Rx Only

cobas® Cdiff

Nucleic acid test for use on the cobas® Liat® System

For in vitro diagnostic use

cobas® Cdiff Nucleic acid test for use on the cobas® Liat® System	20 Tests	P/N: 07454945190
cobas® Cdiff Positive and Negative Control Kit for use on the cobas® Liat® System	5 Sets	P/N: 07454970190

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Intended use

The **cobas**® Cdiff Nucleic acid test for use on the **cobas**® Liat® System is an automated, qualitative in vitro diagnostic test that uses real-time polymerase chain reaction (PCR) for the detection of the toxin B (*tcd*B) gene of toxigenic *Clostridioides difficile* (*C. difficile*) in unformed (liquid or soft) stool specimens obtained from patients suspected of having *C. difficile* infection (CDI). The **cobas**® Cdiff Nucleic acid test for use on the **cobas**® Liat® System is intended for use as an aid in the diagnosis of CDI in humans in conjunction with clinical and epidemiological risk factors.

Summary and explanation of the test

Background: Detection of *C. difficile*

Clostridioides difficile (*C. difficile*) is a gram-positive, anaerobic, spore-forming bacilli that was identified as an etiological agent of antibiotic-associated diarrhea and pseudomembranous colitis in the late 1970s.[1,2] *C. difficile* is the most frequently reported nosocomial pathogen[3] and is believed to be responsible for 15% to 20% of antibiotic-related cases of diarrhea and nearly all cases of antibiotic-associated pseudomembranous colitis.[4] *C. difficile* infection (CDI) incidence has increased four-fold in less than two decades[5] and is associated with severe illness and mortality.[3] Increases in incidence have, in part, been attributed to the emergence of hypervirulent strains such as the BI/O27/North American pulsotype 1 (NAP1) ribotype. Elderly and hospitalized patients with recent antibiotic use are the most at-risk populations for CDI, however CDI frequency is increasing outside the hospital environment as well.[3,6]

Infections are transmitted by spores, and following colonization with toxigenic *C. difficile* individuals may become asymptomatic carriers or develop colonic disease. Clinical features of CDI may range from mild diarrhea to life-threatening pseudomembranous colitis characterized by abdominal pain, profuse diarrhea and systemic symptoms such as fever, anorexia, nausea, and malaise. Despite the dramatic increase in incidence and severity of CDI, metronidazole or vancomycin remain the medical treatments of choice for acute episodes and recurrent infection.[7]

Diagnosis of CDI is usually established by the presence of toxin in stool samples. Most toxigenic strains of *C. difficile* typically produce two protein exotoxins: toxin A and toxin B.[8] A small percentage of toxigenic strains may produce only toxin B.[9] Demonstration of the cytopathic effect on a monolayer of cells by the action of toxin B has been the traditional "gold standard".[10,11] Stool supernatant can be directly incubated on the monolayer of cells; alternatively, *C. difficile* isolates from stool can be cultured in enrichment broth before incubating the supernatant on the cell monolayer (toxigenic culture). Both techniques require at least 48 to 72 hours to obtain a final test result.

Stool culture is not widely performed given the procedural complexity and longer time-to-result described above, and diagnosis is often done with either enzyme immunoassays (EIA) or DNA-based tests.[3,12] Immunoassays for toxin detection are widely used because they can provide positive results in less than 4 hours, but sensitivities are lower compared to culture.[12,13] In contrast, *C. difficile* toxin gene detection with polymerase chain reaction (PCR) is reported to have higher sensitivity and shorter time-to-result than both culture and immunoassays.[3,14-17]

Infection control measures include the prudent use of antimicrobials, prevention of cross-infection, and active surveillance of cases.[18] Thus, there is a great need for highly sensitive and rapid automated detection of *C. difficile*. Molecular methods offer the potential to significantly reduce the detection time, thereby enabling the prompt initiation of antimicrobial treatment and the prompt implementation of infection control measures.[14-16] The **cobas**® Cdiff Nucleic acid test for use on the **cobas**® Liat® System is designed to be a rapid molecular test for the detection of the *C. difficile* toxin B gene in unformed stool specimens obtained from patients suspected of CDI.

Explanation of the test

The **cobas**® Cdiff Nucleic acid test for use on the **cobas**® Liat® System (referred to as "**cobas**® Cdiff" from here on) is a rapid test that fully automates sample preparation, PCR amplification and real-time detection of target DNA sequences on the **cobas**® Liat® Analyzer. The **cobas**® Cdiff test consists of a single-use disposable **cobas**® Cdiff assay tube that contains nucleic acid purification and PCR reagents, as well as an Internal Control (*Bacillus thuringiensis israelensis* or Bti). The **cobas**® Cdiff assay tube hosts the sample preparation and PCR processes. The **cobas**® Cdiff assay tube is self-contained, so the risk of cross-contamination between samples is reduced.

Principles of the procedure

Sample preparation

Organisms within the stool specimen are lysed with a chaotropic agent and proteinase K. Released nucleic acids, including Bti Internal Control DNA, are bound by magnetic glass particles. The particles are washed, and bound nucleic acids are eluted into a small volume of buffer and then mixed with Master Mix and activating co-factor for the PCR reaction.

PCR amplification and TaqMan® detection

The Master Mix reagent contains primer pairs and probes for *C. difficile* toxin B and the Internal Control. If the target nucleic acid sequences are present, amplification with the corresponding primers will occur by a thermostable DNA polymerase, generating PCR products (amplicons). These products are detected by specific TaqMan® probes containing a fluorescent reporter dye and a quencher. Normally, the quencher suppresses the fluorescence of the reporter dye. However, if the PCR product is present, the probe hybridizes to the product and is cleaved by the 5'- to 3'-nuclease activity of the polymerase, thereby separating the reporter dye and quencher. This reaction allows the fluorescence to be emitted from the reporter dye, and the signal is recorded in real time during each PCR cycle by the **cobas**® Liat® Analyzer. This signal is interpreted by the **cobas**® Liat® System Software and reported as final results.

Selective amplification

Selective amplification of target nucleic acid from the specimen is achieved in **cobas**® Cdiff by the use of AmpErase (uracil-*N*-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[19], but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon due to the use of deoxyuridine triphosphate in place of thymidine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contain deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase enzyme prior to amplification of the target DNA. AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step at the alkaline pH of Master Mix, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. AmpErase enzyme is inactive at temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon. **cobas**® Cdiff has been demonstrated to inactivate at least 1000 copies of deoxyuridine-containing *C. difficile* amplicon per PCR.

Reagents and materials

cobas® Cdiff reagents and controls

Table 1: cobas® Cdiff

cobas® Cdiff Nucleic acid test for use on the cobas® Liat® System Store at 2-8°C 20 tests (P/N 07454945190)		
Reagents in cobas® Cdiff Assay Tube	**Reagent ingredients**	**Safety symbol and warning**[a]
cobas® Liat® Cdiff Internal Control	PBS Tween-80 0.01% ProClin® 300 preservative Glycerol EDTA < 1% Bti stock (inactivated)	 **DANGER** H302 + H332 Harmful if swallowed or if inhaled. H314 Causes severe skin burns and eye damage. H317 May cause an allergic skin reaction. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled. H411 Toxic to aquatic life with long lasting effects. H411 Harmful to aquatic life with long lasting effects. P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P272 Contaminated work clothing should not be allowed out of the workplace. P273 Avoid release to the environment. P280 Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P284 Wear respiratory protection. P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention. P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor. P362 + P364 Take off contaminated clothing and wash it before reuse. P391 Collect spillage. P501 Dispose of contents/ container to an approved waste disposal plant.
cobas® Liat® Proteinase K	Tris buffer EDTA Calcium chloride Calcium acetate < 2.0% Proteinase K[b] Glycerine	
cobas® Liat® Magnetic Glass Particles	Magnetic Glass Particles Water	
cobas® Liat® Lysis Buffer	Sodium citrate 3% Polydocanol[b] 42.6% Guanidinium thiocyanate[b] Dithiothreitol	
cobas® Liat® Wash Buffer	Sodium citrate dihydrate 0.05% N-Methylisothiazolone HCl	

cobas® Cdiff Nucleic acid test for use on the cobas® Liat® System Store at 2-8°C 20 tests (P/N 07454945190)		
Reagents in cobas® Cdiff Assay Tube	**Reagent ingredients**	**Safety symbol and warning[a]**
cobas® Liat® Elution Buffer-1	Recombinant Human Serum Albumin Tris-HCl buffer 0.09% Sodium azide	EUH210 Safety data sheet available on request. EUH208 Contains Mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H -isothiazol-3-one (3:1). May produce an allergic reaction. EUH032 Contact with acids liberates very toxic gas. 39450-01-6 Proteinase, Tritirachium album serine 26172-54-3 2-Methyl-2H-isothiazol-3-one hydrochloride 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol
cobas® Liat® Cdiff Master Mix-1	Tricine buffer EDTA DMSO Potassium acetate Potassium hydroxide < 0.01% Upstream and downstream *C. difficile* and Internal Control primers < 0.01% Fluorescent-labeled *C. difficile* and Internal Control probes 0.09% Sodium azide	
cobas® Liat® Cdiff Master Mix-2	DMSO Tween 20 < 0.19% dATP, dCTP, dGTP, dUTP < 0.01% Oligonucleotide aptamer < 0.01% Z05 DNA polymerase (microbial) < 0.02% AmpErase (uracil-*N*-glycosylase) enzyme (microbial) 0.09% Sodium azide	
cobas® Liat® Cdiff Cofactor	Manganese acetate Magnesium acetate Bovine serum albumin from bovine plasma sourced in the United States 0.09% Sodium azide	

[a] Product safety labeling primarily follows EU GHS guidance

[b] Hazardous substance or mixture

Table 2: cobas® Cdiff Positive and Negative Control Kit for use on the cobas® Liat® System

cobas® Cdiff Positive and Negative Control Kit for use on the cobas® Liat® System Store at 15-30°C 5 Sets (P/N 07454970190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning**
Cdiff(+)C (cobas® Liat® Cdiff Positive Control)	Tris buffer EDTA < 0.01% Poly rA RNA (synthetic) 0.05% Sodium azide < 0.01% Non-infectious plasmid DNA (microbial) containing *C. difficile* sequence	5 Vials	N/A
BUF(–)C (cobas® Liat® Negative Control)	Tris buffer EDTA 0.05% Sodium azide < 0.01% Poly rA RNA (synthetic)	5 Vials	N/A

Reagent storage and handling

Table 3: Reagent storage and handling

Reagent	Storage Temperature	Storage Time
cobas® Cdiff Nucleic acid test for use on the **cobas**® Liat® System	2–8°C	Stable until the expiration date indicated
cobas® Cdiff Positive and Negative Control Kit for use on the **cobas**® Liat® System	15-30°C	Stable until the expiration date indicated

Note: Do not freeze reagents.

Reagent expiry date is based on the Coordinated Universal Time (UTC). Local time for reagent expiry could be offset by plus or minus 12 hours, depending on the local time zone relative to UTC.

Additional materials required

Table 4: Additional materials required

Materials	P/N
cobas® PCR Media Uni Swab Sample Kit	07958030190
Disposable gloves, powderless	Any powderless disposable gloves are acceptable.

For more information regarding the materials sold separately, contact your local Roche representative.

Optional material

Table 5: Optional material

Material	P/N
cobas® PCR Replacement Cap Kit	07958056190

For more information regarding the optional material, contact your local Roche representative.

Instrumentation and software required but not provided

Table 6: Instrumentation and software required but not provided

Required Instrumentation and Software, Not Provided
cobas® Liat® Analyzer (P/N 07341920190) • Including **cobas**® Liat® System Software (Core) Version 3.3.0 or higher
cobas® Cdiff Script v1.1 or higher

For more information regarding the instrumentation and software required, contact your local Roche representative.

Precautions and handling requirements

Warnings and precautions

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

- For in vitro diagnostic use only.
- Avoid microbial and DNA contamination of reagents and specimens.
- Safety Data Sheets (SDS) are available upon request from your local Roche representative.
- **cobas**® Liat® Lysis Buffer (LYS reagent) contains guanidine thiocyanate. Do not allow direct contact between guanidine thiocyanate and sodium hypochlorite (bleach) or other highly reactive reagents such as acids or bases. These mixtures can release a noxious gas.
- **cobas**® Liat® Elution Buffer-1 (EB), **cobas**® Liat® Cdiff Master Mix-1 (Cdiff MMX-1), **cobas**® Liat® Cdiff Master Mix-2 (Cdiff MMX-2), **cobas**® Liat® Cdiff Cofactor (Cofactor), BUF(–)C and Cdiff(+)C contain sodium azide.
- For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas**® Liat® Analyzer, consult the current **cobas**® Liat® System User Guide.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink or smoke in work areas.
- Wash hands thoroughly after handling specimens and kit reagents.
- Per institutional policy, wear eye protection, laboratory coats and disposable gloves when handling any reagents. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills occur, dilute with water before wiping dry.
- The addition of AmpErase enzyme into the **cobas**® Liat® Cdiff Master Mix enables selective amplification of target DNA; however, good laboratory practices and careful adherence to the procedures specified in this Instructions-For-Use document are necessary to avoid contamination of reagents and amplification mixtures.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.

Contamination

- Gloves must be worn and must be changed between handling specimens and **cobas**® Cdiff assay tubes or Control vials to prevent contamination. Avoid contaminating gloves when handling specimens and controls. Wear lab gloves, laboratory coats, and eye protection when handling specimens and kit reagents.
- Avoid microbial and ribonuclease contamination of reagents.
- False positive results may occur if carryover of specimens is not prevented during specimen handling.
- Specimens should be handled as infectious using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories[20] and in the CLSI Document M29-A4.[21]

Integrity

- Do not use kits after their expiration dates.
- Do not pool reagents.
- Do not use a damaged **cobas**® Cdiff assay tube or a **cobas**® Cdiff assay tube that has been dropped after removal from its foil pouch.
- Do not reuse **cobas**® Cdiff assay tubes. If a **cobas**® Cdiff assay tube is not housed in a sleeve, or if the tube sample compartment already contains liquid, do NOT use the tube.
- All equipment should be properly maintained according to the manufacturer's instructions.
- All reagent kits should be stored properly. Refer to Table 3.

Disposal

- **cobas**® Cdiff assay tube should be discarded in the appropriate biohazardous waste container as specified by your site specific Environmental Health & Safety standards.
- **cobas**® Cdiff reagents and controls contain sodium azide (see "Warnings and precautions"). Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of solutions containing sodium azide down laboratory sinks, flush the drains with a large volume of cold water to prevent azide buildup.
- Dispose of unused assay tube and waste in accordance with country, federal, state and local regulations.

Spillage and cleaning

- If spills occur on the **cobas**® Liat® Analyzer, follow the appropriate instructions in the **cobas**® Liat® System User Guide to clean.

Specimen collection, transport, and storage

Handle all specimens as if they are capable of transmitting infectious agents.

Specimen collection

The **cobas**® Cdiff should only be used with partially formed or unformed stool specimens. This is defined as a stool specimen that takes the shape of its container. Collect stool specimen in a clean, dry and unused container by following your institution's standard operating procedures.

Specimen transport storage and stability

Unformed stool specimens are stable at 2-30°C for 2 days, or 2-8°C for 9 days before being transferred to **cobas**® PCR Media and tested on the **cobas**® Liat® System (this was demonstrated by testing specimens after consecutive storage at 30°C ± 1°C for 2 days, followed by 2-8°C for 7 days).

Stool specimen re-suspended in **cobas**® PCR Media is stable at 2-30°C for 7 days before testing on the **cobas**® Liat® System.

Transportation of *C. difficile* specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

Test procedure

"Add Lot" workflow

Figure 1: "Add Lot" workflow

1	Start up the system and login
2	Remove Controls and assay tubes from storage
3	Under "Assay" menu, choose "New Lot"
4	Scan the barcode on the Package Insert ID Barcode card
5	Scan and run Negative Control
6	Scan and run Positive Control

Specimen transfer workflow

Figure 2: Specimen transfer workflow

1	Immerse swab into stool specimen
2	Place inoculated swab into **cobas**® PCR Media Tube
3	Break swab shaft at gray notch
4	Cap tube and vortex at least 5 seconds

cobas® Cdiff workflow

Figure 3: cobas® Cdiff workflow

1	Start up the system and login
2	Remove samples and assay tubes from storage
3	On the Main Menu, choose "Run Assay"
4	Scan **cobas**® Cdiff assay tube barcode
5	Scan or enter sample ID
6	Add specimen to **cobas**® Cdiff assay tube using transfer pipette and re-cap the tube
7	Rescan **cobas**® Cdiff assay tube barcode
8	Start run by inserting the **cobas**® Cdiff assay tube
9	Review results*
10	Unload and dispose used **cobas**® Cdiff assay tube

* Refer to current **cobas**® Liat® System User Guide for details of result uploading to DMS and LIS.

Instructions For Use

"Add Lot" procedure

Before using a new lot of **cobas**® Cdiff assay tubes, the "Add Lot" procedure must be performed on the **cobas**® Liat® Analyzer to validate the **cobas**® Cdiff assay tube lot at your site. The procedure includes running a Negative Control sample and a Cdiff Positive Control sample.

Materials needed for "Add Lot"

- New lot of **cobas**® Cdiff Nucleic acid test for use on the **cobas**® Liat® System (two assay tubes and pipettes)
- Package Insert ID Barcode card for the new lot of **cobas**® Cdiff assay tubes
- **cobas**® Liat® Cdiff Positive Control
- **cobas**® Liat® Negative Control
- Barcode card for the **cobas**® Liat® Cdiff Positive Control and the **cobas**® Liat® Negative Control

Note: Refer to the cobas® Liat® System User Guide for detailed operating instructions.

Procedure

1. Press the power on/off button to start the **cobas**® Liat® Analyzer.
2. Select "**Login**" on the screen of the **cobas**® Liat® Analyzer.
3. Enter user name when prompted, select "**Enter**".
4. Enter user password when prompted, select "**Enter**".

Note: You may be prompted to confirm you have read the User Guide (i.e., cobas® Liat® System User Guide).

5. Select "**Assay Menu**" on the main menu of the **cobas**® Liat®Analyzer.
6. Select "**New Lot**" at the bottom of the list.
7. When prompted to **Scan Insert ID**, select "**Scan**" and scan the **cobas**® Cdiff Package Insert ID Barcode card. Ensure that the red scan light is over the entire barcode.

Note: You may be prompted to confirm you have read the Package Insert or Instructions For Use.

8. When prompted to **Scan Negative Control ID**, select "**Scan**" and scan the Negative Control Barcode card included with the control kit. Ensure that the red scan light is over the entire barcode. Next, the **cobas**® Liat® Analyzer will prompt with the message "**Add negative control & scan tube ID.**"
9. Hold a tube of **cobas**® Liat® Negative Control upright and lightly tap on a flat surface to collect liquid at the bottom of the tube.
10. Open up a **cobas**® Cdiff assay tube foil pouch (from the lot to be added) and remove the contents.
11. Use the transfer pipette provided in the pouch to add the **cobas**® Liat® Negative Control to the **cobas**® Cdiff assay tube. Firmly squeeze the bulb of the pipette until the bulb is fully flat, then insert the tip of the pipette into the liquid and draw up the sample by slowly releasing the bulb.

Note: Only use the transfer pipette provided in the cobas® Cdiff assay tube pouch to transfer controls and samples into the cobas® Cdiff assay tube.

12. Carefully remove the cap of the **cobas**® Cdiff assay tube and insert the pipette into the opening. Place the pipette tip near the bottom of the open segment.

13. Slowly squeeze the bulb to empty the contents of the pipette into the **cobas**® Cdiff assay tube. Avoid creating bubbles in the sample. Do not release the pipette bulb while the pipette is still in the **cobas**® Cdiff assay tube.

Note: Do not puncture the cobas® Cdiff assay tube or the seal at the bottom of the sample compartment. If either of these is damaged, discard both the cobas® Cdiff assay tube and the transfer pipette, and restart the testing procedure with a new cobas® Cdiff assay tube and pipette.

14. Screw the cap back onto the **cobas**® Cdiff assay tube. Dispose of the transfer pipette.
15. Select "**Scan**" and place the **cobas**® Cdiff assay tube horizontally on the table beneath the barcode reader so that the red scan light is over the entire barcode. The assay tube entry door on top of the **cobas**® Liat® Analyzer will open automatically once the barcode is read.
16. Remove the **cobas**® Cdiff assay tube sleeve and immediately insert the **cobas**® Cdiff assay tube into the **cobas**® Liat® Analyzer until the assay tube clicks into place.

Note: The cobas® Cdiff assay tube only fits in one way - the grooved side of the cobas® Cdiff assay tube must be on the left while the cap is on top.

17. If the assay tube is not inserted by the time the door closes, rescan the **cobas**® Cdiff assay tube barcode and insert the **cobas**® Cdiff assay tube again. Once the **cobas**® Cdiff assay tube is properly inserted, the **cobas**® Liat® Analyzer will close the door automatically and begin the test.
18. During the test, the **cobas**® Liat® Analyzer displays the running status and estimated time remaining. Once the test is complete, if "**Negative control result accepted**" is displayed, select "**Confirm**". If the result is rejected, repeat the Negative Control run (Steps 8-18). If the repeated control run does not produce the expected results, contact your local Roche representative.
19. When the test is complete, the **cobas**® Liat® Analyzer displays the message "**Remove the assay tube slowly and carefully**" and automatically opens the assay tube entry door. Slowly lift the **cobas**® Cdiff assay tube out of the **cobas**® Liat® Analyzer. Dispose of the used **cobas**® Cdiff assay tube in a biohazardous waste container.
20. Select "**Back**" to proceed with the **cobas**® Liat® Cdiff Positive Control test on the same instrument.
21. Similarly, follow steps 8 to 17 with a **cobas**® Liat® Cdiff Positive Control in place of the **cobas**® Liat® Negative Control.
22. If "**Positive control result accepted. Lot … added…**" is displayed at the end of the run, select "**Confirm**" and then select "**Back**" to return to the main menu. If the result is rejected, repeat the **cobas**® Liat® Cdiff Positive Control test. If repeated control runs do not produce the expected results, contact your local Roche representative.
23. Repeat step 19.
24. Select "**Assay Menu"** to verify the new lot has been added.

After "Add Lot" is completed on one Analyzer use the Tools Menu on the **cobas**® Liat Analyzer with a USB key to transfer the lot information to the other Analyzers at your site. This allows the other Analyzers to use this **cobas**® Cdiff assay tube lot without performing an "Add Lot" on each Analyzer. Follow the instructions in the **cobas**® Liat® System User Guide, and perform an "Export assay lots" on the analyzer on which the "Add Lot" was performed. Then, perform the "Import assay lots" procedure on each of the other analyzers at your site.

Specimen transfer into cobas® PCR Media

1. Stool specimen should be transferred to **cobas**® PCR Media tube and tested within the time frame described in the "Specimen collection, transport, and storage" section. The original stool specimen is also referred to as "primary specimen," and the stool suspension in **cobas®** PCR Media (see steps below) is also referred to as "secondary specimen" in this document.
2. Use the swab provided in the **cobas**® PCR Media Uni Swab Sample Kit to transfer the stool specimen. Without touching the side of the stool container, immerse the tip of the swab fully into the stool specimen, up to the end of the tapered section.

3. Promptly remove and place inoculated swab into the **cobas**® PCR Media tube. Do not test the sample if there is not enough stool to fully submerge the tip of the swab.
4. Break the swab shaft at the gray notch mark, by applying pressure against the side of the **cobas**® PCR Media tube.
5. Cap the tube and vortex the tube for at least 5 seconds.

Note: cobas® Cdiff has been validated for use with the cobas® PCR Media Uni Swab Sample Kit. Other devices or media types have not been validated for use with cobas® Cdiff.

Note: To avoid cross-contamination of stool specimen suspensions in cobas® PCR Media, additional caps for cobas® PCR Media tubes in an alternate color (natural; see "Optional material") should be used to recap specimen suspensions after processing.

Note: cobas® PCR Media tubes contain sufficient volume of cobas® PCR Media for stool suspensions to be assayed multiple times on the cobas® Liat® System. Minimum stool suspension volume to conduct a cobas® Cdiff run is 0.2 mL.

Performing the cobas® Cdiff on clinical specimens

Material needed for running cobas® Cdiff

- **cobas**® Cdiff assay foil pouch which includes the **cobas**® Cdiff assay tube and transfer pipette
- Stool specimens transferred and resuspended in **cobas**® PCR Media (see "Specimen Transfer into **cobas**® PCR Media")

Procedure

1. Ensure that the **cobas**® Liat® Analyzer is powered on.
2. Select "**Login**" on the screen of the **cobas**® Liat® Analyzer.
3. Enter user name when prompted, select "**Enter**".
4. Enter user password when prompted, select "**Enter**".

Note: You may be prompted to confirm you have read the User Guide (i.e., cobas® Liat® System User Guide).

5. From the main menu, select "**Run Assay**".
6. Open up a **cobas**® Cdiff assay tube pouch and take out the assay tube. When prompted to **Scan Tube ID,** select **"Scan"** and place the **cobas**® Cdiff assay tube horizontally on the table beneath the barcode reader so that the red scan light is over the entire barcode.
7. When prompted to **Scan sample ID,** select "**Scan**" to scan the sample barcode. In the case that the sample cannot be scanned, select "**Enter**" to manually enter the sample ID.

Note: Depending on the analyzer configuration, if required to confirm the received patient information, select the "Confirm" button.

8. When prompted, add sample to **cobas**® Cdiff assay tube.
9. Use the transfer pipette provided in the assay tube pouch to transfer secondary specimen. Firmly squeeze the bulb of the pipette until the bulb is fully flat, then insert the tip of the pipette into the liquid and draw up the sample by slowly releasing the bulb.
10. Carefully remove the cap of the **cobas**® Cdiff assay tube and insert the pipette into the opening. Place the pipette tip near the bottom of the open segment.
11. Slowly squeeze the bulb to empty the contents of the pipette into the **cobas**® Cdiff assay tube. Do not release the pipette bulb while the pipette is still in the **cobas**® Cdiff assay tube.
12. Re-cap the **cobas**® Cdiff assay tube and dispose of the transfer pipette.

Note: Avoid cross-contamination of gloves, equipment and work surfaces with the residual contents of the pipette.

13. Select "**Scan"** and rescan the same **cobas**® Cdiff assay tube barcode. The assay tube entry door on top of the **cobas**® Liat® Analyzer will open automatically.
14. Remove the **cobas**® Cdiff assay tube sleeve and immediately insert the **cobas**® Cdiff assay tube into the **cobas**® Liat® Analyzer until the assay tube clicks into place.

Note: The cobas® Cdiff assay tube only fits in one way - the grooved side of the cobas® Cdiff assay tube must be on the left while the cap is on top.

15. If the assay tube is not inserted by the time the door closes, rescan the **cobas**® Cdiff assay tube barcode and insert the **cobas**® Cdiff assay tube again. Once the **cobas**® Cdiff assay tube is properly inserted, the **cobas**® Liat® Analyzer will automatically close the door and begin the test.
16. During the test, the **cobas**® Liat® Analyzer displays the running status and estimated time remaining. Once the test is complete, the **cobas**® Liat® Analyzer displays the message, "**Remove the assay tube slowly and carefully**" and automatically opens the assay tube entry door. Slowly lift the **cobas**® Cdiff assay tube out of the **cobas**® Liat®Analyzer. Dispose of the used **cobas**® Cdiff assay tube in a biohazardous waste container.
17. Select "**Report"** to see the Result Report. If applicable, select "**Print"** to print the report.
18. Select "**Back"**, and then "**Main"** to return to the main menu to perform the next test.

Performing additional control runs

In accordance with local, state, federal and/or accrediting organization requirements, additional control runs may be performed with a lot of **cobas**® Cdiff assay tubes that has already been added through the "Add Lot" procedure. Use the **cobas**® Cdiff Positive and Negative Control Kit for use on the **cobas**® Liat® System to conduct these runs.

Material needed for additional control runs

- **cobas**® Cdiff assay tubes and transfer pipettes
- **cobas**® Liat® Cdiff Positive Control and/or **cobas**® Liat® Negative Control
- Corresponding barcodes for the **cobas**® Liat® Cdiff Positive Control and/or the **cobas**® Liat® Negative Control

Procedure

Use the procedure outlined under the "Performing the **cobas**® Cdiff on clinical specimens" section to perform additional control runs. In step 7, be sure to use the provided control barcodes included in **coba**s® Cdiff Positive and Negative Control Kit to scan as sample ID barcode. Interpretation of results for **cobas**® Cdiff when running additional Cdiff Positive Controls or Negative Controls are shown in Table 9 and Table 10 in the "Interpretation of results" section. Using barcodes other than the control barcodes provided may lead to incorrect control results.

Results

Quality control and validity of results

One **cobas**® Liat® Cdiff Positive Control and one **cobas**® Liat® Negative Control are run during the "Add Lot" procedure described earlier. Valid results must be obtained for both the Positive and Negative Control for the new lot of **cobas**® Cdiff assay tubes to be validated on the instrument. Additional control runs may be performed after the "Add Lot" procedure. Refer to "Performing additional control runs" under Instructions For Use for details.

The **cobas**® Liat® Cdiff Internal Control is packaged inside each **cobas**® Cdiff assay tube and will be run together with each sample during the whole assay workflow.

Positive Control

The **cobas**® Liat® Cdiff Positive Control contains non-infectious DNA plasmids with *C. difficile* target sequence. The **cobas**® Liat® Cdiff Positive Control verifies the integrity of reagents in the **cobas**® Cdiff assay tube and proper function of the **cobas**® Liat Analyzer. If the **cobas**® Liat® Cdiff Positive Control results are frequently invalid, contact your local Roche representative for technical assistance.

Negative Control

The **cobas**® Liat® Negative Control contains no target and monitors potential target contamination in the workflow or environment. If the **cobas**® Liat® Negative Control results are frequently invalid, contact your local Roche representative for technical assistance.

Internal Control

A whole organism Internal Control (Bti) is included in the assay tube and automatically added to all samples at the start of sample preparation. The **cobas**® Liat® Cdiff Internal Control is a chemically-inactivated bacterium that is included in each **cobas**® Cdiff assay tube and processed along with each sample. The Internal Control checks for adequate processing of the target bacteria through all steps of the assay and monitors the presence of inhibitors in the sample preparation and PCR. The **cobas**® Liat® Cdiff Internal Control should be positive in a negative sample and can be negative or positive in a Cdiff positive sample.

Interpretation of results

Note: All specimen and control run validation is determined by the cobas® Liat® System.

Results when running "Add Lot" procedure are interpreted as shown in Table 7.

Table 7: Interpretation of results of cobas® Cdiff when running "Add Lot" Procedure

cobas® Liat® Analyzer Display	Result Report Printout and Interpretation
Negative Control Valid	**Negative Control Valid** Control is negative for the presence of *C. difficile* DNA.
Negative Control Invalid. Repeat Run	**Negative Control Invalid** Result is Invalid. The Negative Control should be re-tested to obtain valid result. Repeat Run.
Positive Control Valid	**Positive Control Valid** Control is positive for the presence of *C. difficile* DNA.
Positive Control Invalid. Repeat Run	**Positive Control Invalid** Result is Invalid. The positive control should be re-tested to obtain valid result. Repeat Run.

Specimen results are interpreted as shown in Table 8.

Table 8: Interpretation of results of cobas® Cdiff when running a clinical specimen

cobas® Liat® Analyzer Display	Result Report Printout and Interpretation
Cdiff Detected	**Cdiff Detected** Specimen is positive for the presence of *C. difficile* DNA.
Cdiff Not Detected	**Cdiff Not Detected*** Specimen is negative for *C. difficile* DNA, or if present, could not be detected.
Assay Invalid	**Assay Invalid**** Result is Invalid. The original specimen should be re-tested to obtain valid result. See "Suggested Re-test Procedure".
Assay Aborted by User	**Assay Aborted by User** Run aborted by user. The original specimen should be re-tested to obtain valid result. See "Suggested Re-test Procedure".
Assay Aborted by System	**Assay Aborted by System** Run aborted by system. The original specimen should be re-tested to obtain valid result. See "Suggested Re-test Procedure".

* A negative result does not preclude the presence of *C. difficile* DNA because results depend on adequate specimen collection, absence of inhibitors, and sufficient DNA to be detected.

** Invalid results may be obtained if the specimen contains excess stool or interference substances that prevent nucleic acid target extraction and/or amplification and detection. See "Procedural limitations" for known interference substances. Insufficient sample volume may also lead to invalid results. The minimum volume of stool/ **cobas**® PCR Media suspension necessary for the **cobas**® Cdiff is 0.2 mL.

Results when running additional controls after following "Add Lot" procedure are interpreted as shown in Table 9 and Table 10.

Table 9: Interpretation of results of cobas® Cdiff when running Positive Control

cobas® Liat® Analyzer Display	Result Report Printout and Interpretation
Positive Control Valid	**Positive Control Valid** Control is positive for the presence of *C. difficile* DNA.
Positive Control Invalid	**Positive Control Invalid** Result is Invalid. The Positive Control should be re-tested to obtain valid result. Repeat Run.

Table 10: Interpretation of results of cobas® Cdiff when running Negative Control

cobas® Liat® Analyzer Display	Result Report Printout and Interpretation
Negative Control Valid	**Negative Control Valid** Control is negative for the presence of *C. difficile* DNA.
Negative Control Invalid	**Negative Control Invalid** Result is Invalid. The Negative Control should be re-tested to obtain valid result. Repeat Run.

Suggested re-test procedure

Invalid and failed/aborted runs can be repeated once using the same secondary sample. If the repeat run is still invalid, a new secondary sample may be prepared from the primary stool specimen. Alternatively, obtain a new primary specimen, if feasible, to conduct **cobas**® Cdiff again.

Procedural limitations

1. **cobas**® Cdiff has only been validated for use with unformed or partially formed stool specimens that have been transferred into the **cobas**® PCR Media tube according to this Instructions-For-Use (also referred to as a Package Insert) document.
2. Reliable results are dependent on adequate specimen collection, transport, storage, and processing. Follow the procedures in this Instructions-For-Use document for **cobas**® Cdiff and the **cobas**® Liat® System User Guide.
3. Detection of *C. difficile* DNA is dependent on the number of organisms present in the specimen and may be affected by specimen collection/processing methods, history of hospitalization, antibiotic treatment regime, and *C. difficile* strains.
4. False negative or invalid results may occur due to interference from various substances. The Internal Control is included in **cobas**® Cdiff to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification. Known interference includes, but may not be limited to the following:
   - Specimens containing greater than 50% (w/v) mucin may generate false negative results.
5. A positive result is indicative of the presence of *C. difficile* DNA and not necessarily viable organisms. Therefore, this test is not recommended for use in treatment monitoring or as a test of cure.
6. Mutations or polymorphisms in primer- or probe-binding regions may affect detection of new or unknown variants, resulting in a false negative result with **cobas**® Cdiff.
7. The predictive value of an assay depends on the prevalence of the disease in any particular population.
8. Use of this product must be limited to personnel trained to the use of the **cobas**® Liat® System.

Non-clinical performance characteristics

Analytical sensitivity

The analytical sensitivity (Limit of Detection or LOD) for the **cobas**® Cdiff was determined by analyzing quantified *C. difficile* cultures diluted to multiple concentration levels in negative stool background suspension in **cobas**® PCR Media. All levels were tested in three replicates each using two unique lots of **cobas**® Cdiff assay tubes. The lowest level with 100% hit rate was tested with additional replicates to confirm the LOD level. If the overall hit rate for that level was less than 95%, the panel level above was tested with additional replicates. The final LOD level was confirmed with at least 21 additional replicates. LOD for this test is defined as the target concentration which can be detected as positive in ≥ 95% of the replicates tested, based on results generated by the worst performing reagent lot.

The results of the analytical sensitivity study are shown in Table 11.

Table 11: cobas® Cdiff Assay LOD (Limit of Detection)

Strain ID	Toxinotype	REA* Type	PFG† Type	Ribotype	Phenotype	LOD (CFU/swab)
ATCC 43255 (VPI 10463)	0	N/A	N/A	87	A+B+CDT-	90
R12087 (CD196)	III	BI	NAP1	27	A+B+CDT+	45

*Restriction endonuclease analysis; †Pulse Field Gel

Detection of *C. difficile* genotypes

The limit of detection of **cobas**® Cdiff on 37 toxigenic strains representing additional toxinotypes was verified by testing three replicates per strain at three times the LOD level (270 CFU/swab) of ATCC 43255. Dilutions and testing samples were prepared in a similar fashion as in the Limit of Detection (LOD) study described above.

All 37 toxigenic strains (Table 12) were detected as 100% positive in this study, confirming that the **cobas**® Cdiff can detect these *C. difficile* toxinotypes.

Table 12: Summary of toxigenic *C. difficile* verification results

	Cdiff Strain	Toxinotype	Ribotype	Hit Rate
1	ATCC# BAA-1382; 630	0	12	100.00%
2	EX 623	I	102	100.00%
3	AC 008	II	103	100.00%
4	2004118; CDC-204118 (NAP-1)	III	27	100.00%
5	SE 844	IIIa	80	100.00%
6	CH6230	IIIc	N/A	100.00%
7	P43	IV	N/A	100.00%
8	55767	IV	23	100.00%
9	2748-06	V	78	100.00%
10	SE 881	V	45	100.00%
11	SE 1203	VI	33	100.00%
12	57267	VII	63	100.00%
13	ATCC# 43598; 1470	VIII	17	100.00%
14	51680	IX	19	100.00%
15	CCUG 8864/STCC20309	X	36	100.00%
16	F15	XII	N/A	100.00%
17	IS 25	XII	56	100.00%
18	R 9367	XIII	70	100.00%
19	R 10870	XIV (new-XIVa)	111	100.00%
20	R 9385	XV (new XIVb)	122	100.00%
21	SUC36	XVI	78	100.00%
22	No 1313	XVII	232	100.00%
23	K095	XVIII	14	100.00%
24	TR13	XIX	N/A	100.00%
25	TR14	XX	N/A	100.00%
26	CH6223	XXI	N/A	100.00%
27	CD07-468	XXII	N/A	100.00%
28	8785	XXIII (New-IXc)	N/A	100.00%
29	597B	XXIV	131	100.00%
30	7325	XXV	27	100.00%
31	7459	XXVI	N/A	100.00%
32	KK2443/2006	XXVII	N/A	100.00%
33	CD08-070	XXVIII	126	100.00%
34	CD07-140	XXIX	56	100.00%
35	ES 130	XXX	N/A	100.00%
36	WA 151	XXXI	N/A	100.00%
37	173070	XXXII	N/A	100.00%

Analytical specificity

To assess the analytical specificity of **cobas**®Cdiff, the following organism panels were tested:

1) 118 bacteria, fungi and viruses that may be found in stool specimens, and one type of human cell (Table 13)

2) 32 *Clostridium* genus organisms, including non-toxigenic *C. difficile* (Table 14)

Clostridium botulinum analytical specificity was predicted using BLAST program against GenBank nucleotide sequence database to mimic PCR amplicon generation step.

All bacteria and human cells were spiked to 1 x 10^6 Units*/mL, and all viruses were spiked to 1 x 10^5 Units*/mL equivalent in stool matrix. Testing was performed with the organisms alone or with two toxigenic *C. difficile* isolates present individually at 3x Limit of Detection (LOD) of **cobas**® Cdiff. Results indicated that none of these organisms interfered with detection of intended Cdiff targets. None produced false positive results when there was no intended *C. difficile* target present.

*Bacteria were quantified in colony forming units (CFU)/mL, human cells were quantified in cells/mL, and viruses were quantified in $TCID_{50}$/mL, except for *Chlamydia trachomatis*, which was quantified in IFU/mL.

Table 13: Microorganisms and human cells tested

Abiotrophia defectiva	*Acinetobacter baumannii*	*Acinetobacter lwoffii*
Aeromonas hydrophila	*Alcaligenes faecalis* ATCC 35655	*Alcaligenes faecalis* subsp. *faecalis* ATCC 15554
Alcaligenes faecalis subsp. *faecalis* ATCC 8750	*Anaerococcus tetradius*	*Bacillus cereus* ATCC 11778
Bacillus cereus ATCC 13472	*Bacteroides caccae*	Bacteroides fragilis
Bacteroides merdae	*Bacteroides stercoris*	*Bifidobacterium adolescentis*
Bifidobacterium longum	*Campylobacter coli ATCC 33559*	*Campylobacter jejuni ATCC 43479*
Campylobacter jejuni Subsp. jejuni ATCC 33292	*Candida albicans*	*Candida catenulata*
Cedecea davisae	*Chlamydia Trachomatis Serovar L2 LGVII454*	*Citrobacter amalonaticus*
Citrobacter freundii	*Citrobacter koseri*	*Citrobacter sedlakii*
Collinsella aerofaciens	*Corynebacterium genitalium*	*Desulfovibrio piger*
Edwardsiella tarda	*Eggerthella lenta*	*Enterobacter aerogenes*
Enterobacter cloacae	*Enterococcus casseliflavus*	*Enterococcus cecorum*
Enterococcus dispar	*Enterococcus faecium van A*	*Enterococcus faecalis Van B*
Enterococcus gallinarum van C	*Enterococcus hirae*	*Enterococcus raffinosus*
Escherichia coli ATCC 11775	*Escherichia coli* ATCC 25922	*Escherichia coli O157:H7* ATCC 700927
Escherichia fergusonii	*Escherichia hermannii*	*Fusobacterium varium*
Gardnerella vaginalis	*Gemella morbillorum*	*Hafnia alvei*
HCT-15 Human Cells	*Helicobacter fennelliae*	*Helicobacter pylori*
Klebsiella oxytoca	*Klebsiella pneumoniae subsp. pneumoniae*	*Lactobacillus acidophilus*
Lactobacillus reuteri	*Lactococcus lactis*	*Leminorella grimontii*
Listeria grayi	*Listeria innocua*	*Listeria monocytogenes* ATCC 15313
Listeria monocytogenes ATCC BAA-839	*Mitsuokella multacida*	*Mobiluncus curtisii*
Moellerella wisconsensis	*Morganella morganii*	*Neisseria gonorrhoeae*
Peptoniphilus asaccharolyticus	*Peptostreptococcus anaerobius*	*Plesiomonas shigelloides*
Porphyromonas asaccharolytica	*Prevotella melaninogenica*	*Proteus mirabilis* ATCC 25933
Proteus mirabilis ATCC 29906	*Proteus penneri*	*Providencia alcalifaciens*
Providencia rettgeri	*Providencia stuartii*	*Pseudomonas aeruginosa* ATCC 35554
Pseudomonas aeruginosa ATCC 33584	*Pseudomonas putida*	*Ruminococcus bromii*
Salmonella enterica serovar Choleraesuis ATCC 7001	*Salmonella enterica subsp. Arizonae* ATCC 13314 *(f.k.a. Salmonella choleraesuis subsp. arizonae)*	*Salmonella enterica subsp. enterica* CMCC 1975
Salmonella enterica subsp. enterica serovar Typhi ATCC 19430	*Salmonella enterica subsp.enterica serovar Typhimurium* ATCC 14028	*Serratia liquefaciens* CMCC 169
Serratia liquefaciens ATCC 27592	*Serratia marcescens* ATCC 13880	*Serratia marcescens* ATCC 8100
Shigella boydii	*Shigella dysenteriae*	*Shigella sonnei*
Staphylococcus aureus	*Staphylococcus epidermidis*	*Stenotrophomonas maltophilia*
Streptococcus agalactiae	*Streptococcus dysgalactiae*	*Streptococcus intermedius*
Streptococcus sp.strain V8 ATCC 12973	*Streptococcus uberis*	*Trabulsiella guamensis*
Veillonella parvula	*Vibrio cholerae*	*Vibrio parahaemolyticus*
Yersinia bercovieri	*Yersinia rohdei*	*Cytomegalovirus* (HHV5)
Human Adenovirus Type 41	*Human Coxsackievirus* A4	*Human Coxsackievirus B4*
Human Echovirus 11	*Human Enterovirus* 71	*Human Rotavirus*
Norovirus GII	-	-

Table 14: *Clostridium* genus organisms, including non-toxigenic *C. difficile*

Clostridium beijerinckii	*Clostridium bifermentans*	*Clostridium bolteae*
*Clostridium botulinum**	*Clostridium butyricum*	*Clostridium chauvoei*
Clostridioides difficile Serogroup B (non-toxigenic)	*Clostridioides difficile Serogroup I (non-toxigenic)*	*Clostridioides difficile (ES 1103) (non-toxigenic Type XIa)***
*Clostridioides difficile (6035/06)(non-toxigenic Type XIa)***	*Clostridioides difficile (F14) (non-toxigenic Type XIb)***	*Clostridium fallax*
Clostridium haemolyticum	*Clostridium histolyticum*	*Clostridium innocuum*
Clostridium methylpentosum	*Clostridium nexile*	*Clostridium novyi*
Clostridium orbiscindens (renamed Flavonifractor plautii)	*Clostridium paraputrificum*	*Clostridium perfringens*
Clostridium ramosum	*Clostridium scindens*	*Clostridium septicum*
Clostridium sordellii	*Clostridium sphenoides*	*Clostridium spiroforme*
Clostridium sporogenes ATCC 15579	*Clostridium sporogenes* CCRI 11128	*Clostridium symbiosum*
Clostridium tertium	*Clostridium tetani*	-

* Based on BLAST program analysis.

Three non-toxigenic Cdiff strains (toxinotype XI) tested during inclusivity study were not detected by the **cobas® Cdiff test are included in this table.

Interference

Thirty eight commonly used medications, as well as fecal fat, whole blood, and mucin, were tested for potential interference effects with **cobas**®Cdiff. All substances were tested at levels above what could be reasonably expected to be collected by a swab in a stool specimen. The amount of interference substance is expressed as concentration in the primary stool specimen. Two *C. difficile* isolates were spiked to 3x Limit of Detection (LOD) of **cobas**® Cdiff and used as targets in the tests. No interference was observed for exogenous substances. For fecal fat, no interference was observed up to 39% (w/v), for whole blood, no interference was observed up to 100% (v/v), and for mucin, no interference was observed up to 50% (w/v). These results are summarized in Table 15. Exogenous substances concentrations higher than listed in Table 15 may generate false negative or invalid results.

Table 15: Results from interference substances testing

Substance	Primary Stool Specimen Concentration
Fecal Fat	0.22% - 39% (w/v)
Whole blood	100% (v/v)
Mucin	50% (w/v)
Aleve	100% (w/v)
Mylanta	100% (w/v)
Anusol	100% (w/v)
Dulcolax	23% (w/v)*
Equate Laxative	50% (w/v)*
Equate Hydrocortisone	100% (w/v)
E-Z-HD Barium Sulfate	100% (w/v)
Fleet	100% (w/v)
Glycerin Suppositories	100% (w/v)
Gravol Suppositories	100% (w/v)
Gynol II Contraceptive	10% (w/v)*
Imodium	100% (w/v)
Kaopectate	100% (w/v)
K-Y Jelly	100% (w/v)
Metronidazole	100% (w/v)
Miconazole	100% (w/v)
Mineral Oil	100% (w/v)
Monistat Cream	100% (w/v)
Monistat Complete Care	100% (w/v)
Nystatin Ointment	100% (w/v)
Palmitic Acid	100% (w/v)
Pedia Lax	100% (w/v)
Pepto Bismol	25% (w/v)*
Witch Hazel	50% (w/v)*
Preparation H Hemorrhoidal Cream	100% (w/v)
Preparation H Hemorrhoidal ointment	100% (w/v)
Dramamine	12.5% (w/v)*
Steric Acid	100% (w/v)
Docusate Sodium	100% (w/v)
Tums	50% (w/v)*
Mesalamine Rectal Suspension	100% (w/v)
Vagisil Anti-itch Cream	12.5% (w/v)*
Vancomycin	100% (w/v)
Vaseline	100% (w/v)
Sun Screen	100% (w/v)
Monistat Vaginal Insert	100% (w/v)
Vaginal Contraceptive Film	100%
Spermicidal Condoms	100%

* These concentrations are higher than what could be reasonably expected from the usage, application, and subsequent carry-over into stool specimens for the corresponding products.

Clinical performance evaluation

Reproducibility

The reproducibility of the **cobas**® Cdiff test was established in a multi-site investigation (2 external sites, 1 internal site) using simulated samples and was evaluated across reagent lot, site, operator and testing day. Reproducibility test panels consisted of 3 panel members: 1 negative specimen and 2 different positive concentrations (~1x LOD, ~3x LOD) of 1 strain of toxigenic *C. difficile*. There were 3 replicates per panel member, and each replicate represented a separate test. For each of 3 reagent lots that were included in the study, panels were tested on 5 nonconsecutive days by 2 different operators at each of the 3 testing sites. The results are summarized in Table 16 and Table 17.

Results

Overall, 818 tests were performed in this study, out of which 798 (97.6%) were valid for the study and included in the final percent agreement analysis. An additional 4 tests (0.5%; 4/818) failed, 12 (1.5%; 12/818) had invalid results and 4 (0.5%; 4/818) were invalidated because of protocol deviations.

Table 16 shows the percent agreement results by panel member concentration. Overall percent agreement was 100% for negative panel members, 98.5% for ~1x LOD panel members, and 99.3% for ~3x LOD panel members.

Table 16: Summary of reproducibility results: percent agreement by panel member

Panel Member		**Negative**	**~1x LOD**	**~3x LOD**
Number of Valid Test Results		262	266	270
Overall Percent Agreement, % (n/N)*				
Overall	**Percent Agreement**	100.0 (262/262)	98.5 (262/266)	99.3 (268/270)
	95% Score CI	(98.6, 100.0)	(96.2, 99.4)	(97.3, 99.8)
Percent Agreement for Component Variables, % (n/N)*				
Reagent Lot	**1**	100.0 (85/85)	100.0 (87/87)	100.0 (90/90)
	2	100.0 (88/88)	100.0 (90/90)	100.0 (90/90)
	3	100.0 (89/89)	95.5 (85/89)	97.8 (88/90)
Site	**1**	100.0 (85/85)	100.0 (88/88)	100.0 (90/90)
	2	100.0 (89/89)	97.8 (87/89)	98.9 (89/90)
	3	100.0 (88/88)	97.8 (87/89)	98.9 (89/90)
Operator	**1**	100.0 (43/43)	100.0 (44/44)	100.0 (45/45)
	2	100.0 (42/42)	100.0 (44/44)	100.0 (45/45)
	3	100.0 (45/45)	97.7 (43/44)	100.0 (45/45)
	4	100.0 (44/44)	97.8 (44/45)	97.8 (44/45)
	5	100.0 (44/44)	100.0 (44/44)	97.8 (44/45)
	6	100.0 (44/44)	95.6 (43/45)	100.0 (45/45)
Testing Day	**1**	100.0 (53/53)	100.0 (54/54)	100.0 (54/54)
	2	100.0 (54/54)	96.3 (52/54)	100.0 (54/54)
	3	100.0 (52/52)	96.0 (48/50)	98.1 (53/54)
	4	100.0 (52/52)	100.0 (54/54)	98.1 (53/54)
	5	100.0 (51/51)	100.0 (54/54)	100.0 (54/54)

Note: CI = confidence interval.

* For the negative panel member: Percent agreement = (number of not detected results/total valid results) x 100. For the positive panel members: Percent agreement = (number of detected results/total valid results) x 100.

Ct values were used to explore the variability that was observed in the reproducibility study for positive panel members. Table 17 presents the standard deviation (SD) and percent coefficient of variation (CV) of Ct values observed both overall and attributed to potential component variables (reagent lot, site, operator, testing day and 'within-run'). 'Within-run' variation refers to the potential variation within a 'study run' that consists of 3 replicate tests performed for a given panel member and that are processed by the same operator on the same analyzer on the same day. For all positive panel members, the overall SD was ≤ 0.60 and the CV was ≤ 1.9% across all components, and the SD was ≤ 0.38 and the CV was ≤ 1.6% for any individual component. There was little overall variability across reagent lot, site, operator, testing day or within-run for both the ~1x LOD and ~3x LOD panel members.

Table 17: Overall mean, Standard Deviation (SD) and Percent Coefficient of Variation (CV) for Ct Values from valid results for positive panel members

			Standard Deviation and Percent Coefficient of Variation											
			Reagent Lot		Site		Operator		Testing Day		Within-Run		Total	
Positive Panel Member	N	Mean Ct	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
~1x LOD	262	31.4	0.13	0.4%	0.26	0.8%	0.00	0.0%	0.13	0.4%	0.51	1.6%	0.60	1.9%
~3x LOD	268	30.0	0.23	0.8%	0.25	0.8%	0.08	0.3%	0.00	0.0%	0.38	1.3%	0.51	1.7%

Note: Ct = Cycle Threshold, SD = Standard Deviation, CV = Percent Coefficient of Variation

Clinical performance

The clinical performance of the **cobas**® Cdiff test was established in a prospective and multi-site investigation of the **cobas**® Cdiff test compared to reference toxigenic culture using unformed stool samples from patients suspected of having *C. difficile* infection. The reference culture was a composite result that combined the results of both direct and broth enrichment culture as outlined in Table 18 below.

Table 18: Derivation of composite reference culture results

Category	Direct Culture	Enrichment Culture	Composite Reference Culture
1	+	+	+
2	+	-	+
3	-	+	+
4	-	-	-

Clinical performance characteristics of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) were calculated by comparing **cobas**® Cdiff test results with composite reference culture. Discrepant analysis using a cleared FDA comparator nucleic acid amplification test (NAAT) was performed on all samples with discordant results between the **cobas**® Cdiff test and composite reference culture. In addition, clinical performance characteristics were determined by comparing **cobas**® Cdiff test results with direct culture alone. The results are summarized in Table 19, Table 20, and Table 21.

Results

In the clinical performance evaluation, 1,013 fresh remnant specimens were prospectively collected across 9 geographically diverse collection sites in the US. Of the 1,013 specimens, 179 were positive by composite reference culture, yielding a prevalence rate of 17.7 % for the study. One hundred forty-seven (14.5%) specimens were positive by direct culture alone. The initial invalid and failed rates of **cobas**® Cdiff testing were 1.4% and 0.2%, respectively. Following 1 retest per invalid or failed result, the final invalid and failed rates were 0.1% and 0%, respectively.

Expected values

The percentage of positive results observed with the **cobas**® Cdiff test in the study population was 17%. Table 19 provides patient demographic characteristics for the clinical performance evaluation. Patients were 47.7% male (n = 483) and 52.3% female (n = 530) with a mean age of 57 years.

Table 19: Demographic characteristics of patients for evaluable specimens (N = 1013)

Characteristic	Statistic	Result
Age (years)	Mean	57
	Range	5-98
Age category (years)		
≤ 5	n (%)	2 (0.2)
6-21	n (%)	42 (4.1)
22-59	n (%)	469 (46.3)
≥ 60	n (%)	500 (49.4)
Sex		
Male	n (%)	483 (47.7)
Female	n (%)	530 (52.3)

Comparison with composite reference culture

In comparison to composite reference culture, the sensitivity of the **cobas**® Cdiff test was 87.2% (156/179), the specificity was 98.1% (818/834). See Table 20 for a detailed performance comparison. Of the 23 specimens with a discordant negative (Not Detected) **cobas**® Cdiff test result relative to the composite reference culture, 19 were negative, 3 were positive, and 1 was invalid by a second FDA-cleared NAAT method. Of the 16 specimens with discordant positive (Detected) **cobas**® Cdiff test results relative to the composite reference culture, 14 were positive and 2 were negative by a second FDA-cleared NAAT method.

Table 20: Comparison of cobas® Cdiff with composite reference culture results

cobas® Cdiff	Composite Reference Culture		Total
	Positive	Negative	
Detected	156	16[a]	172
Not Detected	23[b]	818	841
Total	179	834	1013
Sensitivity	100% x 156/179 = 87.2% (81.5%, 91.3%)	-	-
Specificity	100% x 818/834 = 98.1% (96.9%, 98.8%)	-	-
PPV (95% CI)	100% x 156/172 = 90.7% (85.4%, 94.2%)	-	-
NPV (95% CI)	100% x 818/841 = 97.3% (95.9%, 98.2%)	-	-

Note: CI = (score) confidence interval, PPV = positive predictive value, NPV = negative predictive value.
Discrepant analysis was performed on all 39 specimens with discordant results between the **cobas**® Cdiff test and the composite reference culture, using a cleared FDA comparator NAAT.

[a] Of the 16 specimens with false positive **cobas**® Cdiff test results relative to composite reference culture, 14 were positive, and 2 were negative by that second NAAT method.

[b] Of the 23 specimens with false negative **cobas**® Cdiff test results relative to composite reference culture, 19 were negative, 3 were positive , and 1 had an invalid test result by that second NAAT method.

Comparison with direct culture

In comparison to direct toxigenic culture alone, the Positive Percent Agreement of the **cobas**® Cdiff test was 94.6% (139/147%), the Negative Percent Agreement was 96.2% (833/866). See Table 21 for a detailed performance comparison.

Table 21: Comparison of cobas® Cdiff with direct culture

cobas® Cdiff	Direct Culture		Total
	Positive	Negative	
Detected	139	33[a]	172
Not Detected	8[b]	833	841
Total	147	866	1013
Positive Percent Agreement	100% x 139/147 = 94.6% (89.6%, 97.2%)	-	-
Negative Percent Agreement	100% x 833/866 = 96.2% (94.7%, 97.3%)	-	-

Note: CI = (score) confidence interval

Discrepant analysis, using a cleared FDA comparator NAAT, was performed on all 8 specimens with false negative **cobas**® Cdiff test results and 17 of the 33 specimens with false positive **cobas**® Cdiff test results relative to direct culture alone.

[a] Discrepant testing was performed on 17 of the 33 specimens with false positive **cobas**® Cdiff test results relative to direct culture alone, out of which 14 were positive and 3 were negative by the second NAAT method.

[b] Discrepant testing was performed on all 8 specimens with false negative **cobas**® Cdiff test results relative to direct culture alone, out of which 7 were negative and 1 was positive by the second NAAT method.

Failure codes

The failure codes described in Table 22 can be displayed on the result report based on interpretation and calculation process of the test result.

Table 22: Failure codes and definitions

Failure Code	Sample	Negative Control (Add Lot)	Positive Control (Add Lot)
r0	IC Negative or Invalid. Repeat Run	IC Negative or Invalid. Repeat Run	IC Negative or Invalid. Repeat Run
r1			
r3*			
r4			
x4**	Cdiff Positive while IC Negative or Invalid. Repeat Run	N/A	Cdiff and/or IC Negative or Invalid. Repeat Run
FP	N/A	Cdiff Positive or Invalid. Repeat Run	N/A
g0	N/A	N/A	Cdiff Negative or Invalid. Repeat Run
g1			
g3			
g4			
x5	Sample volume too low	Sample volume too low	Sample volume too low

Note*: Failure code r3 does not appear for Positive and Negative controls.

Note**: Failure code x4 does not appear for Positive Control (Add Lot). For positive control, the x4 failure code can only be triggered when the failure happens during additional positive control runs after “Add Lot” procedure (Refer to “Performing additional control runs”).

For additional Failure codes information, consult the current **cobas**® Liat® System User Guide.

Additional information

Key test features

Sample type	Unformed stool specimens
Amount of sample required	4.3 mL of **cobas**® PCR Media is provided with each **cobas**® PCR Media Uni Swab Sample Kit, a minimum of 0.2 mL is required for a **cobas**® Cdiff.
Test duration	Results are available within ~20 minutes after loading the specimen on the system
Analytical sensitivity	From 45 to 90 CFU/swab depending on isolate.
Specificity	No cross-reactivity with 147 closely related organisms or organisms typically found in stool specimens.
Inclusivity	All known *C. difficile* (Toxinotypes 0 ~ XXXI, except non-Toxigenic Toxinotypes XI) including the BI/ NAP1/027 hyper-virulent epidemic strain

Symbols

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

Table 23: Symbols used in labeling for Roche PCR diagnostic products

Age/DOB Age or Date of Birth	Device not for near-patient testing	QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
SW Ancillary Software	Device not for self-testing	
Assigned Range [copies/mL] Assigned Range (copies/mL)	Distributor *(Note: The applicable country/region may be designated beneath the symbol)*	SN Serial number
Assigned Range [IU/mL] Assigned Range (IU/mL)	Do not re-use	Site Site
EC REP Authorized representative in the European Community	Female	Procedure Standard Standard Procedure
BARCODE Barcode Data Sheet	For IVD performance evaluation only	STERILE EO Sterilized using ethylene oxide
LOT Batch code	GTIN Global Trade Item Number	Store in dark
Biological risks	Importer	Temperature limit
REF Catalogue number	IVD In vitro diagnostic medical device	TDF Test Definition File
CE CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	LLR Lower Limit of Assigned Range	This way up
	Male	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	Manufacturer	UDI Unique Device Identifier
Consult instructions for use	CONTROL − Negative control	ULR Upper Limit of Assigned Range
Σ Contains sufficient for <*n*> tests	NON STERILE Non-sterile	Urine Fill Line Urine Fill Line
CONTENT Content of kit	Patient Name	Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	Patient number	Use-by date
Date of manufacture	Peel here	
Device for near-patient testing	CONTROL + Positive control	
Device for self-testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	

Technical support

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

Manufacturer and distributor

Table 24: Manufacturer and distributor

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

Made in USA

Distributed by

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800-800-5973)

Trademarks and patents

See http://www.roche-diagnostics.us/patents.

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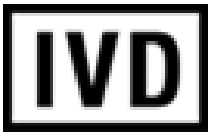

References

1. Bartlett JG, Chang TW, Moon N, Onderdonk AB. Antibiotic-induced lethal enterocolitis in hamsters: studies with eleven agents and evidence to support the pathogenic role of toxin-producing Clostridia. Am J Vet Res. 1978;39(9):1525-1530.
2. Larson HE, Price AB, Honour P, Borrielo SP. Clostridium difficile and the aetiology of pseudomembranous colitis. Lancet. 1978;1(8073):1063-1066.
3. Leffler D.A., Lamont J.T. Clostridium difficile Infection. N Engl J Med 2015; 372:1539-1548.
4. Bartlett J. G. Clinical practice. Antibiotic-associated diarrhea. N Engl J Med. 2002;346(5):334-9.
5. Vindigni S. M, Surawicz C. M. C. difficile Infection: Changing Epidemiology and Management Paradigms. Clinical and Translational Gastroenterology. 2015; 6, e99; doi:10.1038/ctg.2015.24.
6. Hensgens M. P., Keessen E. C., Squire M. M., Riley T. V., Koene M. G., de Boer E. Clostridium difficile infection in the community: a zoonotic disease? Clin Microbiol Infect. 2012;18(7):635-45.
7. Kelly C. P., LaMont J. T. Clostridium difficile--more difficult than ever. N Engl J Med. 2008;359(18):1932-40.
8. Wolfhagen M. J., Torensma R., Fluit A. C., J Verhoef. Toxins A and B of Clostridium difficile. FEMS Microbiol Rev. 1994;13(1):59-64.
9. Johnson S., Sambol S. P., Brazier J. S., Delmee M., Avesani V., Merrigan M. International typing study of toxin A-negative, toxin B-positive Clostridium difficile variants. J Clin Microbiol. 2003;41(4):1543-7.
10. Brecher S. M., Novak-Weekley S. M., E Nagy. Laboratory diagnosis of Clostridium difficile infections: there is light at the end of the colon. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2013;57(8):1175-81.
11. Surawicz C. M., Brandt L. J., Binion D. G., Ananthakrishnan A. N., Curry S. R., H Gilligan P. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. The American journal of gastroenterology. 2013;108(4):478-98; quiz 99.
12. Curry SR. Clostridium difficile. Clinics in Laboratory Medicine. June 2017; 37(2):341-6.
13. Sloan L. M., Duresko B. J., Gustafson D. R., Rosenblatt J. E. Comparison of real-time PCR for detection of the tcdC gene with four toxin immunoassays and culture in diagnosis of Clostridium difficile infection. J Clin Microbiol. 2008;46(6):1996-2001.
14. Deshpande A., Pasupuleti V., Rolston D. D., Jain A., Deshpande N., Pant C. Diagnostic accuracy of real-time polymerase chain reaction in detection of Clostridium difficile in the stool samples of patients with suspected Clostridium difficile Infection: a meta-analysis. Clin Infect Dis. 2011;53(7):e81-90.
15. Kufelnicka A. M., J Kirn T. Effective utilization of evolving methods for the laboratory diagnosis of Clostridium difficile infection. Clin Infect Dis. 2011;52(12):1451-7.
16. Tenover F. C., Baron E. J., Peterson L. R., H Persing D. Laboratory diagnosis of Clostridium difficile infection can molecular amplification methods move us out of uncertainty? J Mol Diagn. 2011;13(6):573-82.
17. Peterson L. R., Mehta M. S., Patel P. A., Hacek D. M., Harazin M., Nagwekar P. Laboratory testing for Clostridium difficile infection: light at the end of the tunnel. Am J Clin Pathol. 2011;136(3):372-80.

18. Monaghan T., Boswell T., Mahida Y. Recent advances in Clostridium difficile-associated disease. Gut. 2008;57(6):850-60.

19. Hardy K, Price C, Szczepura A. Reduction in the rate of methicillin-resistant Staphylococcus aureus acquisition in surgical wards by rapid screening for colonization: a prospective, cross-over study. Clin Microbiol Infect. 2010;16(4):333-339. Epub 2009/07/23.

20. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health. HHS Publication No. (CDC) 21-1112. Biosafety in Microbiological and Biomedical Laboratories. 5th edition. Revised December 2009.

21. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. CLSI document M29-A4 Villanova, PA. Approved guideline-fourth edition. 2014.

Document revision

Document Revision Information	
Doc Rev. 2.0 10/2021	Updated polydocanol concentration in lysis buffer. Updated to support Software 3.3 workflow. Corrected typos and format errors. Updated **Table 8**. Updated the term *Clostridium difficile* to *Clostridioides difficile*. Renamed "**cobas**® Liat® System Operator's Manual" to "**cobas**® Liat® System User Guide". Updated **Interference** section. Updated hazard information, the harmonized symbol page, and distributors addresses. Added Technical support section and Made in statement. Please contact your local Roche Representative if you have any questions.