche Diagnostics GmbH
Sandhofer Str. 116
68305 Mannheim
Germany

IVD

References

1. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 6th 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) 300859, revised June 2020.

2. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline, 4th ed. CLSI Document M29-A4. Wayne, PA: CLSI, 2014.

Document revision

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Doc Rev. 1.0 07/2023	First Publishing

The summary of safety and performance report can be found using the following link: https://ec.europa.eu/tools/eudamed