CLIA Waiver by Application Approval Determination

Decision Summary

A. Document Number

CW240003

B. Parent Document Number

K240217

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 liat CT/NG nucleic acid test

F. Measurand (analyte)

Chlamydia trachomatis (CT) cryptic plasmid DNA and 23S ribosomal RNA
Neisseria gonorrhoeae (NG) pivNG and NGR9 DNA

G. Sample Type(s)

Male urine and female vaginal swabs

H. Type of Test

Qualitative, real-time nucleic acid amplification test (NAAT)

Food and Drug Administration
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Silver Spring, MD 20993-0002
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I. Test System Description

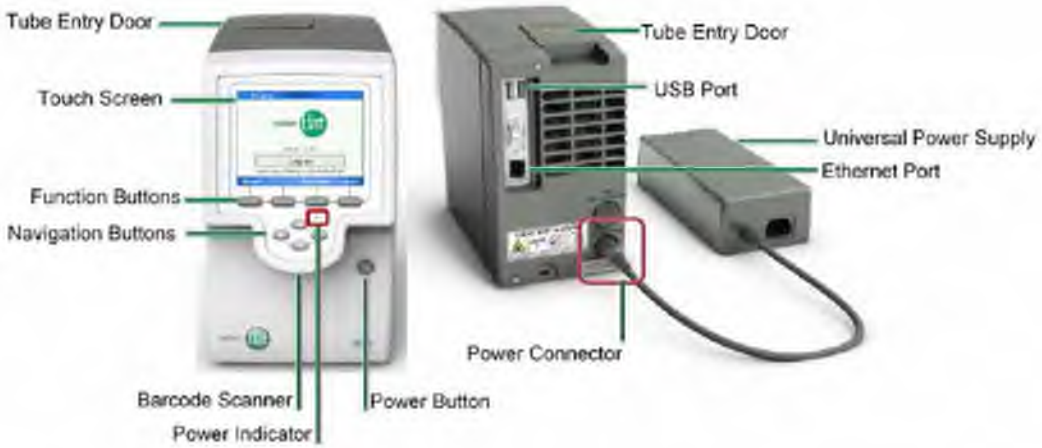
1. Overview

cobas liat system

The cobas liat system can automatically perform qualitative *in vitro* nucleic acid amplification tests. The cobas liat system consists of three main system components: Analyzer, Assay and Accessories. The system components of the cobas liat system are described below.

cobas liat analyzer
The Analyzer is a system component of the cobas liat system and consists of one software subsystem and three hardware units which are the: Infrastructure unit, Thermal, Loading and Motion unit and Detection unit. It is an automated nucleic acid test instrument. In conjunction with an assay tube, the Analyzer performs reagent preparation, target enrichment, inhibitor removal, nucleic acid extraction, polymerase chain reaction (PCR) amplification, real-time detection, and result interpretation to automate the detection or quantification of nucleic acid targets in a biological sample. Figure 1 below shows the cobas liat analyzer.

Figure 1: cobas liat analyzer

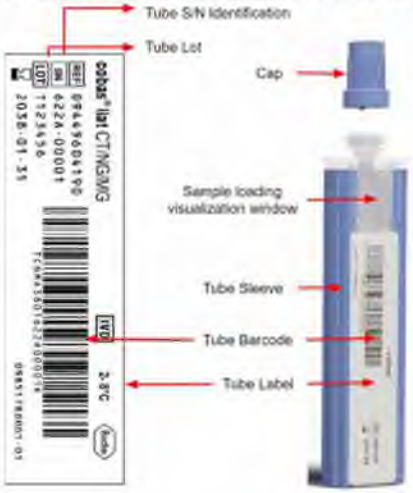


cobas liat Assay Tube
The cobas liat assay uses a flexible tube as a sample processing vessel. It contains all requisite PCR reagents pre-packed in tube segments that are separated by breakable seals. When a cobas liat assay tube containing a raw biological sample is inserted into the cobas liat analyzer, multiple sample processing actuators in the cobas liat analyzer compress the cobas liat assay tube to selectively release the reagents, moving the sample from one segment to the next, and controlling reaction conditions. An embedded microprocessor controls and coordinates these actions to perform all required assay processes, including sample preparation, nucleic acid extraction, target concentration enrichment, inhibitor removal, nucleic acid elution, and real-time RT-PCR. All assay steps are performed within

the closed and self-contained cobas liat assay tube, minimizing cross-contamination between samples.

The detection module monitors the reaction in real-time, while an on-board computer analyzes the collected data and outputs an interpreted result. The latter is displayed in the assay report on the integrated LCD touch screen of the cobas liat analyzer and in an electronic file. The report can be printed directly through a USB or network-connected printer. The results can also be exported to an external server, middleware or data management system (DMS), or to a Laboratory Information System (LIS). Figure 2 below illustrates the cobas liat CT/NG nucleic acid test tube used with the cobas liat analyzer.

Figure 2: cobas liat CT/NG nucleic acid test tube



2. Test System Components

The cobas liat CT/NG nucleic acid test requires the following:

a. A cobas liat CT/NG nucleic acid test tube which contains the following:
   - Internal process control (IPC)
     - Controls for adequate processing of target bacteria through all steps of assay and to monitor the presence of PCR inhibitors.
   - PCR mastermix (assay specific oligonucleotides and polymerase)
   - Co-factor, liat magnetic particles, lysis buffer, wash buffer, and elution buffer
b. Specimen sample stored in cobas PCR Media
c. cobas liat CT/NG/MG Control Kit
   - Contains positive and negative control tubes for use validating new cobas liat CT/NG nucleic acid test tube lots
d. cobas liat System
e. Liat Assay Specific Package (LASP)

Accessories:

- cobas liat Cleaning Tool Kit (CTK)
- Used to manually clean the lens adjacent to the assay tube cavity.
- Contains foam pads, a plastic tool, and alcohol swabs

3. Results

A target result call is determined individually for the target (CT, NG) channel and the internal process control channel based an internal algorithm using signal response. A final result is determined for the targets based on the combination of the target result call, internal process control result call, curve validity (including Ct cutoffs), and the sample input volume.

cobas liat analyzer display	Result Interpretation
CT Not Detected	Valid Negative test for CT (no CT detected)
CT Detected	Valid Positive test for CT (CT present)
CT Invalid	Presence/absence of CT could not be determined. Repeat assay with same sample.
NG Not Detected	Valid Negative test for NG (no NG detected)
NG Detected	Valid Positive test for NG (NG present)
NG Invalid	Presence/absence of NG could not be determined. Repeat assay with same sample.
Assay invalid	Presence/absence of CT/NG could not be determined. Repeat assay with same sample.
Assay aborted by system	Run failed or aborted by system. Repeat assay with same sample.
Assay aborted by script: Script aborted	Run failed or aborted by script. Repeat assay with same sample
Assay aborted by user	Run aborted by user

J. Demonstrating "Simple"

The cobas liat CT/NG nucleic acid test meets the criteria for a "simple" device contained in "Guidance for Industry and FDA Staff: Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices" issued on February 26, 2020.

Specifically, the cobas liat system automates all testing steps and utilizes a unitized self-contained disposable cobas liat tube. The assay utilizes urine and vaginal swabs specimens, without the need for any specimen manipulation. Sample is transferred using a bulb transfer pipette provided with the kits. Running the assay requires no reagent manipulation and no operator intervention during analysis steps. The system requires no troubleshooting or interpretation of multiple or complex error codes, and no electronic or mechanical maintenance is required to be performed by the operator. Results are easy to determine (i.e. reported in English as "Detected", "Not Detected", "Invalid", or "Aborted"), and requires no operator calibration, interpretation, or calculation. A Quick Reference Instruction card, written at no higher than a 7th grade reading level, is also provided. In studies in which intended operators (i.e., test operator with limited or no training or hands-on experience in conducting clinical laboratory testing) performed the assay, the cobas liat CT/NG nucleic acid test demonstrated that untrained users can perform the test and obtain reliable and accurate results.

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

1. Risk Analysis

A comprehensive risk analysis of the cobas liat CT/NG nucleic acid test was performed in accordance with ISO 14971 - Medical Devices - Application of Risk Management to Medical Devices. Device Hazard Analysis and the Failure Mode Effects Analysis (FMEA) methods were used to assess the risk of failure (false positive, false negative or delayed test results) that may occur during use or misuse of the device. Severity and probability for each potential failure mode were assessed to generate an overall risk value. Mitigations (e.g., addition of cautions and warnings to the product labeling) were implemented to reduce any residual risks as far as possible. The risks of failure were re-evaluated after mitigations were implemented.

Based on the hazard analysis, all possible sources of error, which might be at an increased likelihood of occurring when the test system is used in CLIA waived settings by untrained users, were evaluated in flex studies to demonstrate the effectiveness of applicable built-in control measures and assess the insensitivity of the test system to variation under stress conditions. The considered risks included operator errors (human factors), sample and device handling and storage, and environmental factors.

2. Fail-Safe and Failure Alert Mechanisms

The cobas liat CT/NG nucleic acid test for use with the cobas liat system was designed to include features and fail-safe mechanisms built into the system to prevent erroneous results.

Internal Control

The built-in Internal Control monitors all processing steps (sample purification, amplification, and detections) of targets. It also monitors for processing failure, compromised assay tube or the presence of inhibitors.

External Control

Before using a new lot of cobas liat CT/NG nucleic acid test tubes, the "Lot Validation" procedure must be performed on the analyzer to validate the cobas liat CT/NG nucleic acid test tube lot. The procedure includes running a negative control and a positive control in separate runs. After processing is completed for each control, the system will inform the user that the control result has been accepted. The user can now use that specific lot of assay tubes for processing samples.

cobas liat System

The following safety features are implemented in the cobas liat System:

- The analyzer has a built-in auto monitoring system to ensure that it is always functioning optimally. When the analyzer is starting up, a series of initialization diagnostic tests are performed automatically which helps to maintain the health of the analyzer.

- Optical system surveillance that monitors status of detection optics and electronics.
- The system prompts and instructs users on what to scan and when to load the assay tube.
- All the items a user needs to load are barcoded. The system checks barcoding and prevents use of off label assay tubes by verifying barcode validity.
- Assay tubes are self-contained which reduces the risk of cross-contamination between samples.
- Assay tubes are barcoded and allowed to be processed only once on the system.
- The system checks assay tube position and aborts the run if the assay tube was loaded correctly.
- The system flags and invalidates runs automatically where processing errors are detected.
- The system outputs results as a protected file so that results cannot be altered.
- Software installations are protected by cryptographic methods that verify the origin and integrity.

cobas liat System Verifying Fail-Safe and Failure Alert Mechanism
The following built-in controls, lock-out features and failure alert mechanisms have been tested as part of the instrument/software and the system level verification and were shown to be effective, as shown below.

Table 1. Verification of Fail-Safe and Failure Alert Mechanisms

Failure	Description of Failure	Description of Fail-safe Mechanism
Extreme Temperature	Outside the operating range [4°C to 40°C]; temperature <4°C or >40°C	cobas liat analyzer has a built-in sensor checks the internal temperature during the power-on-self test that prevents assays from being run when the system is at a temperature <4°C or >40°C.
Non-level Surface	cobas liat analyzer placed on a surface > 10° tilt angle in transverse (x-axis) or sagittal axis (y-axis)	If the system is at a tilt angle exceeding 10° in either the transverse (x) or sagittal (y) axis it prevents assay run, displays an error message, and aborts the run. The tilt sensor control measure is effective at monitoring the tilt angle failure conditions of the cobas liat analyzer.
Reagents and Hardware Integrity	cobas liat analyzer to prevent execution of runs with expired assay tubes	During the workflow of the sample processing, a customer is required to scan the barcode of the assay tube to be able to start the run. This barcode has the information about the assay, such as unique assay ID and expiration date. The system warns the user if barcode has expired and does not allow an expired assay tube to be loaded.

3. Flex Studies

For cobas liat CT/NG nucleic acid tests, system flex study results were obtained using the cobas liat CT/NG/MG nucleic acid test (K240197) three-target multiplex panel. The assays were developed using the same reagent formulation and design, packaged into two separate products. The product software Assay Specific Packages for each device have the same sample processing workflow, PCR cycling, and calculations. The only difference is the masking of the MG result in the cobas liat CT/NG nucleic acid test. As such, the results of the system flex studies are applicable to both tests. This summary will focus only on the assay's ability to detect CT and NG under the chosen stress conditions.

3.1 Human Factors

a. Improper Tube Storage

The objective of this flex study was to test the effect of improper storage of the cobas liat CT/NG nucleic acid test tube. This study was executed with negative and positive test samples prepared in pooled negative clinical urine specimens stabilized in cobas PCR media. Positive test samples were prepared using culture stocks of CT and NG. The culture stocks were co-formulated in pooled negative urine and pooled negative vaginal swab specimens to approximately 2x LoD. Ten replicates of each test sample per test condition were tested by two operators (five replicates/operator). Control and Test conditions are described in Table 2 below.

Table 2. Conditions for testing improper tube storage

Clinical Sample Matrix	Test Sample	Test Condition	Enclosed in Foil Pouch	Storage Temp (°C)	Time point (Days)	Rationale
Urine (in cobas PCR media)	Analyte Positive (2xLoD) and Negative	0	Yes	2-8	N/A	Control
		1		-20	7	Simulates incorrect storage in freezer/low temp shipping excursion
		2		30	14	Simulates extended storage at room temp (15-30°C)
		3		37	7	Simulates incorrect storage at high temp, or higher shipping temp
		4	No	-20	1	Simulates incorrect storage in freezer after opening package
		5		4	1	Simulates incorrect storage in refrigerator after opening package
		6		30	1	Simulates extended storage at room temp after opening package
		7		37	1	Simulates incorrect storage at high temperature or higher temperature shipping excursion after opening package

Results of testing are listed in Table 3 below:

Table 3. Results of improper tube storage testing

Clinical Specimen Matrix	Test Condition	Negative Specimen No Detection Rate (n/N)		Positive Specimen Detection Rate (n/N)		Invalid Result	False Results
		CT	NG	CT (Avg Ct Value)	NG (Avg Ct Value NGPL, NGR9)		
Urine	0	10/10	10/10	10/10 (34.2)	10/10 (33.0, 32.5)	0	0
	1	10/10	10/10	10/10	10/10	0	0

				(35.4)	(33.9, 32.8)		
	2	10/10	10/10	10/10 (33.6)	10/10 (33.1, 32.2)	0	0
	3	10/10	10/10	10/10 (33.5)	10/10 (32.2, 30.5)	0	0
	4	10/10	10/10	10/10 (34.5)	10/10 (33.5, 32.8)	0	0
	5	10/10	10/10	10/10 (34.0)	10/10 (32.6, 32.2)	0	0
	6	10/10	10/10	10/10 (34.0)	10/10 (32.4, 32.2)	0	0
	7	10/10	10/10	10/10 (33.9)	10/10 (32.4, 32.1)	0	0

Based on the results of this flex study, there was no observed performance impact between improperly storing the assay tubes with or without the foil pouch in the tested temperatures. The results demonstrate that the test is robust against such user errors. The Instructions for Use will specify the following:

- Store assay tubes at 2-8°C
- Do not freeze reagents.

Additionally, any allowable assay tube short-term room temperature storage conditions (≤7 days) will be included in the Instructions for Use labeling. These labeling mitigations are all within the operational limits determined for the product.

Assay Tube Seal Breakage

To simulate operator tampering or mishandling of the cobas liat CT/NG nucleic acid test tube, the assay tube frangible seals between reagent segments were broken and then the assay tubes were tested on the cobas liat analyzer. Control and Test Conditions are described in Table 4 below:

Table 4: Assay tube seal breakage testing conditions

Matrix	Test Sample	Test Condition No.	Test Condition Description
Urine (in cobas PCR media)	Analyte Positive (2x LoD) and Negative	0	Control (no seal break)
		1	Seal break between IC and Liat Magnetic Particle (LMP) segments (IC/LMP Break)
		2	Seal break between LMP and Lysis Buffer (LB) segments (LMP/LB Break)
		3	Seal break between LB and Wash Buffer (WB) segments (LB/WB Break)
		4	Seal break between WB and Elution Buffer (EB) segments (WB/EB Break)
		5	Seal break between EB and Master Mix 1 (MM1) segments (EB/MMX1 Break)
		6	Seal break between MM1 and Cofactor segments (MMX1/Cofactor Break)
		7	Seal break between Cofactor and Master Mix 2 (MM2) segments (Cofactor/MM2 Break)
		8	Seal break between sample and IC segments. Simulates operator inserting a pipette into assay tube to the maximum extendt possible during sample addition

The results of testing are displayed for analyte Negative and Positive Samples below in Table 5.

Table 5: Results of Assay Tube Seal Breakage Testing Conditions

Clinical Sample Matrix	Test Condition	Negative Sample No Detection Rate (n/N)		Test Condition	Positive Sample Detection Rate (n/N)		Invalid Result	False Result
		CT	NG		CT (Avg Ct Value)	NG (Avg Ct Value NGPL, NGR9)		
Urine	0	10/10	10/10	0	10/10 (34.6)	10/10 (32.8, 32.1)	0	0
	1	10/10	10/10	1	10/10 (34.2)	10/10 (32.7, 32.0)	0	0
	2	10/10	10/10	2	9/10 (34.2)	10/10 (32.4, 31.6)	0	1
	2*	10/10	10/10	3[2]	0/0	n/a	10	0
	3[1]	1/1	1/1	4	10/10 (35.0)	10/10 (33.4, 32.6)	9	0
	4	10/10	10/10	5	**8/10** (34.4)	10/10 (33.1, 32.0)	0	2
	5	10/10	10/10	5*	9/10 (34.7)	10/10 (33.9, 32.4)	0	1
	6	10/10	10/10	6[3]	8/9 (34.1)	9/9 (32.5, 31.4)	0	1
	7	10/10	10/10	7	10/10 (34.2)	10/10 (31.7, 30.3)	0	0
	8	10/10	10/10	8	**8/10** (34.7)	10/10 (32.2, 32.0)	0	2
				8*	10/10 (34.2)	10/10 (32.8, 32.3)	0	0

[1] Nine invalid runs occurred for this test condition. The built-in control measures/fail-safe features were effective.
[2] Six aborted runs and four invalid runs occurred for this test condition. The built-in control measures/fail-safe features were effective.
[3] One aborted run occurred for this test condition.
*Repeat runs for condition 2 with negative samples and condition 5 and 8 with positive samples

Based on the results of this flex study, the cobas liat CT/NG nucleic acid test performance, when seals were intentionally broken prior to use, was robust. However, the following was noted:

False negatives did occur with the following assay tube breaks:

- LMP/LB seal break (one sample for CT analyte)
- EB/MM1 seal break (two samples for CT analyte)
- MM1/Co-factor break (one sample for CT analyte)
- Sample input/IC break (two samples for the CT analyte)

While seal breaks did show some loss of performance, the likelihood of an incorrect result is rare, and the risk is tolerable. Furthermore, built-in control measures / fail-safe features for seal breaks between one reagent segment (LB/WB) were effective.

The following labeling mitigations will be included in the Instructions for Use:

- Do not puncture the cobas CT/NG nucleic acid test tube or the seal at the bottom of the sample compartment. If either of these are damaged, discard both the cobas CT/NG nucleic acid test tube and the transfer pipette and restart the testing procedure with a new cobas CT/NG nucleic acid test tube and pipette.
- Do not use a damaged cobas CT/NG nucleic acid test tube.

As an additional design control measure, the liat assay tube is protected against inadvertent damage (such as seal breaks) by a hard plastic outer sleeve/cartridge.

Incorrect Input Sample Volume

The objective of this flex study was to demonstrate the insensitivity of the cobas liat CT/NG assay to the addition of incorrect sample volumes. Control and Test Conditions are described in Table 6 below:

Table 6: Incorrect input sample testing conditions

Matrix	Test Sample	Test Condition No.	Volume (uL)	Rationale
Urine And Vaginal Swab	Analyte Positive (2x LoD) and Negative	0	200	Control Condition, Target sample volume
		1	20	Low sample volume below threshold
		2	150	Low sample volume above volume threshold
		3	400	High sample volume, max sample volume that can be added to assay tube sample segment

The results of testing the analyte Negative and Positive samples are shown below in Table 7.

Table 7: Results of Incorrect Input Sample Testing Conditions

Clinical Sample Matrix	Test Condition	Negative Sample No Detection Rate (n/N)		Test Condition	Positive Sample Detection Rate (n/N)		Invalid Result	False Result
		CT	NG		CT (Avg Ct Value)	NG (Avg Ct Value NGPL, NGR9)		
Urine	0	10/10	10/10	0	10/10 (34.2)	10/10 (32.3, 32.0)	0	0
	1[1]	0/0	0/0	1[2]	10/10 (36.6)	9/9 (35.1, 34.4)	20	0
	2	10/10	10/10	2	10/10	10/10	0	0

					(34.4)	(32.9, 32.2)		
	3	10/10	10/10	3	10/10 (33.6)	10/10 (32.2, 31.9)	0	0
Vaginal Swab	0	10/10	10/10	0	10/10 (33.3)	10/10 (32.0, 31.2)	0	0
	1[1]	0/0	0/0	1[2]	10/10 (34.8)	9/9 (34.4, 33.4)	20	0
	2	10/10	10/10	2	10/10 (33.3)	10/10 (32.5, 31.5)	0	0
	3	10/10	10/10	3	10/10 (33.4)	10/10 (32.0, 31.0)	0	0

[1] The 20 uL sample input volume condition all gave invalid results. When sample input volume was too low, the built-in control measure/fail-safe feature was effective in identifying such error, and the runs were invalidated.
[2] The 20 uL sample input volume condition all gave correct Target Detected results or invalid results. When sample input volume was too low, the built-in control measure/fail-safe feature was effective in identifying such error, and the runs with Target Not Detected results were invalidated.

The study showed that when sample input volume was low, at 20 uL, the built-in control measure / fail-safe feature was effective in identifying such error, and the runs were invalidated rather than potentially reporting a false result. The study demonstrated that sample volume of at least 150 uL is required for the test to perform as expected.

To reduce the occurrence of invalid results due to low sample input volume, each kit includes simple-to-use transfer pipettes with a fixed volume of approximately 200 uL for sample transfer to the cobas liat assay tube.

Assay Tube Hold Time

The objective of this flex study was to test the effect of hold time between addition of the sample to the cobas liat CT/NG assay tube and initiation of the run on the cobas liat analyzer. Control and Test Conditions are described in Table 8 below:

Table 8: Assay Tube Hold Time Testing Conditions

Matrix	Test Sample	Test Condition No.	Temperature (°C)	Time from Sample Addition to Run	Rationale
Urine And Vaginal Swab	Analyte Positive (2x LoD) and Negative	0	20-25	Immediately	Control Condition
		1		2 hours	Simulated deviations from immediate processing
		2		4 hours	
		3		6 hours	

The results of testing the analyte Negative and Positive samples are shown below in Table 9.

Clinical Sample Matrix	Test Condition	Negative Sample No Detection Rate (n/N)		Test Condition	Positive Sample Detection Rate (n/N)		Invalid Result	False Result
		CT	NG		CT (Avg Ct Value)	NG (Avg Ct Value		

						NGPL, NGR9)		
Urine	0	10/10	10/10	0	10/10 (34.1)	10/10 (32.8, 32.1)	0	0
	1	10/10	10/10	1	10/10 (34.4)	10/10 (32.7, 32.1)	0	0
	2	10/10	10/10	2	10/10 (34.4)	10/10 (32.8, 32.1)	0	0
	3	10/10	10/10	3	10/10 (35.0)	10/10 (32.8, 32.2)	0	0
Vaginal Swab	0	10/10	10/10	0	10/10 (34.2)	10/10 (33.2, 31.4)	0	0
	1	10/10	10/10	1	10/10 (34.2)	10/10 (32.6, 31.4)	0	0
	2	10/10	10/10	2	10/10 (33.8)	10/10 (33.2, 31.4)	0	0
	3	10/10	10/10	3	10/10 (33.3)	10/10 (32.9, 31.5)	0	0

Based on the results of this study, there was no observed performance impact between testing the filled assay tubes immediately or after allowing the filled assay tubes to remain at room temperature over the tested time periods. The results demonstrate that the test is robust against such user errors.

The Instructions for Use will specify the following:

Processing of the assay tube must begin within 4 hours of sample addition to the assay tube.

This labeling mitigation is within the operational limits evaluated in this Flex study.

Assay Tube Orientation Post Sample Addition

The objective of this flex study was to demonstrate the insensitivity of the cobas liat CT/NG assay to the orientation of the assay tube following sample addition, and the assay tube being dropped following sample addition. Control and Test Conditions are described in Table 10 below:

Table 10: Assay Tube Orientation Post Sample Addition Testing Conditions

Matrix	Test Sample	Test Condition No.	Test Condition Description	Rationale
Urine And Vaginal Swab	Analyte Positive (2x LoD) and Negative	0	Assay tube (in sleeve held vertically (cap up) after adding sample	Control Condition
		1	Assay tube held horizontally after adding sample	Simulated assay tubes held in different orientations
		2	Assay tube held inverted (cap down) after adding sample, and vigorously shaken to move sample into cap	
		3	Assay tube dropped vertically or horizontally (~3', from workbench to floor) after adding sample	

The results of testing the analyte Negative and Positive samples are shown below in Table 11.

Table 11: Results of Assay Tube Orientation Post Sample Addition Testing

Clinical Sample Matrix	Test Condition	Negative Sample No Detection Rate (n/N)		Test Condition	Positive Sample Detection Rate (n/N)		Invalid Result	False Result
		CT	NG		CT (Avg Ct Value)	NG (Avg Ct Value NGPL, NGR9)		
Urine	0	10/10	10/10	0	10/10 (34.6)	10/10 (32.4, 31.6)	0	0
	1	10/10	10/10	1	10/10 (34.8)	10/10 (32.7, 31.8)	0	0
	2	10/10	10/10	2	10/10 (35.4)	10/10 (33.4, 32.4)	0	0
	3[1]	19/19	19/19	3[3]	16/16 (35.0 35.4)	18/18 (32.7, 32.0) (33.7, 32.4)	3	0
Vaginal Swab	0	10/10	10/10	0	10/10 (34.0)	10/10 (32.9, 31.4)	0	0
	1	10/10	10/10	1	10/10 (34.0)	10/10 (33.2, 31.6)	0	0
	2	10/10	10/10	2	10/10 (33.9)	10/10 (33.5, 31.8)	0	0
	3[2]	16/16	16/16	3[4]	18/18 33.8(34.3)	17/17 (33.2, 32.1) (33.4, 32.1)	4	0

[1] One invalid run occurred for negative samples in this test condition.
[2] One aborted run and three invalid runs occurred for negative samples in this test condition.
[3] Two CT target invalids occurred for positive samples in this test condition.
[4] Two invalid runs occurred for the positive samples in this test condition. One NG target invalid also occurred for this test condition.

Based on the results of this flex study, the cobas liat CT/NG assay performance was insensitive to the cobas liat assay tube being held in horizontal and vertical orientations following sample addition. There is no risk of erroneous result due to holding the cobas liat assay tube vertically, horizontally or shaking the assay tube vigorously after adding sample.

The instructions caution the user not to use a cobas liat CT/NG assay tube that has been dropped after removal from its foil pouch to reduce the occurrence of invalid results.

Sample and Reagent Temperature (Inadequate Temperature Equilibriation)

The objective of this flex study was to evaluate the use of refrigerated samples and/or reagents without prior equilibration to ambient temperature (inadequate temperature equilibration) prior to initiating the cobas liat CT/NG test. Control and Test Conditions are described in Table 12 below:

Table 12: Sample and Reagent Temperature Testing Conditions

Matrix	Test Sample	Test Condition No.	Test Condition Description	Rationale
Urine And Vaginal Swab	Analyte Positive (2x LoD) and Negative	0	Remove sample and assay tube from 2-8°C, and run immediately (Control)	Not allowing tube and sample to equilibriate to ambient temperature
		1	Remove sample and assay tube from 2–8°C, store at ambient room temperature (20-25°C) for at least 30 minutes	Allow tube and sample to equilibrate to ambient temperature.

The results of testing the analyte Negative and Positive samples are shown below in Table 13.

Table 13: Results of Sample and Reagent Temperature Testing Conditions

Clinical Sample Matrix	Test Condition	Negative Sample No Detection Rate (n/N)		Test Condition	Positive Sample Detection Rate (n/N)		Invalid Result	False Result
		CT	NG		CT (Avg Ct Value)	NG (Avg Ct Value NGPL, NGR9)		
Urine	0	10/10	10/10	0	10/10 (34.7)	10/10 (33.6, 32.5)	0	0
	1	10/10	10/10	1	10/10 (34.6)	10/10 (33.1, 32.5)	0	0
Vaginal Swab	0	10/10	10/10	0	10/10 (33.2)	10/10 (32.3, 31.4)	0	0
	1	10/10	10/10	1	10/10 (33.3)	10/10 (32.4, 31.3)	0	0

Based on the results of this flex study, there was no observed performance impact between testing the assay tubes and samples immediately after storage in a refrigerator or after allowing the assay tubes and samples to equilibrate to room temperature. The results demonstrate that the test is robust against such use conditions.

Improper Specimen Storage

The objective of this flex study was to test the effect of improper sample storage temperatures for urine specimens (stabilized in cobas PCR media) on the performance of the cobas liat CT/NG assay. Control and Test Conditions are described in Table 14 below:

Table 14: Improper Specimen Storage Testing Conditions

Matrix	Test Sample	Test Condition No.	Storage Temp (°C)	Time	Test Condition Description
Urine (in cobas PCR media)	Analyte Positive (2x LoD) and Negative	0	2-8	N/A	Control Condition
		1	20-25	1 day	Control condition – room temp storage is an allowed storage temp
		1.03*		3 hours	
		1.04*		4 hours	
		1.03*	32	3 hours	Improper storage at high temp
		1.05*		5 hours	
		1.08*		8 hours	
		1.24*		24 hours	
		2	37	1 day	Improper storage at extreme high temp
		2.03*		3 hours	
		2.05*		5 hours	
		2.08*		8 hours	
		2.24*		24 hours	
		3	-80	1 day	Improper storage at extreme frozen temp
		4	-20	1 day	Improper storage at frozen frozen temp

*Additional timepoints tested for urine samples only

The results of testing are displayed for analyte Negative and Positive Samples below in Table 15.

Table 15: Results of Improper Specimen Storage Testing Conditions

Clinical Sample Matrix	Test Condition	Negative Sample No Detection Rate (n/N)		Test Condition	Positive Sample Detection Rate (n/N)		Invalid Result	False Result
		CT	NG		CT (Avg Ct Value)	NG (Avg Ct Value NGPL, NGR9)		
Urine	0[1]	30/30	30/30	0*[1]	30/30 (34.2)	30/30 (33.1, 32.3)	0	0
	1	10/10	10/10	1	**4/10 (36.0)**	10/10 (32.3, 31.5)	0	6[2]
	1.03	10/10	10/10	1.03*	10/10 (35.1)	10/10 (32.8, 32.5)	0	0
	1.04	10/10	10/10	1.04*	10/10 (35.2)	10/10 (32.7, 32.1)	0	0
	1.03	10/10	10/10	1.03	10/10 (35.1)	10/10 (33.6, 32.6)	0	0
	1.05	10/10	10/10	1.05	**8/10 (36.4)**	10/10 (33.3, 32.4)	0	2
	1.08	10/10	10/10	1.08	**4/10 (36.9)**	10/10 (33.3, 32.6)	0	6
	1.24	10/10	10/10	1.24	**7/10 (36.6)**	10/10 (33.3, 32.7)	0	3
	2	10/10	10/10	2	**6/10 (35.9)**	10/10 (32.3, 31.8)	0	4
	2.03	10/10	10/10	2.03	10/10 (35.2)	10/10 (33.1, 32.6)	0	0
	2.05	10/10	10/10	2.05	9/10 (36.6)	10/10 (33.4, 32.6)	0	1
	2.08	10/10	10/10	2.08	**5/10 (37.3)**	10/10 (33.5, 32.7)	0	5
	2.24	10/10	10/10	2.24*	**6/10 (35.9)**	10/10 (33.2, 32.3)	0	4
	3	10/10	10/10	3	9/10 (34.1)	10/10 (32.6, 31.9)	0	1
	4	10/10	10/10	4	9/10 (34.3)	10/10 (32.8, 31.8)	0	1
Vaginal Swabs	0	10/10	10/10	0	10/10 (34.2)	10/10 (32.4, 31.1)	0	0
	1	10/10	10/10	1	10/10 (34.2)	10/10 (32.8, 31.2)	0	0
	2	10/10	10/10	2	10/10 (33.5)	10/10 (32.6, 31.5)	0	0
	3	10/10	10/10	3	10/10 (34.1)	10/10 (32.7, 31.5)	0	0
	4	10/10	10/10	4	10/10 (33.8)	10/10 (32.7, 31.1)	0	0

*One run was aborted and the repeat run was valid

[1] Control test conditions were repeated on three separate days to allow for evaluation of 3 and 4 hours at 20-25°C room temperature, the 32°C condition, and additional timepoints (3, 5, 8, and 24 hours) at 37°C.
[2]Six positive samples had false results for CT. While this is beyond the specimen storage claim, additional testing was conducted at 20-25°C for 3 and 4 hours
Bold font indicates false test results.

The flex study results demonstrated that test samples in vaginal swab sample matrix (collected in cobas PCR Media) are insensitive to temperatures outside of the room temperature range (i.e., 37°C, - 20°C, and -80°C) for a short duration (i.e., 1 day). Test samples were insensitive to proper sample storage at room temperature (20-25°C), prior to testing, up to 1 day.

Test samples in urine sample matrix (stabilized in cobas PCR Media) are sensitive to improper frozen sample storage temperatures (i.e., -20°C and -80°C) for a short duration (i.e., 1 day). A single sample at either frozen sample storage temperature yielded a false negative CT result. Urine samples are sensitive to improper storage temperature of 32 or 37°C if stored for a duration beyond 3 hours. Test samples were insensitive to proper sample storage at 20-25°C up to 4 hours.

The Instructions for Use will specify the following:

- Urine samples stabilized in cobas PCR Media can be stored up to 4 hours at room temperature or up to 48 hours at 2-8°C, if immediate testing is not possible.
- Vaginal Swab samples collected in cobas PCR Media can be stored up to 24 hours at room temperature or up to 72 hours at 2-8°C, if immediate testing is not possible.

These labeling mitigations are within the operational limits determined for the product.

IC Effectiveness

The objective of this flex study was to evaluate Internal Control (IC) results under process and reagent failure conditions to verify the ability of the Internal Control to monitor the performance of sample preparation and PCR amplification/detection. Control and Test Conditions are described in Table 16 below:

Table 16: IC Effectiveness Test Conditions

Matrix	Test Sample	Test Condition No.	Performance of	Failure Type	Failure Description	Script
Urine and Vaginal Swab (in cobas PCR media)	Analyte Positive (2x LoD) and Negative Sample	0	Control	Control	Normal process (no simulated failures)	CNMA
		1	Sample Preparation	Process Failure	Failure to capture magnetic glass particles during nucleic acid extraction (simulated by not moving the magnet to capture beads during sample preparation step)	CNMG
		2		Reagent Failure	Frangible seal break between adjacent tube segments containing Lysis Buffer and Wash Buffer, allowing mixing of the two reagents	CNMA
		3	PCR amplification and detection	Process Failure	Deviation in PCR temperature from set point (simulated by setting denaturation temp to 80°C)	CNMD
		4		Reagent Failure	Frangible seal break between adjacent tube segments containing Lysis Buffer and Wash Buffer and Elution Buffer, allowing mixing of the three reagents	CNMA

The results of testing are displayed for analyte Negative and Positive Samples below in Tables 17 and 18, respectively.

Table 17: Result Interpretation of IC Effectiveness with Analyte Negative Samples Testing

Matrix	Condition No.	Negative Samples					
		CT		NG		IC	
		# Not Detected	# Assay Invalid	# Not Detected	# Assay Invalid	# Not Detected	# Assay Invalid
Urine	0	10	0	10	0	10	0
	1	0	10	0	10	0	10
	2	0	7	0	7	0	7
	3[1]	0	10	0	10	0	10
	4	0	7	0	7	0	7
Vaginal Swabs	0	10	0	10	0	10	0
	1	0	10	0	10	0	10
	2	0	5	0	5	0	5
	3[2]	0	10	0	10	0	10
	4[3]	0	9	0	9	0	9

[1] Three aborted runs occurred for this test condition.
[2] Five aborted runs occurred for this test condition.
[3] One aborted run occurred for this test condition.

Table 18: Result Interpretation of IC Effectiveness with Analyte Positive Samples Testing

Matrix	Condition No.	Positive Samples							
		CT			NG			IC	
		# Det	# Assay Invalid	# CT Invalid	# Det	# Assay Invalid	# NG Invalid	# Valid	# Invalid
Urine	0	10	0	N/A	10	0	N/A	10	0
	1	0	10	N/A	0	10	N/A	0	10
	2[1]	0	4	N/A	0	4	N/A	0	4
	3	0	10	N/A	0	10	N/A	0	10
	4	0	10	N/A	0	10	N/A	0	10
Vaginal Swabs	0	10	0	0	10	0	N/A	10	0
	1	0	10	0	0	10	N/A	2	8
	2[2]	0	4	4	4	4	N/A	0	8
	3	0	10	0	0	10	N/A	0	10
	4	0	10	0	0	10	N/A	0	10

[1] Six aborted runs occurred for this test condition.
[2] Two aborted runs occurred for this test condition.

The built-in control measures / fail-safe features were demonstrated to be effective for those runs aborted and for which no invalid result (assay or analyte) is displayed.

Based on the results of this study, expected non-erroneous results were observed for the simulated failure test conditions, as indicated by either correct result interpretations, invalid results, or run aborts. No erroneous (false positive or false negative) results were observed. The results of this study demonstrate that the Internal Control is effective to monitor the performance of sample preparation and PCR amplification/detection.

External Control (EC) Effectiveness

The objective of this flex study was to evaluate external control results under process and reagent/assay tube failure conditions to verify the ability of Positive Control (PC) and Negative Control (NC) to monitor the performance of an assay tube lot or to detect systematic errors. Control and Test Conditions are described in Table 19 below:

Matrix	Test Sample	Test Condition No.	Performance of	Failure Type	Failure Description
External Positive Control	Analyte Positive (2x LoD) and Negative Sample	0	Control	Control	Normal process (no simulated failures)
		1	Sample Preparation	Process Failure	Failure to capture magnetic glass particles during nucleic acid extraction (simulated by not moving the magnet to capture beads during sample preparation step)
		2		Reagent Failure	Frangible seal break between adjacent tube segments containing Lysis Buffer and Wash Buffer, allowing mixing of the two reagents
		3	PCR amplification and detection	Process Failure	Deviation in PCR temperature from set point (simulated by setting denaturation temp to 80°C)
		4		Reagent Failure	Frangible seal break between adjacent tube segments containing Lysis Buffer and Wash Buffer and Elution Buffer, allowing mixing of the three reagents

The results of testing are displayed for analyte Negative and Positive Samples below in Table 20 and 21, respectively.

Table 20: Result Interpretation of EC Effectiveness with Negative Control (NC) Testing

Failure Description	Result Interpretation		Run Status
	NC Valid	NC Invalid	Aborted Runs
Normal process (no simulated failures)	10	0	0
Failure to capture magnetic glass particles during nucleic acid extraction (simulated by not moving the magnet to capture beads during sample preparation step)	0	10	0
Frangible seal break between adjacent tube segments containing Lysis Buffer and Wash Buffer, allowing mixing of the two reagents	0	8	2
Deviation in PCR temperature from set point (simulated by setting denaturation temp to 80°C)	0	10	0
Frangible seal break between adjacent tube segments containing Lysis Buffer and Wash Buffer and Elution Buffer, allowing mixing of the three reagents	0	9	1
Run NC to simulate low level contamination (~3x LoD of CT, NG)	0	10	0

Table 21: Result Interpretation of EC Effectiveness with Positive Control (PC) Testing

Failure Description	Result Interpretation		Run Status
	PC Valid	PC Invalid	Aborted Runs
Normal process (no simulated failures)	10	0	0
Failure to capture magnetic glass particles during nucleic acid extraction (simulated by not moving the magnet to capture beads during sample preparation step)	0	10	0
Frangible seal break between adjacent tube segments containing Lysis Buffer and Wash Buffer, allowing mixing of the two reagents	0	6	4
Deviation in PCR temperature from set point (simulated by setting denaturation temp to 80°C)	0	10	0
Frangible seal break between adjacent tube segments containing Lysis Buffer and Wash Buffer and Elution Buffer, allowing mixing of the three reagents	0	7	3

The built-in control measures / fail-safe features were demonstrated to be effective for those runs aborted and for which no invalid result (assay or analyte) is displayed.

Based on the results of this study, the PC and NC were able to detect all simulated failure conditions tested, as indicated by invalid or aborted control results. The results demonstrate that the external controls are effective to monitor the performance of an assay tube lot or to detect systematic errors.

Urine Sample Volume

The objective of this flex study was to test the effect of improper urine sample transfer to the cobas PCR Media tube on the performance of the cobas liat CT/NG assay. Control and Test Conditions are described in Table 22 below:

Table 22: Urine Sample Volume Testing Conditions

Test Condition No.	Test Condition Description	Rationale
0	Control (~5 mL of urine)	Control Condition
1.1	Over-filled with ~6 mL of urine	Improperly collected sample (over-filled +9% above upper most black fill line [~5.5 mL] on cobas PCR media tube label window)
1.2	Over-filled with ~8 mL of urine	Improperly collected sample (over-filled +45% above upper most black fill line Note: Tube capacity is 13 mL with ~4.5 mL of cobas PCR media
2.1	Under-filled with ~4 mL of urine	Improperly collected sample (under-filled -11% below lower most black fill line [~4.5 mL] on cobas PCR media tube label window)
2.2	Under-filled with ~2 mL of urine	Improperly collected sample (under-filled -56% below lower most black fill line
2.3	Under-filled with ~1 mL of urine	Improperly collected sample (under-filled -78% below lower most black fill line
3	Invert sample 0 times	Improper mixing of samples – Not mixing enough (0 times) or mix too much (>5 times) per cobas PCR urine sample kit PI
4	Invert sample 10 times	

The results of testing are displayed for analyte Negative and Positive Samples below in Table 23.

Table 23: Results of Improper Urine Sample Volume and Mixing Testing

Clinical Sample Matrix	Test Condition	Negative Sample No Detection Rate (n/N)		Positive Sample Detection Rate (n/N)		Invalid Result	False Result
		CT	NG	CT (Avg Ct Value)	NG (Avg Ct Value NGPL, NGR9)		
Urine	0	10/10	10/10	10/10 (33.9)	10/10 (32.7, 32.2)	0	0
	1.1	10/10	10/10	10/10 (34.0)	10/10 (33.3, 32.4)	0	0
	1.2	10/10	10/10	10/10 (34.2)	10/10 (33.3, 32.5)	0	0
	2.1	10/10	10/10	10/10 (34.5)	10/10 (33.9, 33.0)	0	0
	2.2	10/10	10/10	10/10 (35.0)	10/10 (34.4, 33.6)	0	0
	2.3	10/10	10/10	10/10 (36.4)	**8/10 (35.2, 33.9)**	**0**	**2**
	3	10/10	10/10	10/10 (33.9)	10/10 (33.1, 31.9)	0	0
	4	10/10	10/10	10/10 (33.8)	10/10 (33.1, 31.9)	0	0

Bold font indicates test results not meeting acceptance criteria.

Based on the results of this study, there was no significant observed performance impact between proper and improper urine sample transfer to the cobas PCR Media tube (or mixing) when urine sample volume filled into the cobas PCR Media tube ranged from ~2 mL to ~8 mL. The results demonstrate that the test is robust against improper urine volume additions below the fill line (~2.0 - 4.5 mL) and above the fill line (~5.5 - 8.0 mL). Urine volume additions less than 2.0 mL may result in false negative results. Additionally, over-mixing (tube inverted 10 times) and no mixing did not impact performance.

To help ensure proper urine sample transfer to the cobas PCR Media tube and proper mixing, the cobas PCR Urine Sample Kit Package Insert contains simple instructions (with pictograms) as follows:

- The correct volume of urine has been added when the fluid level is between the two black lines (~5.5 and ~4.5ml, respectively) on the tube label.
- Invert the tube 5 times to mix.

Incorrect Swab Elution

The objective of this flex study was to test the effect of incorrectly eluting the swab in cobas PCR Media prior to initiating the cobas liat CT/NG test. Control and Test Conditions are described in Table 24 below:

Table 24: Incorrect Swab Elution Testing Conditions

Matrix	Test Sample	Test Condition No.	Test Condition Description	Rationale
Vaginal Swab	Analyte Positive (2x LoD) and Negative	0	Rotate swab clockwise for 30" in specimen and then transfer the swab to cobas PCR media, breaking the swab into the tube and capping it	Control Condition
		1	Dip swab once in cobas PCR media and remove	Operator may not properly elute sample

The results of testing are displayed for analyte Negative and Positive Samples below in Table 25.

Table 25: Results of incorrect swab elution testing study

Clinical Sample Matrix	Test Condition	Negative Sample No Detection Rate (n/N)		Test Condition	Positive Sample Detection Rate (n/N)		Invalid Result	False Result
		CT	NG		CT (Avg Ct Value)	NG (Avg Ct Value NGPL, NGR9)		
Vaginal Swab	0	10/10	10/10	0	10/10 (32.9)	10/10 (32.3, 30.7)	0	0
	1	10/10	10/10	1	10/10 (34.6)	10/10 (33.2, 31.5)	0	0

Based on the results of this flex study, there was no observed performance impact between leaving the vaginal swab in cobas PCR Media and removing the vaginal swab from cobas PCR Media immediately after dipping. The results demonstrate that the test is robust against such use conditions.

Bubbles in Sample Chamber

The objective of this flex study was to demonstrate the insensitivity of the cobas liat CT/NG to the presence of bubbles in the sample chamber. Control and Test Conditions are described in Table 26 below:

Table 26: Bubbles in Sample Chamber Testing Conditions

Matrix	Test Sample	Test Condition No.	Test Condition Description	Rationale
Urine And Vaginal Swab	Analyte Positive (2x LoD) and Negative	0	None	Control Condition
		1	Bubbles in Sample Chamber	Operator may inadvertently create bubbles when transferring the sample to the sample chamber

The results of testing are displayed for analyte Negative and Positive Samples below in Table 27.

Table 27: Results of Bubbles in Sample Chamber Testing Conditions

Clinical Sample Matrix	Test Condition	Negative Sample No Detection Rate (n/N)		Test Condition	Positive Sample Detection Rate (n/N)		Invalid Result	False Result
		CT	NG		CT (Avg Ct Value)	NG (Avg Ct Value NGPL, NGR9)		
Urine	0	10/10	10/10	0	10/10 (34.2)	10/10 (32.6, 32.0)	0	0
	1	10/10	10/10	1	10/10 (33.9)	10/10 (32.7, 31.9)	0	0
Vaginal Swab	0	10/10	10/10	0	10/10 (34.1)	10/10 (32.9, 31.8)	0	0
	1	10/10[1]	10/10	1	10/10[1] (33.2)	10/10 (32.3, 31.4)	1	0

Based on the results of this study, the cobas liat CT/NG assay performance was insensitive to bubbles in the cobas liat assay tube sample chamber. There was no observed performance impact between control samples and samples with bubbles.

Delay in Sample Testing

The objective of this flex study was to test the effect of a delay in sample testing. Control and Test Conditions are described in Table 28 below:

Table 28: Delay in Sample Testing Conditions

Matrix	Test Sample	Test Condition No.	Storage Temp (°C)	Timepoint	Rationale
Vaginal Swab	Analyte Positive (2x LoD) and Negative	0	2-8	N/A	Sample run immediately
		1	20-25	48 hours	Sample stored at room temp and testing delayed 2 days
		2	2-8	96 hours	Sample refrigerated and testing delayed 4 days
Urine		0	2-8	N/A	Sample run immediately
		1	20-25	48 hours	Sample stored at room temp and testing delayed 2 days
		1.1		6 hours	Sample stored at room temp and testing delayed 6 hours
		2	2-8	96 hours	Sample refrigerated and testing delayed 4 days
		2.1		72 hours	Sample refrigerated and testing delayed 3 days

The results of testing are displayed for analyte Negative and Positive Samples below in Table 29.

Table 29: Results of Delay in Sample Testing Study

Clinical Sample Matrix	Test Condition	Negative Sample No Detection Rate (n/N)		Test Condition	Positive Sample Detection Rate (n/N)		Invalid Result	False Result
		CT	NG		CT (Avg Ct Value)	NG (Avg Ct Value NGPL, NGR9)		
Vaginal Swab	0	10/10	10/10	0	10/10 (33.6)	10/10 (32.7, 31.0)	0	0
	1	10/10	10/10	1	10/10 (33.4)	10/10 (32.8, 30.8)	0	0
	2	10/10	10/10	2	10/10 (33.2)	10/10 (32.5, 30.9)	0	0
Urine	0[1]	20/20	20/20	0[1]	20/20 (34.5)	20/20 (33.1, 32.6)	0	0
	1	10/10	10/10	1	**5/10 (35.4)**	10/10 (33.3, 32.6)	0	5
	1.1	10/10	10/10	1.1	10/10 (35.4)	10/10 (33.0, 32.2)	0	0
	2	10/10	10/10	2	**7/10 (36.2)**	10/10 (33.1, 32.3)	0	3
	2.1	10/10	10/10	2.1	10/10 (34.5)	10/10 (32.6, 32.1)	0	0

[1] The control condition for urine has 20 results as the 1.1 and 2.1 test conditions were run on a different day. Hence, there are a total of 20 control condition results.
Bold text indicates false results.

Based on the results of this study, test performance with urine samples (stabilized in cobas PCR Media) is robust when testing is delayed up to 3 days and when samples are refrigerated (2-8°C). However, when testing is delayed and samples are stored at room temperature (20- 25°C), false negative results do occur when samples are stored beyond the intended storage claim (i.e., up to 3 hours at 30°C).

Test performance with vaginal swab samples (collected in cobas PCR Media) is robust to delays of up to 4 days when samples are stored refrigerated (2-8°C) or up to 2 days when samples are stored at room temperature (20-25°C).

To reduce the risk of erroneous results, the Instructions for Use will specify the following:

- Urine samples stabilized in cobas PCR Media can be stored up to 4 hours at room temperature or up to 48 hours at 2-8°C if immediate testing is not possible.
- Vaginal Swab samples collected in cobas PCR Media can be stored up to 24 hours at room temperature or up to 72 hours at 2-8°C if immediate testing is not possible.

These labeling mitigations are within the operational limits determined for the product.

Incorrect Collection Kit Storage

The objective of this flex study was to test the effect of incorrectly stored collection kits (commercially available cobas PCR Urine Sample Kit and cobas PCR Media Uni Swab Sample Kit) on the performance of the cobas liat CT/NG assay. Control and Test Conditions are described in Table 30 below:

Table 30: Incorrect Collection Kit Storage Testing Conditions

Matrix	Test Sample	Test Condition No.	Storage Temp (°C)	Timepoint	Rationale
Urine And Vaginal Swab	Analyte Positive (2xLoD) and Negative	0	15-30	0	Control condition
		1	45	1 week	Collection kit storage temperature outside IFU specified range
		2	-20		
		2.2		2 weeks*	

*Included two freeze-thaw cycles

The results of testing are displayed for analyte Negative and Positive Samples below in Table 31.

Table 31: Results of Incorrect Collection Kit Storage Testing Conditions

Clinical Sample Matrix	Test Condition	Negative Sample No Detection Rate (n/N)		Test Condition	Positive Sample Detection Rate (n/N)		Invalid Result	False Result
		CT	NG		CT (Avg Ct Value)	NG (Avg Ct Value NGPL, NGR9)		
Urine	0	10/10	10/10	0	10/10 (33.9)	10/10 (33.2, 32.2)	0	0
	1	10/10	10/10	1	**9/10 (32.2)**	10/10 (32.2, 31.6)	0	1
	2	10/10	10/10	2	**8/10 (33.1)**	10/10 (33.1, 31.8)	0	2
	2.1[1]	10/10	10/10	2.1[1]	**9/10 (33.0)**	10/10 (33.0, 32.0)	0	1
	2.2[2]	10/10	10/10	2.2[2]	10/10 (33.1)	10/10 (33.1, 31.9)	0	0
Vaginal Swab	0	10/10	10/10	0	10/10 (32.7)	10/10 (32.7, 31.4)	0	0
	1	10/10	10/10	1	10/10 (33.0)	10/10 (33.0, 31.7)	0	0
	2	10/10	10/10	2	10/10 (32.3)	10/10 (32.3, 31.1)	0	0

[1] Repeat testing for previous timepoint that did not meet acceptance criteria.
[2] Additional tested timepoint (2 weeks at -20°C) with 2 freeze thaws due to initial observations at 1 week
Bold font indicates false results.

No significant change to assay performance was observed when the cobas PCR Media Uni Swab Sample Kit (for vaginal swabs) was stored at correct (stored at 15-30°C) or incorrect (e.g., -20°C and 45°C) storage conditions.

Assay performance was however impacted when the cobas PCR Media Urine Sample Kit was stored at out of specification temperatures (e.g., -20°C and 45°C) for up to one week; false results were reported for analyte positive urine samples. However, when the cobas PCR Media Urine Sample Kit was stored at -20°C for two weeks and underwent two freeze-thaw cycles, assay performance was not impacted. Therefore, this suggests storage of the kit at -20°C has a low risk of a false result in urine.

To help ensure proper storage of the collection kit, the cobas PCR Media tube, collection kit packet label (contains symbol), the cobas PCR Urine Sample Kit Package Insert, and cobas PCR Media Uni Swab Sample Kit Package Insert contain instructions as follows:

Store at 15°C to 30°C.

3.2 Environmental Conditions

Unless otherwise specified, the following design was used for reference testing and preparation of contrived samples for all environmental condition flex studies.

Reference condition
To set up the reference condition, six analyzers were placed in a regulated bio-safety level 2 laboratory at Roche Diagnostics International in Rotkreuz, Switzerland. On each cobas liat analyzer, five cobas liat CT/NG nucleic acid assay runs were performed with co-formulated positive samples in clinical urine matrix. The environmental conditions were following the recommended ranges stated in the cobas liat analyzer User Guide. Additionally, two negative samples were run on three cobas liat analyzers.

Preparation of contrived samples
Analyte positive contrived samples were prepared by co-spiking all three analytes (CT/NG) into analyte negative urine clinical matrix to a final concentration of 2x LoD for each analyte. Two analyte-negative and five analyte-positive samples were tested per instrument.

To support the cobas liat CT/NG test, this summary of study results will focus solely on CT and NG results.

Extreme Temperatures
This study was conducted to validate the insensitivity of cobas liat CT/NG nucleic acid test when performed on the cobas liat analyzer to the temperature operating range. The cobas liat analyzer has a built-in sensor, which checks the internal temperature during the power-on self-test and creates a fail-safe mechanism that prevents assays from being run when the system is at a temperature < 4°C or > 40°C. In addition, the cobas liat analyzer user's manual, environmental conditions section, requires the operation of the cobas liat analyzer between 15°C and 32°C. The study tested if (1) at temperatures within the operating range of 4°C to 40°C but outside of the user manual's recommended temperature range, assays yielded the expected results, and (2) at temperatures outside of these limits, the cobas liat analyzer prevented assays from being run.

Temperatures outside of the operating range of 4°C to 40°C were not tested; previously executed testing was leveraged. Temperatures within the operating range were tested. To set up the temperature within limit conditions, three cobas liat analyzers were placed in a temperature-controlled chamber set at the test temperature (5 °C, 39 °C) and allowed to equilibrate over two hours.

Testing and control conditions are summarized in Table 32 below.

Table 32: Temperature Extreme Testing Conditions

Sample	Test Condition No.	Temp (°C)	Rationale	Tested During
5 Analyte Positive (2x LoD) and 2 Negative Samples in Urine Clinical Matrix Per cobas liat System	1	<4	Low temp outside limit	Previously tested
	2	5	Low temp within limit	Current study
	3	22	Controlled reference condition	
	4	39	High temp within limit	
	5	>40	High temp above limit	Previously tested

The results of testing are displayed below in Table 33.

Table 33: Results of Temperature Extreme Testing Conditions

	Negative Sample No Detection Rate (n/N)			Positive Sample Detection Rate (n/N)			Invalid Result	False Result
Clinical Sample Matrix	Test Condition	CT	NG	Test Condition	CT	NG		
Urine	2	6/6	6/6	2	**14/15**	15/15	0	1
	4	6/6	6/6	4	15/15	15/15	0	0

The study demonstrated that cobas liat CT/NG nucleic acid test performance was largely insensitive to different ambient temperatures within the operating range. However, the control condition did have a single sample across the six instruments and 30 total replicates that reported a false result for the CT analyte. Additionally, a single sample reported a false result for the CT analyte at the lower temperature within the operating range (i.e., 5°C).

The internal temperature sensor control measure is effective at preventing assays from being run when the system is at a temperature outside the operating range of <4°C to >40°C. As such, there is a low risk of erroneous results due to operating the cobas liat analyzer in different temperature environments.

Extreme Humidity

This study was conducted to evaluate the effect of humidity on the cobas liat CT/NG nucleic acid test when performed on the cobas liat analyzer. The cobas liat analyzer user's manual, environmental conditions section, requires the operation of the cobas liat analyzer between 15% to 80% relative humidity. The studies were performed within the

specified relative humidity operating ranges for the cobas liat analyzer (15% - 80%) as well as outside the recommended ranges (10%, 90% and 96%). To set up each humidity test condition, three cobas liat analyzers equilibrated for two hours in an environmental chamber configured for the corresponding relative humidity condition.

Testing and control conditions are summarized in Table 34 below.

Table 34: Humidity Extreme Testing Conditions

Sample	Test Condition No.	Relative Humidity (%rH)	Rationale
5 Analyte Positive (2x LoD) and 2 Negative Samples in Urine Clinical Matrix Per cobas liat System	1	25-45	Controlled reference condition
	2	10*	Low rH outside of specified range
	3	15	Low rH within specified range
	4	80	High rH within specified range
	5	90	High rH outside specified range
	6	96	

rH = relative Humidity

*The lower rH to be tested was 5% rH, however the lowest possible rH that could be set in the climate chamber was 10% rH. Therefore, lowest tested was 10% rH.

The results of testing are displayed below in Table 35.

Table 35: Results of Humidity Extreme Testing Conditions

Clinical Sample Matrix	Negative Sample No Detection Rate (n/N)			Positive Sample Detection Rate (n/N)			Invalid Result	False Result
	Test Condition	CT	NG	Test Condition	CT	NG		
	2	6/6	6/6	2	14/15	15/15	0	0
	4	6/6	6/6	4	**15/15**	15/15	0	0
	5	6/6	6/6	5	14/15	15/15	0	1
	6	6/6	6/6	6	**28/30**	**30/30**	0	2

The study demonstrated that cobas liat CT/NG nucleic acid test performance was insensitive to relative humidity environments within and outside of the specified 15% - 80% rH range. The cobas liat CT/NG nucleic acid test yielded expected results. As such, there is a low risk of erroneous results due to operating the cobas liat analyzer in environments of different relative humidity. Note, the cobas liat analyzer User Guide and QRG, users are instructed to run the test at 15%-80% relative humidity.

Extreme Altitude

This study was conducted to evaluate the effect of extreme altitude on the cobas liat CT/NG nucleic acid test analyzer. The cobas liat analyzer user's manual and product specifications specifies that the maximum operating altitude should be 2000 m above sea level. This study evaluated if the assay would yield expected results at altitudes within and outside of the specified limits.

Testing and control conditions are summarized in Table 36 below.

Table 36: Altitude (Barometric Pressure) Extreme Testing Conditions

Sample	Test Condition No.	MASL	Rationale
Positive Control and Negative Control	1	429 masl	Normal altitude/ Controlled reference condition Location: Roche Diagnositcs Rotkreuz
	2	3040 masl	Extreme altitude condition. Location: Measuring station of Mount Titlis, CH

MASL = meters above sea level

The same analyzer was used for both the controlled reference condition and high-altitude testing. The controlled reference condition was tested with at least 15 replicates to match the high-altitude testing condition. The controlled reference condition was not tested using 5 replicates across 6 analyzers as stated in the protocol.

The results of testing are displayed below in Table 37.

Table 37: Results of Altitude (Baromatric Pressure) Extreme Testing Conditions

Negative Control No Detection Rate (n/N)			Positive Control Detection Rate (n/N)			Invalid Result	False Result
Test Condition	CT	NG	Test Condition	CT	NG		
1	2/2	2/2	1	19/19	17/19	0	2
2	6/6	6/6	2	19/19	17/19	0	2

Initial testing of the cobas liat CT/NG nucleic acid test with diluted positive control sample for both the control condition (429 m) and stress condition (3,040 m) returned false negative results for NG target. However, additional replicates produced with undiluted positive control sample yielded expected results on cobas liat analyzer at altitudes within and outside the limits. Based on the analysis, there was no associated trend from the CT target as well as the internal process control (IPC) to suggest that the false negative result was due to the condition tested. Given the reference and extreme condition both gave the same observed result for NG target, the likelihood of an erroneous result is not greater under stress conditions and considered low risk. Additionally, the cobas liat analyzer User Guide specifically recommends using the analyzer at maximal altitude of 2000 masl.

Non-level Surface Testing

This study was conducted to evaluate the insensitivity of cobas liat CT/NG nucleic acid test when performed on the cobas liat analyzer to operating on non-level surfaces and the effectiveness of tilt sensor control measures, which detects the tilt angle of the cobas liat analyzer. If it exceeds 10° in either the transverse (x) or sagittal (y) axis before executing an assay, the cobas liat analyzer will output an error message and abort the run. If the

system senses a tilt of more than 10° during the run execution, it displays a warning, but does not abort the run. In addition, the cobas liat analyzer user's manual instructs the user to install the cobas liat analyzer on a level surface.

Testing and control conditions are summarized in Table 38.

Table 38: Non-level Surface Testing Conditions

Sample	Test Condition No.	Tilt Angle	Rationale	Tested During
5 Analyte Positive (2x LoD) and 2 Negative Samples in Urine Clinical Matrix Per cobas liat System	1	9°	Tilt angle in sagittal (y-axis) axis **inside** the limits	Previously performed
	2		Tilt angle in transverse (x-axis) axis **inside** the limits	
	3	11°	Tilt angle in sagittal (y-axis) axis **outside** the limits. Tilt **before** run start so run will be aborted.	
	4		Tilt angle in transverse (x-axis) axis **outside** the limits. Tilt **after** run start so run will be aborted.	
	5		Tilt angle in sagittal (y-axis) axis **outside** the limits. Tilt **before** run start so run will not be aborted.	Conducted in current study
	6		Tilt angle in transverse (x-axis) axis **outside** the limits. Tilt **before** run start so run will not be aborted.	
	7	0°	Controlled reference condition	

For test condition 5 and 6 (Table 38), three cobas liat analyzers were tested with five runs each (a total of 15 runs per condition) with co-formulated positive sample in urine clinical matrix. Additionally, for each condition, two negative samples were run on each cobas liat analyzer.

The results of testing are displayed below in Table 39.

Table 39: Non-level Surface Testing Results

Negative Control No Detection Rate (n/N)			Positive Control Detection Rate (n/N)			Invalid Result	False Result
Test Condition	CT	NG	Test Condition	CT	NG		
5	0/6	0/6	5	14/15	15/15	0	1
6	0/6	0/6	6	14/15	15/15	0	1
7	0/6	0/6	7	29/30*	30/30	0	1

*A single run had a valid result and amplification/detection for NG, but the CT target dropped out.

The study demonstrated that cobas liat CT/NG nucleic acid test performance was insensitive when operating the cobas liat analyzer with an angle tilt. The cobas liat CT/NG nucleic acid test yielded expected results on cobas liat analyzer when operating on non-level surfaces and the tilt sensor control measure is effective at monitoring the tilt angle failure conditions of the cobas liat analyzer. A run will be aborted if tilt happens outside the limits before the start of a run or display the appropriate warning message if tilt happens outside the limits during a run. In addition, the cobas liat analyzer User

Guide advises the operator to place the analyzer on a level surface; therefore, there is a very low risk for erroneous result.

Sunlight Exposure

This study was conducted to evaluate the insensitivity of cobas liat CT/NG nucleic acid test performed on the cobas liat analyzer under sunlight exposure. The cobas liat analyzer User Guide advises the operator to place the analyzer away from direct sunlight. The control reference and test conditions are summarized in Table 40 below.

Table 40: Sunlight Exposure Testing Conditions

Sample	Test Condition No.	Sunlight	Rationale
5 Analyte Positive (2x LoD) and 2 Negative Samples in Urine Clinical Matrix Per cobas liat System	1	No direct sunlight	Controlled reference condition
	2	Direct sunlight through the fan-slits	Instrument is exposed to sunlight

To create a direct sunlight condition, the cobas liat analyzers were placed on a table, with the fan-slits facing the sun. Five replicates were tested on three cobas liat analyzers with coformulated positive sample in urine background. Additionally, two negative samples were run on each cobas liat analyzer.

Results of testing are summarized in Table 41 below.

Negative Control No Detection Rate (n/N)			Positive Control Detection Rate (n/N)			Invalid Result	False Result
Test Condition	CT	NG	Test Condition	CT	NG		
2	6/6	6/6	2	14/15	14/14[1]	0	1

[1]NG invalid: Sunlight interfered with the photometer channel detecting the NG target, which was correctly invalidated by the cobas liat system.

The study demonstrated that cobas liat CT/NG nucleic acid test performance was insensitive to sunlight exposure on the cobas liat system. In addition, the cobas liat analyzer User Guide advises the operator to place the analyzer away from direct sunlight. As such, there is a very low risk for erroneous result.

Vibrational Effects

This study was conducted to evaluate the insensitivity of cobas liat CT/NG nucleic acid test performed on the cobas liat analyzer to operate under vibrational conditions. The cobas liat analyzer User Guide instructs to install the analyzer in a vibration-free environment. The control reference and test conditions are summarized in Table 42 below.

Table 42: Vibrational Effects Testing Conditions

Sample	Test Condition No.	Vibration	Rationale
5 Analyte Positive (2x LoD) and 2 Negative Samples in Urine Clinical Matrix per cobas liat System	1	No vibration	Controlled reference condition
	2	Highest table vibration (20913x g/14000 rpm)	Simulating a strong centrifuge nearby (20913x g/14000 rpm)

To create a vibrating environment for the cobas liat analyzer, the three tested instruments were placed on a table next to a large centrifuge. The cobas liat CT/NG nucleic acid test runs and the centrifuge were started simultaneously. In total, the centrifuge went through four centrifugation cycles with uneven loading (to create additional vibration) during each assay run. The cobas liat CT/NG nucleic acid test runs were performed with co-formulated positive sample in urine background. Additionally, two negative samples were run on each cobas liat analyzer.

The results of testing are summarized in Table 43 below.

Table 43: Vibrational Effects Testing Conditions Results

Negative Control No Detection Rate (n/N)			Positive Control Detection Rate (n/N)			Invalid Result	False Result
Test Condition	CT	NG	Test Condition	CT	NG		
2	6/6	6/6	2	15/15	15/15	0	0

The study demonstrated that cobas liat CT/NG assay performance was insensitive to vibrational conditions. In addition, the cobas liat analyzer User Guide advises the operator to place the analyzer in an environment with no perceptible vibration. As such, there is a very low risk for erroneous result.

Poor Airflow

This study was conducted to evaluate the insensitivity of cobas liat CT/NG nucleic acid test when performed on the cobas liat analyzer to poor airflow. The cobas liat analyzer has a fan that regulates the internal temperature inside the cobas liat analyzer. Additionally, the cobas liat analyzer User Guide advises the operator to leave at least 10 cm of space at the rear of the analyzer for airflow and to prevent blocking the vents of the analyzer. The control reference and test conditions are summarized in Table 44 below.

Table 44: Effect of Poor Airflow Testing Conditions

Sample	Test Condition No.	Vibration	Rationale
5 Analyte Positive (2xLoD) and 2 Negative Samples in Urine Clinical Matrix per cobas liat System	1	cobas liat analyzers placed with 30 cm gap to back wall and 30 cm gap to the side	Controlled reference condition (optimal airflow to both the back and side of the instrument)
	2	cobas liat analyzers placed with 30 cm gap to back wall and 1 cm gap to the side	Good airflow to the back, bad airflow to the side
	3	cobas liat analyzers placed with ~ 5 cm gap to back wall and 1 cm gap to the side	Bad airflow to the back and side of the instrument

To set up each ventilation test condition, five runs on three cobas liat analyzer (15 runs per condition) were tested with co-formulated positive sample in urine clinical matrix. Additionally, for each condition, two negative samples were run on each cobas liat analyzer.

The results of testing are summarized in Table 45 below.

Negative Control No Detection Rate (n/N)			Positive Control Detection Rate (n/N)			Invalid Result	False Result
Test Condition	CT	NG	Test Condition	CT	NG		
2	6/6	6/6	2	14/15	14/15	0	1
3	6/6	6/6	3	14/15	15/15	0	1

The study demonstrated that cobas liat CT/NG nucleic acid test performance was insensitive to poor airflow conditions. In addition, the cobas liat analyzer User Guide specifies the space needed to reduce the risk for erroneous results:

- Allow at least 10 cm (4 in) rear clearance for ventilation needed for a cobas liat analyzer to operate with adequate ventilation.
- Dust-free environment with adequate ventilation

Drafty Conditions

This study was conducted to evaluate the insensitivity of cobas liat CT/NG nucleic acid test performed on the cobas liat analyzer operating under drafty conditions. The control reference and test conditions are summarized in Table 46 below.

Table 46: Drafty Effects Testing Conditions

Sample	Test Condition No.	Drafty Condition	Rationale
5 Analyte Positive (2x LoD) and 2 Negative Samples in Urine Clinical Matrix per cobas liat System	1	Instrument is facing empty space (no draft)	Controlled reference condition
	2	Instrument is facing an operational fan, blowing air at the back of the instrument	Simulate drafty condition

To create a drafty environment for the cobas liat analyzer, the three instruments were placed on a table facing an operational fan, blowing wind (measured wind speed between 0.75 and 3.2 m/s) to the backside of the instruments. On each instrument, five runs were performed with co-formulated positive sample in urine background. Additionally, two negative samples were run on each cobas liat analyzer.

The results of testing are summarized in Table 47 below.

Table 47: Results of Drafty Testing Conditions

Negative Control No Detection Rate (n/N)			Positive Control Detection Rate (n/N)			Invalid Result	False Result
Test Condition	CT	NG	Test Condition	CT	NG		
2	6/6	6/6	2[1]	15/15	15/15	0	0

[1] As with the relative humidity testing, an investigation into the false negatives produced in condition 2 (see CT target) suggested that the positive sample was at the borderline of the LoD. New contrived samples were prepared with adjusted dilutions and evaluated both at the reference condition (repeat testing) and at the test condition 2.

The study demonstrated that cobas liat CT/NG nucleic acid test performance was insensitive to drafty conditions. The cobas liat CT/NG nucleic acid test yielded expected results. As such, there is an insignificant risk of erroneous results.

Movement of Instrument During Testing

This study was conducted to evaluate the insensitivity of cobas liat CT/NG nucleic acid test to cobas liat analyzer movement while in operation. To verify the assay performance of the test system under the movement condition, the cobas liat analyzer was placed on the platform of an orbital shaker. The cobas liat CT/NG nucleic acid test run was initiated, and the orbital shaker was immediately began orbiting at a low rpm as the cobas liat CT/NG nucleic acid test was processed. The orbiting continued until the conclusion of the cobas liat CT/NG nucleic acid test and a result was generated. The control reference and test conditions are summarized in Table 48 below.

Table 48: Effects of Instrument Movement Testing Conditions

Sample	Test Condition No.	Movement condition	Rationale
5 Analyte Positive (2x LoD) and 2 Negative Samples in Urine Clinical Matrix per cobas liat System	1	No movement*	Controlled reference condition
	2	Movement	Movement condition using orbital shaker at low rpm (~120-200 rpm/orbital path length .125 inches)

To set up the movement condition, three cobas liat analyzers were placed on the platform of an orbital shaker. The cobas liat CT/NG nucleic acid test runs were initiated and then the orbital shaker was immediately powered on at low rpm. The orbital shaker was allowed to rotate at low rpm until the completion of each assay run. Five replicates on each instrument were tested. Additionally, two negative samples were run on each cobas liat analyzer.

The results of testing are summarized in Table 49 below.

Table 49: Results of Movement Testing Conditions

Negative Control No Detection Rate (n/N)			Positive Control Detection Rate (n/N)			Invalid Result	False Result
Test Condition	CT	NG	Test Condition	CT	NG		
2	6/6	6/6	2	15/15	15/15	0	0

The study demonstrates that the cobas liat assay performance is insensitive to analyzer movement during operation. As such, there is a low risk of erroneous results due to operating the cobas liat analyzer in environments while in motion. Additionally, the cobas liat analyzer User Guide advises the operator to install the analyzer and use in a vibration-free environment.

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

Two studies were conducted that demonstrated there is an insignificant risk of an erroneous result when using the cobas liat CT/NG nucleic acid test:

i. A prospective clinical study
ii. Device performance with analyte concentrations near the cutoff

1. Clinical Study

a) Study Sites and Duration

A prospective clinical study was conducted from December 13th, 2022, to August 18th, 2023, to establish the performance of cobas liat CT/NG nucleic acid test when used by untrained operators in a CLIA-waived environment. Male urine for 2340 males and vaginal swabs from 2512 females were collected and tested at 13 geographically diverse intended use clinical sites across the US. The results obtained with the cobas CT/NG assay were compared to a Patient Infected Status (PIS) or Composite Comparator Algorithm (CCA) result derived from a combination of FDA-cleared NAATs for both analytes (described in more detail under the section "Clinical Comparator Method Used").

b) Operators

A total of 42 operators took part in cobas liat CT/NG testing, of which, 37 represented POC CLIA-waived operators. Five of the 42 operators had experience in a moderate complexity laboratory. The operators consisted of research specialists/assistants, nurses,

lab technicians (only in title; operator did not have any instrument/sample testing experience when study was initiated), research/study coordinators, administrative managers, and other patient care providers. Selected operators may or may not have had prior experience using commercially available assays on the cobas liat system; however, no more than one operator participating in the clinical study at each site have had prior cobas liat system experience. The participating operators were provided with the assay's Instructions for Use (IFU), Quick Reference Instructions, and the cobas liat system User Guide. No training was provided to the operators.

c) Subjects

A total of 4852 subjects (2512 females and 2340 males) were enrolled in the study from clinical POC settings in geographically diverse locations throughout the US. Patients were classified as symptomatic if they self-reported 1 or more symptoms indicative of a potential urogenital infection from CT and NG, or based on the discretion of the examining clinician.

d) Samples

Prospectively enrolled female subjects provided 4 vaginal swab specimens. Prospectively enrolled male subjects provided a urine specimen that was aliquoted into collection devices for three FDA-cleared comparator NAAT tests and into cobas PCR Media for testing with the cobas liat CT/NG nucleic acid test.

Of the evaluable subjects, 2304 male subjects provided 2302 male urine specimens (2 subjects provided vaginal swab specimens) and 2476 females provided 1240 clinician-collected vaginal swabs and 1236 self-collected vaginal swabs for evaluation with the cobas liat CT/NG nucleic acid test.

In total, the following sample results were included in the calculations of clinical performance estimates:

Specimen Type	CT Results	NG Results
Male Urine	2296	2301
VS CC	1239	1238
VS SC	1234	1234

VS CC = Vaginal Swab Clinician-Collected
VS SC = Vaginal Swab Self-Collected

In addition, a total of 253 archived vaginal and male urine specimens, prospectively collected, were infected and tested in this clinical study due to low NG prevalence. These archived specimens were distributed to untrained operators at the participating clinical sites for testing on cobas liat CT/NG as part of the normal clinical workflow.

e) Clinical Comparator Method Used

The clinical performance of cobas liat CT/NG nucleic acid test was evaluated by comparing the results from collected specimen types to a Patient Infection Status (PIS, infected or not infected) or Composite Comparator Algorithm (CCA, positive or negative) result for each analyte. Male urine samples were tested with three FDA cleared NAATs (Nucleic Acid Amplification Test) for each analyte (CT and NG); a concordant result for two out of three NAATs determined the final PIS status. Vaginal swabs (clinician-collected and self-collected) were tested with three NAATs for each analyte; a concordant result for two out of three NAATs tests determined the CCA result for CT and NG. If NAAT1 and NAAT2 were discordant then NAAT3 was performed to be the tiebreaker between the first two discordant results.

f) Results and Analysis

The cobas liat CT/NG nucleic acid test results of each analyte from each sample type (male urine and vaginal swab) were compared to the PIS/CCA result to determine the clinical performance of the assay. The cobas liat CT/NG nucleic acid test positive percent agreement (PPA) and negative percent agreement (NPA) estimates or sensitivity and specificity estimates were calculated separately for each specimen type and each analyte, respectively.

Tabulation of the total number of samples (N) and the number of true positive (TP), false positive (FP), true negative (TN), and false negative (FN) cobas liat CT/NG results, against the CCA/PIS result, are shown and separated by each target pathogen, specimen type, and symptom status (Symp = Symptomatic; Asymp = Asymptomatic).

Table 50: CT Clinical Performance

Specimen Type	Symptom Status	N	TP	FP	TN	FN	Sensitivity		Specificity	
							Estimate (95% CI)	n/N	Estimate (95% CI)	n/N
Male Urine	Symp	808	55	1	751	1	98.2% (90.6%, 99.7%)	55/56	99.9% (99.3%, 100.0%)	751/752
	Asymp	1488	53	1	1432	2	96.4% (87.7%, 99.0%)	53/55	99.9% (99.6%, 100.0%)	14332/1433
	Total	2296	108	2	2183	3	97.3% (92.4, 99.1%)	108/111	99.9% (99.7%, 100.0%)	2182/2185
Specimen Type	Symptom Status	N	TP	FP	TN	FN	PPA		NPA	
							Estimate (95% CI)	n/N	Estimate (95% CI)	n/N
Vaginal Swabs	Symp	1116	60	3	1052	1	98.4% (91.3%, 99.7%)	60/61	99.7% (99.2%, 99.9%)	1052/1055
	Asymp	1357	47	2	1307	1	97.9% (89.1%, 99.6%)	47/48	99.8% (99.4%, 100.0)	1307/1309
	Total	2473	107	5	2359	2	98.2% (93.6%, 99.5%)	107/109	99.8% (99.5%, 99.9%)	2359/2364

Table 51: NG Clinical Performance in Male Urine and Vaginal Swabs against the PIS/CCA result

Specimen Type	Symptom Status	N	TP	FP	TN	FN	Sensitivity		Specificity	
							Estimate (95% CI)	n/N	Estimate (95% CI)	n/N
Prospective Male Urine	Symp	813	68	0	745	0	100.0% (94.7%, 100.0%)	68/68	100.0% (99.5%, 100.0%)	745/745
	Asymp	1488	11	3	1474	0	100.0% (74.1%, 100.0%)	11/11	99.8% (99.4%, 99.9%)	1474/1477
	Total	2301	79	3	2219	0	100.0% (95.4%, 100.0%)	79/79	99.9% (99.6%, 100.0%)	2219/2222
Archived Male Urine	Symp	125	77	0	48	0	100.0% (95.2%, 100.0%)	77/77	100.0% (92.6%, 100.0%)	48/48
	Asymp	38	5	0	33	0	100.0% (56.6%, 100.0%)	5/5	100.0% (89.6%, 100.0%)	33/33
	Total	163	82	0	81	0	100.0% (95.5%, 100.0%)	82/82	100.0% (95.5%, 100.0%)	81/81
Total Male Urine	Symp	938	145	0	793	0	100.0% (97.4%, 100.0%)	145/145	100.0% (99.5%, 100.0%)	793/793
	Asymp	1526	16	3	1507	0	100.0% (80.6%, 100.0%)	16/16	99.8% (99.4%, 99.9%)	1507/1510
	Total	2464	161	3	2300	0	100.0% (97.7, 100.0%)	161/161	99.9% (99.6%, 100.0%)	2300/2303
Specimen Type	Symptom Status	N	TP	FP	TN	FN	PPA		NPA	
							Estimate (95% CI)	n/N	Estimate (95% CI)	n/N
Prospective Vaginal Swabs	Symp	1115	22	2	1089	2	91.7% (74.2%, 97.7%)	22/24	99.8% (99.3%, 99.9%)	1089/1091
	Asymp	1357	18	2	1337	0	100.0% (82.4%, 100.0%)	18/18	99.9% (99.5%, 100.0%)	1337/1339
	Total	2472	40	4	2426	2	95.2% (84.2%, 98.7%)	40/42	99.8% (99.6%, 99.9%)	2426/2430
Archived Vaginal Swabs	Symp	42	20	0	22	0	100.0% (83.9%, 100.0%)	20/20	100.0% (83.9%, 100.0%)	22/22
	Asymp	48	25	0	23	0	100.0% (86.7%, 100.0%)	25/25	100.0% (86.7%, 100.0%)	23/23
	Total	90	45	0	45	0	100.0% (92.1%, 100.0)	45/45	100.0% (92.1%, 100.0%)	45/45
Total Vaginal Swabs	Symp	1157	42	2	1111	2	95.5% (84.9%, 98.7%)	42/44	99.8% (99.5%, 100.0%)	1111/1113
	Asymp	1405	43	2	1360	0	100% (91.8%, 100.0%)	43/43	99.9% (99.5%, 100.0%)	1360/1362
	Total	2562	85	4	2471	2	97.7% (92.0%, 99.4%)	85/87	99.8% (99.6%, 99.9%)	2471/2475

2. Device performance with analyte concentrations near the cutoff

Performance with analyte concentrations near the assay LoD was evaluated as part of the reproducibility study. Testing was performed at three external sites with a minimum of three cobas liat analyzers per site. Operators at all three external sites were chosen to represent typical POC operators with limited or no training or hands-on experience conducting laboratory testing. Two operators at each site each tested one panel per specimen type per day (one complete panel consists of three panel members each tested in triplicate) for a total of 15 days. Each panel, per specimen type, consisted of a negative panel member (negative for all analytes), a low positive panel member (1-2x LoD), and a moderate positive (3-5x

LoD) panel member with each positive panel member being co-formulated with all analytes. The following tables display only those panel members near the analyte cutoff (i.e., 1-2x LoD). A summary of percent agreement with expected results for each panel member by site and overall is presented in the following tables.

Table 52: CT- Summary of Performance Near Assay Cutoff for Each Site

Specimen Type	Panel Member Concentration	Percent Agreement with Expected Results (n/N) (95% Confidence Interval)			
		Site 1	Site 2	Site 3	Overall
Vaginal Swab	1-2x LoD	100% (90/90) (95.9% - 100.0%)	100% (89/89) (95.9% – 100.0%)	100% (90/90) (95.9% - 100.0%)	100% (269/269) (98.6% - 100.0%)
	Negative	100% (90/90) (95.9% - 100.0%)	100% (83/83) (95.6 – 100.0%)	100% (90/90) (95.9% - 100.0%)	100% (263/263) (98.6% - 100.0%)
Male Urine	1-2x LoD	87.8% (79/90) (79.4% - 93.0%)	93.3% (83/89) (86.1% - 96.9%)	91.1% (82/90) (83.4% - 95.4%)	90.7% (244/269) (86.6% - 93.6%)
	Negative	100% (90/90) (95.9% - 100.0%)	100% (80/80) (95.6% – 100.0%)	100% (90/90) (95.9% - 100.0%)	100% 260/260 (98.5% - 100.0%)

LoD: limit of detection

Table 53: NG- Summary of Performance Near Assay Cutoff for Each Site

Specimen Type	Panel Member Concentration	Percent Agreement with Expected Results (n/N) (95% Confidence Interval)			
		Site 1	Site 2	Site 3	Overall
Vaginal Swab	1-2x LoD	100% (90/90) (95.9% - 100.0%)	100% (89/89) (95.9% – 100.0%)	100% (90/90) (95.9% - 100.0%)	100% (269/269) (98.6% - 100.0%)
	Negative	100% (90/90) (95.9% - 100.0%)	100% (83/83) (95.6 – 100.0%)	100% (90/90) (95.9% - 100.0%)	100% (263/263) (98.6% - 100.0%)
Male Urine	1-2x LoD	100% (90/90) (95.9% - 100.0%)	98.9% (88/89) (93.9% – 99.8%)	100% (90/90) (95.9% - 100.0%)	99.6% (268/269) (97.9% - 99.9%)
	Negative	100% (90/90) (95.9% - 100.0%)	100% (80/80) (95.6% – 100.0%)	100% (90/90) (95.9% - 100.0%)	100% 260/260 (98.5% - 100.0%)

LoD: limit of detection

An investigation into the reproducibility results for the CT analyte suggested there may have been a dilution error in the preparation of the CT analyte in urine matrix reproducibility panel member. Therefore, a supplemental precision study was performed at one site across three lots, days, operators and instruments for cobas liat CT/NG for the detection of CT in urine from urine panels prepared at negative, 1x-2x and 3x-5x LoD concentration levels. There were six total POC performing the testing. Each operator tested one panel per day for five non-consecutive days for each lot (one complete panel consisted of three panel members). Table 54 below summarizes the results for panel members near the analyte cutoff (i.e., 1-2x LoD):

Table 54: Summary of Within-Laboratory Precision study for CT in Urine Matrix

Panel Member Concentration	Operator	n/N[1]	Agreement with Expected Results (%)
1-2x LoD	1	44/45	97.8%
	2	44/44	100.0%
	3	45/45	100.0%
	4	44/44	100.0 %
	5	45/45	100.0%
	6	45/45	100.0%
Negative	1	43/44	97.7%
	2	45/45	100.0%
	3	45/45	100.0%
	4	45/45	100.0%
	5	45/45	100.0%
	6	44/44	100.0%

[1]n is the number of tests with expected results. N is the total number of valid tests

The results of these studies demonstrate that untrained users could perform the cobas liat CT/NG nucleic acid test accurately, including testing samples with organism concentrations near the assay LoD.

3. Operator Questionnaire

Upon completing their participation within the clinical study, operators were provided a questionnaire to assess the ease-of-use. Ease-of- use agreement scores were high, ranging from 4.0 to 4.5 (out of 5). Separately, operators were assessed for proficiency for reporting the correct positive result, negative result, test status, conducting the correct response on targets that cannot be assessed. The overall operator proficiency score across these five responses indicate that the test is easy to use and the written instructions are clear.

M. Labeling for Waived Devices

The labeling consists of:

1. Package Insert
2. Quick Reference Instructions
3. Instruction Manual
4. Instructions for the two specimen collection kits (urine and vaginal swabs)

The following elements are appropriately present:

- The QRI is written in simple language and contains graphics which visually aid the user in processing samples.

- The labeling identifies the system as CLIA Waived.
- A statement informing the user that the test procedure must be followed as written to maintain the CLIA waived status is present.
- The QRI includes instructions for performing Quality Control testing.
- Technical support telephone number is prominently displayed.
- All appropriate cautions regarding sample handling and processing are present.
- The labeling includes the statement that a Certificate of Waiver is required to perform the test in a waived setting.
- 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.