October 22, 2022

Roche Molecular Systems, Inc.
Rita Hoady
Senior Manager, Regulatory Affairs
4300 Hacienda Drive
Pleasanton, California 94588-2722

Re: K213804
Trade/Device Name: cobas SARS-CoV-2 Qualitative for use on the cobas 6800/8800 Systems
Regulation Number: 21 CFR 866.3981
Regulation Name: Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The SARS-Cov-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test
Regulatory Class: Class II
Product Code: QQX
Dated: December 3, 2021
Received: December 6, 2021

Dear Rita Hoady:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's

requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Assistant Director
Viral Respiratory and HPV Branch Division
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K213804

Device Name
cobas SARS-CoV-2 Qualitative for use on the cobas 6800/8800 Systems

Indications for Use *(Describe)*
cobas SARS-CoV-2 Qualitative for use on the cobas 6800/8800 Systems is a real-time RT-PCR test intended for the qualitative detection of nucleic acids from SARS-CoV-2 in nasal and nasopharyngeal specimens collected from symptomatic individuals suspected of COVID-19 by their healthcare provider.
Results are for the detection of SARS-CoV-2 RNA. 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 pathogens.
Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Results are meant to be used in conjunction with clinical observations, patient history, recent exposures and epidemiological information, and laboratory data, in accordance with the guidelines provided by the relevant 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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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cobas SARS-CoV-2 Qualitative
510(k) Summary

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

Submitter Name	Roche Molecular Systems, Inc.
Address	4300 Hacienda Drive Pleasanton, CA 94588-2722
Contact	Rita Hoady Phone: (925) 487-1055 Fax: (925) 225-0207 Email: rita.hoady@roche.com
Date Prepared	December 2, 2021
Proprietary Name	**cobas** SARS-CoV-2 Qualitative for use on **cobas** 6800/8800 Systems
Classification Name	Device to detect and identify nucleic acid targets in respiratory specimens from microbial agents that cause the SARS-CoV-2 respiratory infection and other microbial agents when in a multi-target test
Product Codes	21 CFR 866.3981
Predicate Devices	BioFire COVID-19 Test 2 (K211079)
Establishment Registration	Roche Molecular Systems, Inc. (2243471)

1. DEVICE DESCRIPTION

cobas SARS-CoV-2 Qualitative is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas** 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas** 6800/8800 Systems software(s), which assigns test results for all tests. Results can be reviewed directly on the system screen and printed as a report.

Nucleic acid from patient samples and added internal control RNA (RNA IC) molecules are simultaneously extracted. Nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and

potential PCR inhibitors, are removed with subsequent wash steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way.

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

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

The **cobas** SARS-CoV-2 Qualitative master mix contains detection probes which are specific for the coronavirus type SARS-CoV-2, members of the Sarbecovirus subgenus, and the RNA Internal Control nucleic acid. The coronavirus and RNA Internal Control detection probes are each labeled with unique fluorescent dyes that act as a reporter. Each probe also has a second dye which acts as a quencher. When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Each reporter dye is measured at defined wavelengths, which enables simultaneous detection and discrimination of the amplified coronavirus target and the RNA Internal Control. The master mix includes deoxyuridine triphosphate (dUTP), instead of 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.

2. INDICATIONS FOR USE

cobas SARS-CoV-2 Qualitative for use on the **cobas** 6800/8800 Systems is a real-time RT-PCR test intended for the qualitative detection of nucleic acids from SARS-CoV-2 in nasal and nasopharyngeal specimens collected from symptomatic individuals suspected of COVID-19 by their healthcare provider.

Results are for the detection of SARS-CoV-2 RNA. 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 pathogens.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Results are meant to be used in conjunction with clinical observations, patient history, recent exposures and epidemiological information, and laboratory data, in accordance with the guidelines provided by the relevant 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.

3. TECHNOLOGICAL CHARACTERISTICS

The primary technological characteristics and intended use of the RMS **cobas** SARS-CoV-2 Qualitative for use on the **cobas** 6800/8800 Systems are substantially equivalent to other legally marketed nucleic acid amplification tests intended for the qualitative detection of SARS-CoV-2 virus (SARS-CoV-2).

As indicated in Table 1, **cobas** SARS-CoV-2 Qualitative for use on the **cobas** 6800/8800 Systems is substantially equivalent to significant characteristics of the identified predicate device, BioFire COVID-19 Test 2 (K211079).

Table 1: Comparison of the cobas SARS-CoV-2 Qualitative for use on the cobas 6800/8800 Systems with the Predicate Device

	Submitted Device: cobas SARS-CoV-2 Qualitative	**Predicate Device: BioFire COVID-19 Test 2 (K211079)**
Regulation Number	21 CFR 866.3981	Same
Regulation Name	Device to detect and identify nucleic acid targets in respiratory specimens from microbial agents that cause the SARS-CoV-2 respiratory infection and other microbial agents when in a multi-target test	Same
Product Code	QQX	Same
Intended Use	**cobas** SARS-CoV-2 Qualitative for use on the **cobas** 6800/8800 Systems is a real-time RT-PCR test intended for the qualitative detection of nucleic acids from SARS-CoV-2 in nasal and nasopharyngeal specimens collected from symptomatic individuals suspected of COVID-19 by their healthcare provider. Results are for the detection of SARS-CoV-2 RNA. 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 pathogens. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Results are meant to be used in conjunction with clinical observations, patient history, recent exposures and epidemiological information, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities. **cobas** SARS-CoV-2 Qualitative 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.	The BioFire COVID-19 Test 2 is a qualitative nested multiplexed RT-PCR in vitro diagnostic test intended for use with the BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems. The BioFire COVID-19 Test 2 detects nucleic acids from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in nasopharyngeal swabs (NPS) from symptomatic individuals suspected of COVID-19 by their healthcare provider. Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in NPS 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 co-infection with other pathogens. Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities. The BioFire COVID-19 Test 2 is intended for use by trained medical and laboratory professionals in a laboratory setting or under the supervision of a trained laboratory professional.
Conditions for use	For prescription use	Same

	Submitted Device: cobas SARS-CoV-2 Qualitative	**Predicate Device: BioFire COVID-19 Test 2 (K211079)**
Sample Types	Nasopharyngeal swab specimen Nasal swab specimen	Nasopharyngeal swab specimen
Analyte Targets	SARS-CoV-2	SARS-CoV-2
Sample Preparation Procedure	Automated by **cobas** 6800/8800 Systems	Automated by BioFire FilmArray 2.0 or BioFire FilmArray Torch systems
Amplification Technology	Real-time PCR	PCR-based multiplexed nucleic acid test
Detection Chemistry	Paired reporter and quencher fluorescence labeled probes (TaqMan Technology) using fluorescence resonance energy transfer (FRET)	Two Step Nested multiplex PCR: 1. Reverse transcription, followed by a multiplexed first stage PCR reaction (PCR1). Multiple simultaneous second-stage PCR reactions (PCR2) to amplify sequences within the PCR1 products using fluorescence double stranded binding dye. Endpoint melting curve data to detect target-specific amplicons
Controls used	Sample processing control (IC) Positive and negative control	Two process controls: 1. RNA Process Control (IC) PCR2 Control (A positive result indicates that PCR2 was successful)
Result Analysis	Based on PCR cycle threshold analysis	Endpoint melting curve data to detect target-specific amplicons

4. SPECIAL CONTROLS/STANDARDS/GUIDANCE REFERENCED

Class II Special Controls as per 21 CFR 866.3981.

5. NON-CLINICAL PERFORMANCE EVALUATION

5.1. Analytical sensitivity (Limit of Detection)

The Limit of Detection (LoD) for cobas SARS-CoV-2 Qualitative was determined using an inactivated quantified SARS-CoV-2 virus (WHO International Standard for SARS-CoV-2, NIBSC code: 20/146). LoD is defined as the lowest concentration of SARS-CoV-2 RNA that can be detected at a rate of at least 95%. A total of 5 concentration levels (500, 250, 125, 62.5, and 31.25 IU/ml) were prepared by diluting the SARS-CoV-2 target in negative simulated clinical matrix stabilized in UTM. Three independent dilution series with three lots of reagents were tested with a total of 24 replicates per concentration.

The concentration level with observed hit rates greater than or equal to 95% was determined to be the LoD for each of the two targets (SARS-CoV-2 and pan-Sarbecovirus) as described in Table 2 and Table 3.

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

Viral Strain	Kit lot	Hit rate ≥ 95% [IU/mL]	Mean Ct at ≥ 95% Hit rate
WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146)	Lot 1	**250 (24/24)**	33.2
	Lot 2	**125 (23/24)**	34.1
	Lot 3	**250 (23/24**	33.2

The LoD was confirmed at 250 IU/mL for SARS-CoV-2 (Target 1). For all three reagent lots, at least 23/24 replicates detected the target at 250 IU/ml.

Table 3: Summary of LoD for pan-Sarbecovirus using WHO International Standard (NIBSC code: 20/146)

Viral Strain	Kit lot	Hit rate ≥ 95% [IU/mL]	Mean Ct at ≥ 95% Hit rate
WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146)	Lot 1	**125 (24/24)**	35.2
	Lot 2	**125 (24/24)**	36.0
	Lot 3	**125 (23/24)**	34.8

5.2. Inclusivity

The inclusivity of cobas SARS-CoV-2 Qualitative for the detection of SARS-CoV-2 was confirmed by testing nine SARS-CoV-2 strains, including six variant strains. The lowest target analyte at which all four tested replicates were positive are reported in Table 4. In silico analysis of additional SARS-CoV-2 sequences indicates that >99.99 % of sequences for SARS-CoV-2 have no changes in primer/probe binding sites at both target regions simultaneously. All known sequences are predicted to be detected by at least one of the two target regions.

Table 4: Summary of inclusivity

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

5.3. Precision

Within-laboratory precision was examined using a panel of SARS-CoV-2 (USA-WA1/2020, heat-inactivated) cultures diluted in simulated clinical matrix in universal transport media. Sources of variability were examined with a panel consisting of three concentration levels, using three lots of **cobas** SARS-CoV-2 Qualitative reagents and three instruments over a course of 15 instrument days (2 runs/day x 3 instruments x 5 days/instrument) for a total of 30 runs containing a total of 90 replicates per concentration. A description of the precision panel and the observed positivity rates are shown in Table 5. All negative panel members tested negative throughout the study. Analysis of standard deviation and percent coefficient of variation (CV) of the Ct values from tests performed on positive panel members (see Table 6) yielded overall CV percentage ranging from 1.1% to 2.2% for **cobas** SARS-CoV-2 Qualitative.

Table 5: Summary of within laboratory precision

Target	Panel Member	Level (x LoD)	Positive Results	Total Results	Positivity %	Two-sided 95% CI Lower Bound	Two-sided 95% CI Upper Bound
Target 1 (SARS-CoV-2)	Weak positive	~0.3x	9	90	10%	5%	18%
	Low positive	~1.0x	82	90	91%	83%	96%
	Moderate positive	~3.0x	90	90	100%	96%	100%
	Weak positive	~0.3x	31	90	34%	25%	45%

Target	Panel Member	Level (x LoD)	Positive Results	Total Results	Positivity %	Two-sided 95% CI Lower Bound	Two-sided 95% CI Upper Bound
Target 2 (pan-Sarbecovirus)	Low positive	~1.0x	84	90	93%	86%	97%
	Moderate positive	~3.0x	90	90	100%	96%	100%
N/A	Negative	Blank	0	90	0%	0%	4%

Table 6: Overall mean, standard deviation, and percent coefficient of variation for Ct values by positive panel member

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

5.4. Reproducibility

The reproducibility of **cobas** SARS-CoV-2 Qualitative was evaluated across multiple variables that theoretically could affect reported results, including: reagent lot, testing site/instrument, day, and run. The evaluation was conducted at 3 testing sites, using 3 reagent lots, with a 4-member panel of positive and negative samples resulting in a total number 216 tests per concentration (not including controls). The positive panel members contained SARS-CoV-2 viral culture material [WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146)] at 3 different concentrations in universal transport medium (UTM) based simulated clinical matrix. Each site tested two reagent lots for 6 days. Two runs were performed each day and 3 replicates of each panel member were performed for each run. An overall SARS-CoV-2 positive result was determined by a positive detection in either or both of the SARS-CoV-2 or/and pan-Sarbecovirus channels. The evaluation results are summarized in Table 7.

The system showed a 99.1% negative percent agreement with a 95% CI of 96.7% 99.9%. The test results showed good lot-to-lot, instrument-to-instrument (site), day-to-day, and between run variation for the ~0.3x LoD, ~1x LoD, and ~3x LoD panel members ().

Table 7: Overall mean estimate, standard deviations, and coefficients of variation (%) for cycle threshold values by viral target and expected viral concentration (positive panel members)

Viral Target	Panel Member Concen-tration	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	0	214/216[c]	99.1	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
SARS-CoV-2	~0.3x LoD	45/216	20.8	33.6	0.00	0.0	0.00	0.0	0.11	0.3	0.00	0.0	0.35	1.1	0.37	1.1
SARS-CoV-2	~1x LoD	196/216	90.7	33.2	0.00	0.0	0.09	0.3	0.00	0.0	0.17	0.5	0.37	1.1	0.42	1.3
SARS-CoV-2	~3x LoD	216/216	100.0	32.2	0.05	0.2	0.02	0.1	0.00	0.0	0.03	0.1	0.24	0.8	0.25	0.8
pan-Sarbecovirus	~0.3x LoD	158/216	73.1	36.5	0.18	0.5	0.00	0.0	0.00	0.0	0.00	0.0	0.71	2.0	0.74	2.0
pan-Sarbecovirus	~1x LoD	214/216	99.1	35.4	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.67	1.9	0.67	1.9
pan-Sarbecovirus	~3x LoD	216/216	100.0	34.1	0.11	0.3	0.05	0.2	0.00	0.0	0.00	0.0	0.32	0.9	0.34	1.0

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

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

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

[b] Percent agreement with expected results.

[c] 2 negative panel members were tested positive. Sequencing showed that one of these samples was positive and the other was negative. The Ct values and the curve analysis of these samples may suggest a low level of contamination during specimen handling.

5.5. Analytical specificity/cross-reactivity

A panel of 47 viruses, bacteria, and fungi (including those commonly found in respiratory tract) and pooled human nasal wash were tested with **cobas** SARS-CoV-2 Qualitative to assess analytical specificity. The organisms listed in Table 8 were spiked at concentrations of 1 x 10^5 units/mL for viruses and1 x 10^6 units/mL for other organisms, unless otherwise noted.

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

Additional *in silico* analysis conducted with other coronaviruses and respiratory flora indicated no concerns with the test performance by predicting any false-negative or false-positive results.

Table 8: Cross-reactivity test results

Microorganism	Concentration
Human coronavirus 229E	1.0E+05 TCID50/mL
Human coronavirus OC43	1.0E+05 TCID50/mL
Human coronavirus HKU1	1.0E+05 TCID50/mL
Human coronavirus NL63	1.0E+05 TCID50/mL
MERS coronavirus	1.0E+05 genomic equivalent/mL
SARS coronavirus	1.0E+05 PFU/mL
Adenovirus B (Type 34)	1.0E+05 TCID50/mL
Bocavirus	1.0E+05 cp/mL
Cytomegalovirus	1.0E+05 TCID50/mL
Epstein Barr virus	1.0E+05 cp/mL
Human Metapneumovirus (hMPV)	1.0E+05 TCID50/mL
Measles virus	1.0E+05 TCID50/mL
Mumps virus	1.0E+05 TCID50/mL
Parainfluenza virus Type 1	1.0E+05 TCID50/mL
Parainfluenza virus Type 2	1.0E+05 TCID50/mL
Parainfluenza virus Type 3	1.0E+05 TCID50/mL
Parainfluenza virus Type 4	1.0E+05 TCID50/mL
Influenza A (H1N1)	1.0E+05 TCID50/mL
Influenza A virus (H1N1-2009, H1N3, H3N2)	1.0E+05 TCID50/mL
Influenza B	1.0E+05 TCID50/mL
Enterovirus E (Type 1)	1.0E+05 TCID50/mL

Microorganism	Concentration
Parechovirus	1.0E+05 TCID50/mL
Respiratory syncytial virus	1.0E+05 PFU/mL
Rhinovirus	1.0E+05 TCID50/mL
Candida albicans	1.0E+06 CFU/mL
Chlamydia pneumoniae	1.0E+06 TCID50/mL
Corynebacterium diphtheriae	1.0E+06 CFU/mL
Escherichia coli	1.0E+06 CFU/mL
Haemophilus influenzae	1.0E+06 CFU/mL
Lactobacillus gasseri	1.0E+06 CFU/mL
Legionella pneumophila	1.0E+06 CFU/mL
Legionella jordanis (non-pneumophila)	1.0E+06 CFU/mL
Moraxella catarrhalis	1.0E+06 CFU/mL
Mycobacterium tuberculosis	1.0E+06 cells/mL
Neisseria elongata	1.0E+06 CFU/mL
Neisseria meningitidis	1.0E+06 CFU/mL
Pseudomonas aeruginosa	1.0E+06 CFU/mL
Pneumocystis jirovecii	1:20 of Patient Sample
Staphylococcus aureus	1.0E+06 CFU/mL
Staphylococcus epidermidis	1.0E+06 CFU/mL
Streptococcus pneumoniae	1.0E+06 CFU/mL
Streptococcus pyrogenes	1.0E+06 CFU/mL
Streptococcus salivarius	1.0E+06 CFU/mL
Bordetella pertussis	1.0E+06 CFU/mL
Mycoplasma pneumoniae	1.0E+06 CFU/mL

5.6. Interference

The effect of exogenous substances potentially secreted into respiratory specimens was evaluated (Table 9). Each potentially interfering substance was tested at or above clinically relevant levels in negative simulated clinical matrix stabilized in universal transport media in absence and presence of SARS-CoV-2 target (spiked at ~3x LoD).

None of the substances interfered with the test performance by generating false-negative, false-positive or invalid results at the concentrations tested, as shown below.

Table 9: List of exogenous substances tested for interference

Substance*	Product Name	Concentration
Oxymetazoline	Afrin Nasal Spray	0.011 mg/mL
Galphimia glauca, Luffa operculata, Sabadilla	Zicam nasal spray	0.023 mg/mL
Lidocaine and Phenylephrine	Liposomal NUMB520 Spray	2.68 mg/mL
Budesonide	Budesonide Nasal spray	0.039 mg/mL
Phenol	Chloraseptic	0.47 mg/mL
Fluticasone propionate	Flovent DisKus	166.67 µg/mL
Mupirocin	Mupirocin onitment UPS (each gram contacin 20 mgs)	0.20 mg/mL
Zanamivir	Relenza (Inhalation powder)	0.0015 mg/mL
Oseltamivir	Antiviral drug – Tamiflu	0.0073 mg/mL
Benzocaine and Menthol	Cepacol (Sore throat Lozenges)	5.00 mg/mL
Tobramycin	Tobramycin ophthalmic solution	0.018 mg/mL
Petroleum Jelly	Vaseline	1% (w/v)
Nicotine	Snuff Tobacco	1% (w/v)
Camphor-synthetic eucalyptus oil and menthol ointment	Analgesic ointment (Vicks@VapoRubR)	1% (w/v)
0.65% NaCl, Phenylcarbino, Benzalkonium chloride	Saline Nasal Spray with Preservatives	1% (w/v)

* FluMist was not evaluated to assess potential interference.

Endogenous substances that may be present in respiratory specimens were tested for interference (Table 10). Each potentially interfering substance was tested at or above clinically relevant levels in negative simulated clinical matrix stabilized in universal transport media in absence and presence SARS-CoV-2 target (spiked at ~3x LoD).

None of the substances interfered with the test performance by generating false-negative, false-positive or invalid results at the concentrations tested, as shown below.

Table 10: List of endogenous substances tested for interference

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

Substance	Concentration
Human Whole Blood	5% (v/v)

5.7. Matrix equivalency

Equivalence between simulated and real clinical matrix was evaluated using the nasophyrangeal and nasal swabs. The WHO International Standard was used to formulate panels to a target concentration of approximately 3x LoD (above LoD), 1x LoD (at LoD) and 0.3x LoD (below LoD) into pooled negative clinical samples of each sample type (NPS, NS and simulated), stabilized in universal transport media. Twenty-five replicates per concentration were tested for each sample type. All replicates tested at the 1x LoD and 3x LoD concentrations were positive for SARS-CoV-2 for all three sample types. Simulated clinical matrix, nasopharyngeal swabs and nasal swab sample types are acceptable for use with **cobas** SARS-CoV-2 Qualitative.

5.8. Collection media equivalency – UTM-RT, cobas PCR Media and 0.9% physiological saline

Equivalence between different collection media (UTM-RT, **cobas** PCR Media, and saline) was evaluated using the WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146). The WHO International Standard was used to formulate to a target concentration of approximately 2x LoD (low positive) and 4x LoD (moderate positive) into paired individual negative clinical samples, stabilized either in Universal Transport Media (UTM-RT), **cobas** PCR Media (CPM), or 0.9% physiological saline (NaCl). At least 20 replicates per low positive sample and 10 replicates per moderate positive sample were tested for each collection media type. All replicates tested were positive for SARS-CoV-2 in all the three collection media types. UTM-RT, **cobas** PCR Media, and 0.9% physiological saline are acceptable for use with **cobas** SARS-CoV-2 Qualitative. **cobas**® PCR Media, and 0.9% physiological saline were not evaluated in the clinical study.

6. CLINICAL PERFORMANCE EVALUATION

6.1. Performance with clinical specimens

The performance of **cobas** SARS-CoV-2 Qualitative was evaluated in a multi-center study with three external testing sites evaluating prospectively collected clinical specimens in UTM-RT or UVT from individuals with signs and symptoms of respiratory infection. Participants from 12

geographically distributed enrollment centers each provided nasopharyngeal swab (NPS) and nasal swab (NS, anterior nares) specimens as part of a dual collection where (a) the collection order (first specimen collected) was alternated between the NPS and NS specimen, and (b) the collection method for NS specimens was also alternated with 50% of the NS specimens were self-collected on-site with healthcare provider (HCP) instructions, while the other 50% were collected by the healthcare provider. The study used a composite comparator method wherein laboratory sites used up to three highly sensitive EUA SARS-CoV-2 molecular assays, testing NPS specimen from each subject. The composite comparator result was defined as the concordant results from two comparator assays (test A and test B). In case of discordance between the initial two comparator assays, the sample was tested by a third assay (test C) and the result of the third test determined the composite comparator result. The composite comparator result was indetermined when valid results could not be obtained from two assays (i.e., insufficient volume for repeat testing of invalid/failed results).

From March to June 2021, a total of 1,154 participants were enrolled, of which samples from 968 participants were included in the evaluation. Samples from 186 participants were not included: 184 specimens were excluded due to issues associated with specimen shipments and/or being unable to complete testing within the times identified by manufacturer's instructions, and two subjects were excluded for being previously enrolled in the study (exclusion criteria). When self-reporting COVID-19 vaccination status, 207 (21.4%) of the 968 participants were fully vaccinated.

Of the 968 participants, 961 contributed a NPS specimen which resulted in 942 participants with a confirmed infected status. For NPS, 4 specimens had failed/invalid **cobas** SARS-CoV-2 Qualitative results, resulting in 938 evaluable NPS results. For NS, 8 specimens were invalid/missing **cobas** SARS-CoV-2 Qualitative NS results, resulting in 934 evaluable results.

When compared with the NPS composite comparator result, **cobas** SARS-CoV-2 Qualitative yielded a positive percent agreement (PPA) of 98.7% for NPS and 97.4% for NS specimens. The negative percent agreement (NPA) was 99.7% and 99.9% for NPS and NS specimens, respectively (Table 11).

Table 11: Summary of clinical performance of cobas SARS-CoV-2 Qualitative for nasopharyngeal (NPS) and nasal swabs (NS) versus the NPS composite comparator

Specimen Type	Total (N)	PPA	PPA 2-sided 95% Score CI	NPA	NPA 2-sided 95% Score CI
Nasopharyngeal (NPS)	938	98.7% (77/78)	(93.1 %, 99.8 %)	99.7% (857/860)	(99.0 %, 99.9 %)
Nasal Swab (NS)*	934	97.4% (76/78)	(91.1 %, 99.3 %)	99.9% (855/856)	(99.3 %, 100.0 %)

*Healthcare provider-collected nasal swab specimens and nasal swab specimens self-collected on-site with healthcare provider instructions

7. CONCLUSIONS

The conclusions drawn from the non-clinical and clinical studies demonstrate that the device is substantially equivalent to the predicate device.

8. REFERENCES

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