CLIA Waiver by Application Approval Determination

Decision Summary

A. **Document Number**

CW220015

B. Parent Document Number

K223783

C. CLIA Waiver Type:

Dual 510(k) and CLIA Waiver by Application (Dual Submission)

D. Applicant

Roche Molecular Systems, Inc.

E. Proprietary and Established Names

cobas SARS-CoV-2 Nucleic acid test for use on the cobas Liat System

F. Measurand (analyte)

SARS-CoV-2 virus RNA

G. Sample Type(s)

Nasopharyngeal Swabs (NPS) and Anterior nasal swabs (NS)

H. Type of Test

This is a real-time RT-PCR nucleic acid amplification assay for the qualitative detection of SARS-CoV-2 RNA.

I. Test System Description

1. Overview

The cobas SARS-CoV-2 Nucleic acid test for use on the cobas Liat System is an automated *in vitro* diagnostic test for the qualitative detection of SARS-CoV-2 RNA in nasopharyngeal swab (NPS) and anterior nasal swab (NS) specimens eluted in transport media.

The assay targets both the ORF1 a/b non-structural region and nucleocapsid protein gene that are unique to SARS-CoV-2. An Internal Process Control (IPC) is also included. The IPC is present to control for adequate processing of the target virus and to monitor for the presence of inhibitors in the sample preparation and RT-PCR.

The assay utilizes a single-use disposable cobas assay tube (cobas Liat Tube) that holds the sample purification and PCR reagents and hosts the sample preparation and PCR processes. The cobas Liat tube is a flexible tube used as a sample vessel. It contains all required unit dose reagents pre-packed in tube segments, separated by peelable seals, in the order of reagent use.

The cobas SARS-CoV-2 assay uses silica magnetic particle-based nucleic acid extraction and TaqMan probe-based real-time PCR amplification and detection. The cobas Liat Analyzer automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in biological samples. During the testing process, multiple sample processing actuators of the cobas Liat Analyzer compress the cobas Liat Tube to selectively release reagents from tube segments, move the sample from one segment to another, and control reaction volume, temperature, and incubation time. The cobas Liat Analyzer software controls and coordinates these actions to perform all required assay processes, including sample preparation, nucleic acid extraction, target enrichment, inhibitor removal, nucleic acid elution, and real-time PCR. All assay steps are performed within the closed and self-contained cobas SARS-CoV-2 assay tube.

2. Test System Components

   a. The cobas SARS-CoV-2 kit includes:
      - 20 cobas SARS-CoV-2 test assay tubes (P/N 09408592190)
      - 2 cobas transfer pipette packs (12 pipettes/pack - P/N 9329676001)
      - 1 Package Insert Barcode card

   b. The cobas SARS-CoV-2 Quality Control Kit (P/N 09408835190) is provided separately, and includes:
      - cobas SARS-CoV-2 Positive Control (b)(4) (P/N 09212078001)
      - cobas Dilution UTM (negative control) (b)(4) (P/N 08053669001)
      - 8 transfer pipettes
      - 1 Control Kit Barcode

J. Demonstrating "Simple"

- The cobas Liat System automates all nucleic acid test (NAT) processes, including reagent preparation, target enrichment, inhibitor removal, nucleic acid extraction, amplification, real-time detection, and result interpretation in a rapid manner.
- The assay utilizes NPS and NS specimens collected in transport medium without the need for any specimen manipulation. A provided fixed volume pipette is used to transfer the sample to the assay tube. The tube is capped and remains closed for the entire test process. No further materials need to be added or removed from the tube.

- Running the assay requires no reagent manipulation. The assay tube contains all assay reagents pre-packed in tube segments separated by seals.
- The assay tube is designed such that it can only be inserted in the cobas Liat Analyzer in one direction.
- The test does not require any operator intervention during the analysis step.
- The cobas Liat Analyzer performs automated analysis of test results, which are reported on the cobas Liat Analyzer screen as "SARS-CoV-2 Detected", "SARS-CoV-2 Not Detected", or "Assay Invalid".
- No technical or specialized training is required for troubleshooting or error code interpretation. If an error code is shown, simple on-screen instructions are provided to the operator for next steps.
- The system requires no electronic or mechanical maintenance tasks by the operator. The analyzer performs self-diagnostics during startup (initialization) and utilizes an advanced error diagnostics system to monitor the analyzer's performance during an assay. Under normal operation, the analyzer alerts the operator if a malfunction or error is detected.
- The analyzer requires no adjustment or calibration from the operator.
- The Quick Reference instructions are written at a 7th grade comprehension level.

K. Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-safe Mechanisms

1. Risk Analysis

   Risk analysis was performed by the firm according to the principles of risk minimization as found in the standard EN ISO 14971 *Medical Devices – Application of risk management to medical devices*. Potential sources of errors that could adversely affect system performance were identified and mitigated first through system design and then through additional cautions in the labeling. All residual risks of harm to the patient or operator were evaluated in flex studies.

2. Fail-Safe and Failure Alert Mechanisms

   The cobas Liat System is designed with a variety of fail-safe and/or failure alert mechanisms to prevent operator and instrument errors, as listed below.

Table 1. Fail-safe and failure alert mechanisms built into the cobas Liat System

#	Description
1	Operator permissions restrict access to specific instrument functions/test capabilities
2	On-screen instructions
3	Use of barcodes to automate assay selection and provide traceability
4	Lock-out feature to prevent use of expired or previously used reagents
5	Controls to ensure correct tube insertion
6	Sample volume detection
7	Electronic error diagnostic and recovery to prevent out-of-specification operation
8	System self-checks for instrument subsystems

9	Auto-calibration and monitoring
10	Automated data acquisition, analysis, and result interpretation
11	Internal Process Control to monitor the test procedure for each individual sample
12	Mandatory "Add Lot" procedure for new reagent lots

3. Flex Studies

The cobas SARS-CoV-2 on the cobas Liat System is performed according to the same workflow as the previously cleared and CLIA waived tests on the Liat System (e.g., cobas Influenza A/B Nucleic acid test (K223591/CW220014), and cobas Influenza A/B & RSV Nucleic acid test (K153544/CW150018)) and the physical properties of the Liat Analyzer are shared between all assays. Therefore, some of the previously conducted flex studies, considered not to be assay specific, demonstrating operational robustness of the cobas Liat System, were not repeated and the previously generated data were leveraged for this application.

The following flex studies were conducted previously and may be referenced in the CLIA Waiver Decision Summaries for CW140014, CW150013, and CW150018:

a. Variation in operating temperature (environmental temperature above/below specified range).
b. Variation in humidity levels (environmental humidity above/below specified range).
c. Variation in altitude and atmospheric pressure (operation above specified altitude).
d. Operation of the instrument on non-level surface (instrument tilt (x-, y-tilt).
e. Movement of the Liat Analyzer during analysis.
f. Variable Sample Volume (input sample above/below the specified volume).
g. Assay tube orientation post sample addition.
h. Broken or compromised seals within the Liat tubes (due to mishandling prior to testing)
i. Improper storage of Liat tubes at temperatures outside of specified range (i.e., Liat tubes in foil pouches stored at -20°C or at 37°C, and Liat tubes without foil pouch, stored at -20°C,4°C,25°C, and 37°C).

Additional flex studies, deemed as assay specific, were performed to evaluate the robustness (i.e., risk of erroneous results) of the cobas SARS-CoV-2 assay when subjected to potential variations in workflow and control effectiveness that may reasonably be expected to occur with untrained operators in the intended use CLIA waived setting. Test conditions were designed based on a risk analysis of the complete test system and included conditions intended to verify the effectiveness of built-in controls. These studies are described below.

a. Evaluation of Assay Tube Hold Time after Sample Addition

The purpose of this study was to show the insensitivity of the cobas SARS-CoV-2 assay to delay between addition of the sample to the cobas SARS-CoV-2 Assay Tube and initiation of the run on the Liat Analyzer. To perform these studies, co-formulated panel consisting of low positive (~3x LoD) samples containing inactivated SARS-CoV-2 viral cultures in pooled matrix and negative samples (unspiked matrix) were used. Three matrices were evaluated: NPS specimens collected in Universal Transport Media (UTM), NPS specimens collected in M4RT media, NPS specimens collected in 0.85% saline, and NPS specimens collected in 0.90% saline The samples were added to the assay tube and run (a) immediately, (b) after 2 hours, and (c) after 4 hours of storage at ambient temperature (~30°C) in a designated incubator. For each condition, five (5) replicates of the each sample negative samples (NPS in each media type) and five (5) replicates of low positive (~3x LoD) samples (NPS in each media type) were tested. All samples generated valid and expected results. The results are summarized in **Table 2**.

Table 2. Assay Tube Hold Time Results

Sample	Hold Time at ~30°C (Hour)	% Positive Results			
		NPS UTM	NPS M4RT	NPS Saline 0.85%	NPS Saline 0.90%
Negative	0	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/5)
	2	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/5)
	4	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/5)
Positive	0	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)
	2	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)
	4	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)

The results demonstrated that the cobas SARS-CoV-2 assay can tolerate hold time between addition of the sample to the assay tube and initiation of the run on the Liat System for up to 4 hours at ambient temperature in a designated incubator (~30°C).

b. Internal Processing Control (IPC) Effectiveness

This study evaluated the effectiveness of the Internal Process Controls (IPC) to monitor the performance of the **cobas** SARS-CoV-2 assay sample processing and PCR amplification/detection under simulated process and reagent failures. To perform these studies, a panel consisting of ~3x LoD concentrations of inactivated SARS-CoV-2 viral cultures in pooled negative clinical NPS collected in UTM was used. For each condition, five (5) replicates of negative and five (5) replicates of positive samples were tested. The test conditions evaluated are described in Table 3. The summary of results are shown in Table 4.

Table 3. Test Condition for IPC Effectiveness

Monitored Function	Failure Conditions
Control	Control: all functions performed as expected
Broken seal separating Lysis Buffer and Wash Buffer.	Reagent Failure: Break off frangible seal between the assay tube segments containing Lysis Buffer and Wash Buffer, and allowing mixing of the two reagents
Broken seal separating Wash Buffer and Elution Buffer.	Reagent Failure: Break of frangible seal between the assay tube segments containing Wash Buffer and Elution Buffer, and allowing mixing of the two reagents
Failure in magnetic beads capture during sample preparation	Process Failure: Failure to capture magnetic beads during nucleic acid extraction (simulated by not moving the magnet to capture beads during sample preparation step)
Failure of temperature control during in PCR Amplification and Detection	Process Failure: Deviation in PCR temperature (simulated by setting denaturation temp to 80ºC)

Table 4. IPC Effectiveness Results

Test Sample	Testing Condition	SARS-CoV-2 Result	IPC Result
Negative Sample (Negative NPS)	Control	Not Detected (5/5)	Detected (5/5)
	Lys-WB Seal broken	Invalid (5/5)	Invalid (5/5)
	WB-EB Seal broken	Invalid (5/5)	Invalid (5/5)
	Sample prep bead failure	Invalid (5/5)	Invalid (5/5)
	Denaturation @ 80ºC	Invalid (5/5)	Invalid (5/5)
Contrived Positive Sample (3x LoD targets in Negative NPS)	Control	Detected (5/5)	Detected (5/5)
	Lys-WB Seal broken	Invalid (5/5)	Invalid (5/5)
	WB-EB Seal broken	Invalid (5/5)	Invalid (5/5)
	Sample prep bead failure	Invalid (5/5)	Invalid (5/5)
	Denaturation @ 80ºC	Invalid (5/5)	Invalid (5/5)

All tests from each stress condition generated invalid results. Results indicate that under failure mode testing conditions, the IPC appears to be effective in monitoring the performance of sample processing and PCR amplification/detection failure conditions.

c. External Control Effectiveness

This study evaluated the external positive and negative control results under the cobas SARS-CoV-2 assay process and reagent/assay tube failure conditions in order to substantiate the ability of Positive Controls (PC) and Negative Controls (NC) to monitor such processes. The same processes as listed above in Table 3 were evaluated by running PC and NC in five (5) replicates under the simulated conditions.

Table 5. External Control Effectiveness Results

Test Sample	Testing condition	SARS-CoV-2 Result	IPC Result
Negative Control (NC)	Control	Not Detected (5/5)	100% (5/5)
	Bead Failure	Invalid (5/5)	100% (5/5)
	Lysis/wash seal break	Invalid (5/5)	100% (5/5)
	Denaturation temp set to 80°C	Invalid (5/5)	100% (5/5)
	Wash/EB seal break	Invalid (5/5)	100% (5/5)
	Control stored at 65°C for 48hrs	Invalid (5/5)	100% (5/5)
	Contaminated NC	Invalid (5/5)	100% (5/5)
Positive Control	Control	Detected (5/5)	100% (5/5)
	Bead Failure	Invalid (5/5)	100% (5/5)
	Lysis/wash seal break	Invalid (5/5)	100% (5/5)
	Denaturation temp set to 80°C	Invalid (5/5)	100% (5/5)
	Wash/EB seal break	Invalid (5/5)	100% (5/5)
	Control stored at 65°C for 48hrs	Invalid (5/5)	100% (5/5)

The NC & PC both failed (generated invalid results) under the reagent and process failure modes tested. All runs with the simulated failure conditions yielded invalid results for the targets while the Internal Control (IC) remained valid. Thus, demonstrating that the Positive and Negative controls are effective in monitoring the process and reagent/assay tube failure conditions.

Overall, the flex studies demonstrated that the cobas SARS-CoV-2 assay is robust to foreseeable user-dependent errors or potential reagent failure. The combination of built-in assay controls, the fail-safe and failure alert mechanisms, and the external controls are

effective in preventing the generation of erroneous results due to operator error and/or use of the cobas Liat System outside the specified operating environmental conditions.

L. Demonstrating "Insignificant Risk of an Erroneous Result" –Accuracy

1. Comparison Study

a. Study Design

i. Study Sites and Duration

Clinical performance characteristics of the cobas SARS-CoV-2 assay were evaluated in a multi-site prospective study during February to June 2022 winter respiratory season in the U.S. Ten (10) sites throughout the U.S. participated in the clinical study. The sites consisted of emergency rooms, urgent care clinics, outpatient clinics, physicians' offices and drive through COVID-19 testing sites. All the sites qualified as representative of CLIA waived intended use sites for this device.

ii. Operators

There were a total of 30 operators representative of intended CLIA waived users across the ten (10) clinical testing sites, with between 2 to 5 operators per site. The participants consisted of medical staff personnel providing patient care and included medical assistants, nurses, office managers, study coordinators, phlebotomists, and others. The test operators who participated in the study were untrained in the use of the cobas SARS-CoV-2 assay and had no hands-on experience with conducting diagnostic testing in a clinical laboratory.

Each operator collected the patient sample and tested it on the cobas SARS-CoV-2 assay using the Liat Analyzer. Of the 30 operators that took part in the study, 15 tested at least 5 positive samples and 5 negative samples (as determined by the comparator test).

iii. Instructions for Use

The operators were provided the cobas SARS-CoV-2 Quick Reference Instructions, and the cobas Liat System User Guide. Instructions for Use (IFU) were available to the operators. No other materials or instructions were provided and the operators received no training in the use of the test.

iv. Subjects (Patients)

Specimens for the prospective clinical study were collected under informed consent or, if the subject was < 18 years of age, with parental permission and assent of the participant. The subjects were enrolled according to the study protocol Inclusion or Exclusion criteria.

v. Samples

The clinical performance of the cobas SARS-CoV-2 Nucleic acid test for use on the Liat System was evaluated using a combination of prospectively collected fresh and frozen specimens as described below.

Prospective clinical specimens were collected and tested from February to June 2022. One nasal swab (NS) (either self- or clinician-collected) and one nasopharyngeal swab (NPS) were collected from each patient and tested using the cobas SARS-CoV-2 assay at all study sites. The swabs were collected from each subject using standard collection methods. The specimens were tested fresh at the clinical site with cobas SARS-CoV-2 by the intended use operators following the Quick Reference Instructions. The same samples were shipped to the reference testing laboratory, where they were tested on the comparator methods following the product's IFUs.

Based on the submitted information, 1828 evaluable NPS samples and 1826 evaluable NS samples were included in the analysis for the performance evaluation of the cobas SARS-CoV-2 assay. Of these, 627 NPS specimens were collected from individuals with signs and symptoms of respiratory tract infection and 1201 were from asymptomatic individuals (413 suspected of SARS-CoV-2 infection due to recent exposure or other reasons and 788 from individuals without symptoms or other reasons to suspect COVID-19). Among the NS specimens tested in the study, 626 were collected from individuals with signs and symptoms of respiratory tract infection and 1198 were from asymptomatic individuals (411 suspected of SARS-CoV-2 infection due to recent exposure or other reasons and 787 from individuals without symptoms or other reasons to suspect COVID-19).

For each specimen type (NPS and NS), 23 each frozen SARS-CoV-2-positive and -negative specimens from 92 symptomatic individuals, prospectively and sequentially between (March–June 2021) were distributed to 3 of the 10 sites and worked into the daily workflow of sites for testing with cobas SARS-CoV-2 test.

vi. Comparative Method (CM)

A composite comparator method (CCM) consisting of three highly sensitive FDA EUA authorized molecular RT-PCR assays was used for demonstrating the clinical performance of the cobas SARS-CoV-2 assay. The composite comparator result was defined based on concordant results from at least two of the three comparator assays.

b. Clinical Study Results

i. Clinical Performance of the cobas SARS-CoV-2 assay in Symptomatic Patients

The clinical performance of the cobas SARS-CoV-2 assay, when used by untrained operators, testing specimens from patients with symptoms of respiratory tract infection, testing NPS and NS specimens, is shown in Tables 6 and 7 below, respectively.

Performance with NPS Specimens

Table 6. Clinical performance of cobas SARS-CoV-2 on the cobas Liat System with **NPS** specimens (Symptomatic Patients)

	Composite Comparator Method SARS-CoV-2 Result	
cobas SARS-CoV-2 on cobas Liat System	Positive	Negative
Positive	125	3
Negative	6	539
PPA = 95.4% (95% CI: 90.4% - 97.9%) NPA = 99.5% (95% CI: 98.4% - 99.8%)		

Performance with NS Specimens

Table 7. Clinical performance of cobas SARS-CoV-2 on the cobas Liat System with **NS** specimens (Symptomatic Patients)

	Composite Comparator Method SARS-CoV-2 Result	
cobas SARS-CoV-2 on cobas Liat System	Positive	Negative
Positive	129	1
Negative	5	539
PPA = 96.3% (95% CI: 91.6% - 98.4%) NPA = 99.8% (95% CI: 98.96% - 99.97%)		

ii. Clinical Performance of the cobas SARS-CoV-2 assay in Asymptomatic Subjects

The clinical performance of the cobas SARS-CoV-2 assay, when used by untrained operators, testing specimens from patients without symptoms of respiratory tract infection, testing NPS and NS specimens, is shown in Tables 8 and 9, respectively.

Performance with NPS Specimens -

Table 8. Clinical performance of cobas SARS-CoV-2 on the cobas Liat System with **NPS** specimens (Asymptomatic Patients)

	Composite Comparator Method SARS-CoV-2 Result	
cobas SARS-CoV-2 on cobas Liat System	Positive	Negative
Positive	52	5
Negative	2	1142
PPA =96.3% (95% CI: 87.5% - 98.98%) NPA = 99.6% (95% CI: 98.98% - 99.8%)		

Performance with NS Specimens

Table 9. Clinical performance of cobas SARS-CoV-2 on the cobas Liat System with **NS** specimens (Asymptomatic Patients)

	Composite Comparator Method SARS-CoV-2 Result	
cobas SARS-CoV-2 on cobas Liat System	Positive	Negative
Positive	45	1
Negative	5	1147
PPA =90.0% (95% CI: 78.6% - 95.7%) NPA = 99.9% (95% CI: 99.5% - 99.98%)		

iii. Invalid Rate for Clinical Evaluation Samples

A total of 1929 NPS testes were run altogether, of which 22 tests were non-evaluable (4 were failed test,4 were invalid test and 14 tests had protocol deviations) and were not included in the final calculations. NPS tests demonstrated 1.14% of invalid rate with a 95% score CI: 0.75% - 1.72%.

A total of 1923 NS testes were run altogether, of which 21 tests were non-evaluable (9 were failed test,5 were invalid test and 7 tests had protocol deviations) and were not included in the final calculations. NS tests demonstrated 1.09% of invalid rate with a 95% score CI: 0.72% - 1.66%.

2. Device Performance with Analyte Concentrations Near the Cutoff

The performance of the cobas SARS-CoV-2 assay with samples at virus concentration near the assay cutoff, was evaluated during a reproducibility study conducted in support of the 510(k) for this device, which included a sample with low concentration of the virus (1x-2x LoD) and a negative sample. Both samples were prepared in simulated negative matrix. The positive sample was spiked with inactivated SARS-CoV-2 with 90 results at each site. The study was conducted at three CLIA waived sites with six untrained operators (two operators/site). Testing was conducted over 5 days using 3 lots of reagents and each sample was tested in three replicates on each day. Each site utilized a minimum of three Liat Analyzers, and all replicates for each panel member were tested on the same analyzer. A total of 270 tests were generated for each panel member.

The summary of qualitative results are shown in **Table 10**

Table 10. Reproducibility Study- Qualitative Results

Target	Panel Conc.	% Agreement with Expected Results/ (n Agreement/N Tested) (95% CI)			
		Site 1 (APR)	Site 2 (IND)	Site 3 (URM)	Overall
SARS-CoV-2	Low Positive (1x-2x LoD)	100% (89/89)	100% (90/90)	97.7% (85/87)	99.24% (260/266)
	Moderate positive (3x-5x LoD	100.0% (88/88)	100.0% (90/90)	100.0% (90/90)	100.0% (268/268)
	Negative	100% (90/90)	100% (88/88)	98.9% (89/90)	99.6% (267/268)

Overall, 802 valid tests results; 8 samples did not generate valid results and were excluded from percent agreement analysis.

The cobas SARS-CoV-2 Nucleic acid test for use on the cobas Liat System demonstrated that samples at concentrations near the assay cutoff generate accurate results when tested by untrained users in CLIA waived environments.

3. Operator Questionnaire

Upon completion of the Clinical and Near the Cutoff Studies, the operators at each site were asked to complete an Operator Questionnaire that asked them to rate the ease of use of the test procedure and answer proficiency questions related to cobas SARS-CoV-2 result interpretation. The proficiency portion of the questionnaire included seven (7) images of the cobas Liat System display and asked if the displayed result for SARS-CoV-2 was positive, negative, or could not be assessed. The ease-of-use questionnaire asked the operators to reply to a series of 8 questions related to ease of use of the test using an agreement scale (1= strongly disagree to 5= strongly agree). Overall, the operators found the test to be easy to use with an average score of 4.5.

M. Labeling for Waived Devices

The labeling consists of:

1. cobas SARS-CoV-2 for use on the Liat System Instructions for Use
2. cobas SARS-CoV-2 for use on the Liat System Quick Reference Instructions
3. cobas Liat System User Guide

2. The following elements are appropriately present:

- The cobas Liat System User Guide specifies the environmental operating conditions under which testing may be performed.
- The cobas Liat System User Guide and cobas SARS-CoV-2 for use on the Liat System Instructions for Use are clear and easy to understand.
- The cobas SARS-CoV-2 for use on the Liat System Instructions for Use and Quick Reference Instructions (QRI) identify the test as CLIA Waived.
- The QRI is written at the 7th grade reading level and the graphics depict the possible test results.
- The QC procedure is included in the QRI.
- The cobas SARS-CoV-2 for use on the Liat System Instructions for Use:
    - Indicate that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test.
    - Include step-by-step instructions for performing the test.
    - Include safety considerations applicable for untrained users.
    - Specify the actions to be taken if an invalid test result is obtained.
    - Include a summary of the studies performed to support CLIA Waiver.
    - Include appropriate warnings and/or limitations pertaining to clinical interpretation of test results.

- Include recommendations for Quality Control testing including the source of appropriate control materials and the frequency of testing.
- The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

N. Conclusion:

The submitted information in this CLIA waiver application supports a CLIA waiver approval decision.