Rx Only

cobas® Cdiff Test

for use on the cobas® 4800 System

For in vitro diagnostic use

IVD

cobas® 4800 System Sample Preparation Kit	240 Tests 960 Tests	P/N: 05235782190 P/N: 05235804190
cobas® 4800 System Lysis Kit 1	240 Tests 960 Tests	P/N: 06768253190 P/N: 06768270190
cobas® 4800 System Wash Buffer Kit	240 Tests 960 Tests	P/N: 05235863190 P/N: 05235871190
cobas® 4800 System Internal Control Kit 1	20 Runs	P/N: 06768318190
cobas® 4800 Cdiff Amplification/Detection Kit	80 Tests 240 Tests	P/N: 06768237190 P/N: 06768261190
cobas® 4800 Cdiff Controls and Cofactor Kit	10 Runs	P/N: 06768300190

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Intended use

The **cobas**® Cdiff Test on the **cobas**® 4800 system is an automated, qualitative in vitro diagnostic test, that utilizes real-time polymerase chain reaction (PCR), for the direct detection of the toxin B (*tcd*B) gene of toxigenic *Clostridium difficile (C. difficile*) in unformed (liquid or soft) stool specimens obtained from patients suspected of having *C. difficile* infection (CDI). The **cobas**® Cdiff Test 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 / principles of the procedure

Background: screening of *C. difficile*

C. difficile is a Gram-positive, anaerobic, spore-forming bacillus that was identified as an etiologic agent of antibiotic-associated pseudomembranous colitis in the late 1970s.[1,2] It is believed to be responsible for 15-20% of antibiotic-related cases of diarrhea and nearly all cases of antibiotic-associated pseudomembranous colitis.[3] Over the last decade, the incidence of *C. difficile*-associated infection (CDI) has progressively increased and is now a significant clinical problem in developed countries. Whereas incidence rates ranged from 30-40 cases per 100,000 population in acute care hospitals in the United States, the incidence rose to more than 80 per 100,000 in 2005.[4] Outbreaks of CDI have been previously described.[5] The direct cost associated with CDI was $6,326 per case in the US.[6]

Increases in incidence have in part been attributed to the emergence of a purportedly hypervirulent strain, classified as ribotype 027/North American pulsotype 1 (NAP1) and toxinotype III. Toxigenic strains of *C. difficile* typically produce two toxins: toxin A (an enterotoxin) and toxin B (a cytotoxin).[7] A small percentage of strains only produce toxin B.[8]

Increased virulence has recently been described in strains that produce another toxin, termed binary toxin, and carry a deletion in the negative regulator gene *tcd*C.[9,10] The latter strains were reported to be more virulent in vitro and appear to cause more morbidity and mortality in humans.[11,12]

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.

Diagnosis of CDI is usually established by demonstration of the presence of toxins A and/or B in stool samples. Demonstration of the cytopathic effect on a monolayer of cells, by the action of toxin B, is considered by many to be the "gold standard."[13, 14] Demonstration of the cytopathic effect can be achieved by direct incubation of stool supernatant on the monolayer of cells; alternatively, *C. difficile* isolates can be grown in selective broth and the supernatant obtained for subsequent incubation on the cell monolayer (toxigenic culture).[15] Both techniques require a minimum of 48 to 72 hours for a final result. Immunoassays for the detection of toxins A and B are widely used because they provide positive results in less than 4 hours; however, sensitivities are significantly lower compared to tissue culture.[16] Compared with clinical criteria supporting CDI, PCR was reported to have a sensitivity, specificity, and positive and negative predictive values of 93.3%, 97.4%, 75.5%, and 99.4%, respectively, with a turnaround time of < 4 hours.[15] PCR is considered the optimum rapid single test for detection of *C. difficile* toxin.[17-20] Despite the dramatic

increase in incidence and severity of CDI, metronidazole or vancomycin remain the medical treatment of choice for acute episodes and recurrent infection.[21]

Infection control measures include the prudent use of antimicrobials, prevention of cross-infection, and active surveillance of cases.[22] Repeat "test of cure" testing is not advised since toxins may be present for prolonged times without clinical symptoms.

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.[17-20]

Explanation of the test

The **cobas**® Cdiff Test contains two major processes: (1) automated sample preparation to extract nucleic acids from the unformed stool specimens; (2) PCR amplification of target DNA sequences using *C. difficile* specific primers, and real-time detection of cleaved fluorescent-labeled *C. difficile* specific oligonucleotide detection probes. An Internal Control, containing unrelated randomized DNA sequence, is added to all samples prior to automated sample preparation and is amplified and detected simultaneously with each sample to monitor the entire process.

Principles of the procedure

Sample preparation

Sample preparation for the **cobas**® Cdiff Test is automated with the use of the **cobas x** 480 instrument. Organisms are lysed with chaotropic agent, proteinase K, and SDS reagents. Released nucleic acids, along with added Internal Control DNA, are bound by magnetic glass particles. They are washed and then eluted into a small volume of buffer. The instrument then takes an aliquot of the eluted material and sets up the PCR reaction with an activated Master Mix.

PCR amplification and TaqMan® detection

The PCR cycling steps and detection of target signal occurs in the **cobas z** 480 analyzer. The Master Mix reagent contains primer pairs and probes for two targets: toxin B and 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 dye and a quencher. Normally, the quencher suppresses the fluorescence of the dye. However, if the PCR product is present, the probe hybridizes to the product and gets cleaved by the 5' to 3' nuclease activity of the polymerase. This reaction allows the fluorescence to be emitted from the dye, and the signal is recorded in real time during each PCR cycle by the **cobas z** 480 analyzer. The signal is interpreted by the **cobas**® 4800 System Software and reported as final results.

Selective amplification

Selective amplification of target nucleic acid from the specimen is achieved in the **cobas**® Cdiff Test 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,[23] 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 55ºC, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon. The **cobas**® Cdiff Test has been demonstrated to inactivate at least 1000 copies of deoxyuridine-containing *C. difficile* amplicon per PCR.

Materials and reagents

Materials and reagents provided

Kit/Cassettes	**Components and Reagent Ingredients**	**Quantity per Test**	**Safety Symbol and Warning***
cobas® 4800 System Sample Preparation Kit 240 Tests (P/N: 05235782190)	**MGP** (**cobas**® 4800 System Magnetic Glass Particles) Magnetic Glass Particles 93% Isopropanol	10 x 4.5 mL	 Danger H225 Highly flammable liquid and vapour. H319 Causes serious eye irritation. H336 May cause drowsiness or dizziness. P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233 Keep container tightly closed. P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P280 Wear protective gloves/ eye protection/ face protection. P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. P370 + P378 In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.
	EB (**cobas**® 4800 System Elution Buffer) Tris buffer 0.09% Sodium azide	10 x 18 mL	N/A

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 System Sample Preparation Kit 960 Tests (P/N: 05235804190)	**MGP** (**cobas**® 4800 System Magnetic Glass Particles) Magnetic Glass Particles 93% Isopropanol	10 x 13.5 mL	 Danger H225 Highly flammable liquid and vapour. H319 Causes serious eye irritation. H336 May cause drowsiness or dizziness. P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233 Keep container tightly closed. P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P280 Wear protective gloves/ eye protection/ face protection. P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. P370 + P378 In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.
	EB (**cobas**® 4800 System Elution Buffer) Tris buffer 0.09% Sodium azide	10 x 18 mL	N/A

cobas® 4800 System Lysis Kit 1 240 Tests (P/N: 06768253190)	**LYS-1** (**cobas**® 4800 System Lysis Buffer-1) Sodium citrate 5% Polydocanol 42.6% Guanidinium thiocyanate Dithiothreitol	10 x 10 mL	 Danger H302 + H332 Harmful if swallowed or if inhaled. H317 May cause an allergic skin reaction. H318 Causes serious eye damage. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled. H412 Harmful to aquatic life with long lasting effects. EUH032 Contact with acids liberates very toxic gas. P261 Avoid breathing dust/fume/gas/ mist/vapours/spray. P280 Wear protective gloves/eye protection/face protection. P284 Wear respiratory protection. P304 + P340 + P312 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell. 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. P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
	PK (**cobas**® 4800 System Proteinase K) Tris buffer EDTA Calcium chloride Calcium acetate < 2.0% Proteinase K Glycerine	10 x 0.9 mL	
	SDS (**cobas**® 4800 System SDS Reagent) Tris-HCl buffer Sodium dodecyl sulfate 0.09% Sodium azide	10 x 3 mL	

cobas® 4800 System Lysis Kit 1 960 Tests (P/N: 06768270190)	**LYS-1** (**cobas**® 4800 System Lysis Buffer-1) Sodium citrate 5% Polydocanol 42.6% Guanidinium thiocyanate Dithiothreitol	10 x 36 mL	 Danger H302 + H332 Harmful if swallowed or if inhaled. H317 May cause an allergic skin reaction. H318 Causes serious eye damage. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled. H412 Harmful to aquatic life with long lasting effects. EUH032 Contact with acids liberates very toxic gas. P261 Avoid breathing dust/fume/gas/ mist/vapours/spray. P280 Wear protective gloves/eye protection/face protection. P284 Wear respiratory protection. P304 + P340 + P312 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell. 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. P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
	PK (**cobas**® 4800 System Proteinase K) Tris buffer EDTA Calcium chloride Calcium acetate < 2.0% Proteinase K Glycerine	20 x 1.2 mL	
	SDS (**cobas**® 4800 System SDS Reagent) Tris-HCl buffer Sodium dodecyl sulfate 0.09% Sodium azide	10 x 9 mL	

cobas® 4800 System Wash Buffer Kit 240 Tests (P/N: 05235863190)	**WB** (**cobas**® 4800 System Wash Buffer) Sodium citrate dihydrate 0.05% N-Methylisothiazolone HCl	10 x 55 mL	N/A
cobas® 4800 System Wash Buffer Kit 960 Tests (P/N: 05235871190)	**WB** (**cobas**® 4800 System Wash Buffer) Sodium citrate dihydrate 0.05% N-Methylisothiazolone HCl	10 x 200 mL	N/A
cobas® 4800 System Internal Control Kit 1 20 Runs (P/N: 06768318190)	**IC-1** (**cobas**® 4800 IC-1) Tris buffer EDTA < 0.01% Poly rA RNA (synthetic) 0.05% Sodium azide < 0.01% Non-infectious, synthetic internal control DNA encapsulated in Lambda bacteriophage coat protein	20 x 0.5 mL	N/A
cobas® 4800 Cdiff Amplification/Detection Kit 80 Tests (P/N: 06768237190)	**Cdiff MMX** (**cobas**® Cdiff Master Mix) Tricine buffer EDTA DMSO Potassium acetate Potassium hydroxide Tween 20 < 0.19% dATP, dCTP, dGTP, dUTP < 0.01% Upstream and downstream *C. difficile* and Internal Control primers < 0.01% Fluorescent-labeled *C. difficile* and Internal Control probes < 0.01% Oligonucleotide aptamer < 0.01% Z05 DNA polymerase (microbial) < 0.02% AmpErase (uracil-N-glycosylase) enzyme (microbial) 0.09% Sodium azide	10 x 0.3 mL	N/A

cobas® 4800 Cdiff Amplification/Detection Kit 240 Tests (P/N: 06768261190)	**Cdiff MMX** (**cobas**® Cdiff Master Mix) Tricine buffer EDTA DMSO Potassium acetate Potassium hydroxide Tween 20 < 0.19% dATP, dCTP, dGTP, dUTP < 0.01% Upstream and downstream *C. difficile* and Internal Control primers < 0.01% Fluorescent-labeled *C. difficile* and Internal Control probes < 0.01% Oligonucleotide aptamer < 0.01% Z05 DNA polymerase (microbial) < 0.02% AmpErase (uracil-N-glycosylase) enzyme (microbial) 0.09% Sodium azide	10 x 0.7 mL	N/A
cobas® 4800 Cdiff Controls and Cofactor Kit 10 Runs (P/N: 06768300190)	**Cdiff (+) C** (**cobas**® 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	10 x 0.5 mL	N/A
	(–) C (**cobas**® 4800 System Negative Control) Tris buffer EDTA 0.05% Sodium azide < 0.01% Poly rA RNA (synthetic)	10 x 0.5 mL	N/A
	Cofactor-3 (**cobas**® 4800 Cofactor-3) Manganese acetate Magnesium acetate Bovine serum albumin from bovine plasma sourced in the United States 0.09% Sodium azide	10 x 1.7 mL	N/A

* Product safety labeling primarily follows EU GHS guidance

Reagent storage and handling

Reagent	Storage Temperature	Storage Time
cobas® 4800 System Sample Preparation Kit	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Lysis Kit 1	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Internal Control Kit 1	2–8°C	Stable until the expiration date indicated
cobas® 4800 Cdiff Amplification/Detection Kit	2–8°C	Stable until the expiration date indicated
cobas® 4800 Cdiff Controls and Cofactor Kit	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Wash Buffer Kit	15–25°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 but not provided

Materials	P/N
CORE Tips, 1000 µL, rack of 96	04639642001
50 mL Reagent Reservoir	05232732001
200 mL Reagent Reservoir	05232759001
cobas® 4800 System Extraction (deep well) Plate	05232716001
cobas® 4800 System AD (microwell) Plate 0.3 mL and Sealing Film	05232724001
Sealing foil applicator	04900383001
24-position carrier	04639502001
Solid waste bag	05530873001 (small) or 04691989001 (large)
Hamilton STAR Plastic Chute	04639669001
cobas® PCR Media and Swab Sample Kit	07051891190
cobas® PCR Media Uni Swab Sample Kit	07958030190
Disposable gloves, powderless	Any powderless disposable gloves are acceptable.
Vortex Mixer (single tube)	Any vortex mixer is acceptable.
Centrifuge equipped with a swinging bucket rotor with minimum RCF of 1500	Any appropriate centrifuge is acceptable.

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

Optional materials

Materials	P/N
Sealing mat or deep well plate cover	Roche 04789288001 or Hamilton 6474-01
Caps, neutral color (for recapping post-run specimens)	Roche P/N 07958056190; for recapping post-run specimens in 13 mL Round Base tubes.

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

Instrumentation and software required but not provided

Required Instrumentation and Software, Not Provided
cobas® 4800 System **cobas x** 480 instrument **cobas z** 480 analyzer Control Unit
cobas® 4800 System **cobas**® Cdiff AP Software Version 1.0.1 or higher
cobas® 4800 System Application Software (Core) Version 2.1.0 or higher

For more information regarding the materials sold separately, 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 office.
- LYS-1 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.
- MGP contains isopropanol and is highly flammable. Keep away from open flames and potential spark producing environments.
- Prevent exposure of MGP to sources of magnetic field.
- EB, Cdiff MMX, SDS, Cofactor-3, (–)C, Cdiff (+)C and IC-1 contain sodium azide.
- For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas x** 480 instrument or **cobas z** 480 analyzer, consult the appropriate **cobas**® 4800 System - System Manual. If contamination is suspected, perform cleaning and weekly maintenance as described in the appropriate **cobas**® 4800 System - System Manual.

Note: For specific instructions, see "Specimen Collection, Transport, and Storage".

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands thoroughly after handling specimens and kit reagents.
- 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.
- 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 reagents 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*[24] and in the CLSI Document M29-A4.[25]

Integrity

- Do not use kits after their expiration dates.
- Do not pool reagents.
- Do not use disposable items beyond their expiration date.
- All disposable items are for one time use. Do not reuse.
- All equipment should be properly maintained according to the manufacturer's instructions.

Disposal

- **cobas**® 4800 system reagents and **cobas**® Cdiff Test specific reagents 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 reagents and waste in accordance with country, federal, state and local regulations.

Note: For disposal of liquid waste, refer to the appropriate cobas® 4800 System – System Operator's Manual.

Spillage and cleaning

- LYS-1 reagent contains guanidine thiocyanate. If liquid containing guanidine thiocyanate is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, FIRST clean the affected area with laboratory detergent and water, and then with 0.5% sodium hypochlorite.
- If spills occur on the **cobas**® 4800 instrument, follow the instructions in the appropriate **cobas**® 4800 System - System Manual to clean.
- Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas x** 480 instrument or **cobas z** 480 analyzer. Clean the **cobas x** 480 instrument or **cobas z** 480 analyzer according to procedures described in the appropriate **cobas**® 4800 System - System Manual.

Specimen collection, transport, and storage

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

Specimen collection

Collect unformed stool specimen in a sterile container. Specimens should be collected following the procedure documented in 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 7 days, and at -20°C for 60 days before testing on the **cobas**® 4800 System (this was demonstrated by testing specimens after consecutive storage at 30 ± 1°C for 2 days, followed by 2-8°C for 5 days, followed by -20°C for 60 days).

Stool specimen mixed with the **cobas**® PCR Media is stable at 2-8°C for 60 days or at 30°C for 7 days before testing on the **cobas**® 4800 System.

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

Instructions for use

Running the test

Figure 1: cobas® Cdiff workflow

1	Start up the system.
2	Perform instrument maintenance.
3	Remove samples and reagents from storage.
4	Start run: • Load carriers with samples.
5	With LIS: confirm work order Without LIS: create work order
6	Load consumables (deepwell plate, microwell plate, tip racks) and reagents
7	Start sample preparation run
8	Unload and seal microwell plate
9	Remove samples, used reagents, and deepwell plate.
10	Load microwell plate into analyzer
11	Review results
12	With LIS: send results to LIS
13	Unload analyzer

Test procedure

All reagents except Cdiff MMX and Cofactor-3 must be at ambient temperature prior to loading on the **cobas x** 480 instrument. The Cdiff MMX and Cofactor-3 reagents may be taken directly from 2-8°C storage as they will equilibrate to ambient temperature on board the **cobas x** 480 instrument by the time they are used in the process.

Note: Refer to the appropriate cobas® 4800 System Operator's Manual Software for cobas® Cdiff Test for detailed operating instructions.

Run size

The **cobas**® 4800 System is designed to support mixed-batch format with tests that share the same automated specimen extraction process and PCR profile for amplification and detection. The generic **cobas**® 4800 System Sample Preparation Kit, generic **cobas**® 4800 System Lysis Kit 1 and generic **cobas**® 4800 System Wash Buffer Kit are available in two kit sizes, each sufficient for 10 runs of up to either 24 or 96 samples, which include the controls and specimens for all assays to be run. The **cobas**® 4800 Cdiff Amplification/Detection Kit is available in two sizes, each sufficient to test up to either 80 or 240 samples, which include Cdiff controls and specimens to be run. Multiple vials of the **cobas**® 4800 Cdiff Master Mix reagent can be used as appropriate in one run, as long as they are the same kit size. The generic **cobas**® 4800 System Internal Control Kit 1 and the **cobas**® 4800 Cdiff Controls and Cofactor Kit are available in a single kit size, and can support all run configurations. For each run containing *C. difficile* specimens, one **cobas**® 4800 Cdiff Positive Control and one **cobas**® 4800 System Negative Control must be

used (see "Quality Control"). For a single test run, the maximum number of samples allowed is 94 specimens and 2 controls.

Note: Although not an optimal use of reagents, a generic 96-Test reagent can be used for a run containing 1-22 total specimens. However, different sizes of the cobas® 4800 System Wash Buffer (WB) Kit, cobas® 4800 System Sample Preparation Kit and cobas® 4800 System Lysis Kit 1 cannot be mixed. For example, if a 96-Test WB reagent bottle is scanned at the start of the run, 96-Test size reagents from the other two kits must also be used.

Note: Although not an optimal use of reagents, a 24-Test cobas® 4800 Cdiff MMX can be used for a run containing 1-6 C. difficile specimens. See the appropriate cobas® 4800 System Operator's Manual for cobas® Cdiff Test and "Loading the reagents" section for details on how to change kit size.

Workflow

The **cobas**® Cdiff Test is performed using the full workflow within the **cobas**® 4800 Software. It consists of sample preparation on the **cobas x** 480 instrument followed by amplification/detection on the **cobas z** 480 analyzer. The run can be Cdiff only, or mixed-batch format with tests that share the same automated specimen extraction process and PCR profile for amplification and detection. The software will display tests that are compatible for mixed batching with the **cobas**® Cdiff Test at the test selection step. Refer to the "**Performing a full workflow run**" section in the appropriate **cobas**® 4800 System Operator's Manual for **cobas**® Cdiff Test for details.

Specimen transfer into cobas® PCR media tube

1. Use **one** polyester swab to transfer stool to the **cobas**® PCR Media tube. 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, then 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.

2. Break the swab shaft at the gray notch mark, by applying pressure against the side of the tube. Cap tube and vortex for a minimum of 5 seconds. Uncap and place tube(s) on 24-position sample carrier rack(s) for processing. Discard the caps.

Note: The cobas® Cdiff Test has been validated for use with the cobas® PCR Media and Swab Sample Kit and cobas® PCR Media Uni Swab Sample Kit. Do not use other devices or media types.

Note: Use only one polyester swab to transfer stool. Excess stool transferred to the cobas® PCR Media tube may cause clots and/or invalid results.

Note: Stool specimens must be transferred into cobas® PCR Media tubes which are labeled with a proper barcode for processing on the cobas x 480 instrument. Consult the appropriate cobas® 4800 System - System Manual for proper barcoding procedures and the list of acceptable barcodes for the cobas® 4800 System.

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

Note: cobas® PCR Media contains sufficient volume for the stool suspension to be assayed multiple times on the cobas® 4800 System. Minimum stool suspension volume to conduct a cobas® Cdiff run is 3 mL in the cobas® PCR Media tube.

Performing the cobas® Cdiff Test

Note: Refer to the cobas® 4800 System Operator's Manual for cobas® Cdiff Test for more information on performing mixed batched runs.

1. Perform the system startup and maintenance procedures by following the instructions in the appropriate **cobas**® 4800 System - System Manual in the "Instrument Maintenance" section.
2. Collect all reagents and consumables needed. Reagents must be at room temperature by the time the run is started with the exception of **cobas**® Cdiff MMX and Cofactor-3 reagents.

Note: All reagents and reagent reservoirs are barcoded and designed for one time use. The cobas® 4800 Software tracks the use of the reagents and reagent reservoirs and rejects previously used reagents or reagent reservoirs.

3. Start a new run and define the work order for the run. There are three ways to create a work order:
   - By using the sample editor before sample rack is loaded into **cobas x** 480 instrument ("Editor" button on the right of the main menu). Work orders can be saved, edited and reloaded if necessary.
   - By following the software wizard for the new run and loading specimens into **cobas x** 480 instrument when prompted. The specimen barcodes will be automatically scanned, and the requested results for each specimen must be defined.
   - By using your institution's LIS system.

   Refer to the appropriate **cobas**® 4800 System Operator's Manual for **cobas**® Cdiff Test for more details. When selecting the requested results, check "Cdiff".
4. Load samples and define/select work order or use LIS as appropriate. The "Unload sample carriers after transferring to deep well plate" option is selected by default. This allows the operator to retrieve the remaining stool suspension specimens as soon as possible after they are aliquoted for processing by **cobas x** 480 instrument. Stool suspension containers should be re-capped with fresh closure (see "Optional Materials") if storage is needed.
5. Follow the software wizard guide and load consumables. Do not load or remove individual tips into a partially used tip rack, as the software tracks the number of tips that are left. If there are not enough tips for the run to be conducted, the software will alert the user.
6. Load the sample preparation reagents into the barcoded reagent reservoirs. The reagent reservoirs are available in two sizes: 200 mL and 50 mL. Follow the software wizard guide to select the correct reagent reservoir size. The reagent reservoir barcodes must face to the right of the carrier. Use the "scan-scan-pour-place" method to load sample preparation reagents:
   - Scan the reagent bottle barcode
   - Scan the reagent reservoir barcode
   - Pour the reagent into the reservoir
   - Place the filled reagent reservoir into the designated position on the reagent carrier

Note: The cobas® 4800 System has an internal clock to monitor the length of time the reagents are on-board. Once the WB is scanned, 1 hour is allowed to complete the loading process and click on the Start button. A countdown timer is displayed on the Workplace Tab. The system will not allow the run to start if the on-board timer has expired.

Note: To assure the accurate transfer of MGP, vortex or vigorously shake the MGP vial <u>immediately prior</u> to dispensing into the reagent reservoir.

7. Load amplification/detection reagents (Cdiff MMX and Cofactor-3), Proteinase K (PK) and controls [Cdiff (+) C, IC and (–) C] directly onto the reagent carrier. In order to prevent contamination, it is required to change gloves after handling positive controls.

Note: The software wizard will calculate the optimal number and size of cobas® Cdiff MMX reagent to use. This will be reflected in the "Kit size" column on the MMX and Cofactor loading screen. To use a different size of cobas® Cdiff MMX reagent, click the "Change kit size" button.

8. After a successful sample preparation run, the "Sample Preparation results" button and the Unload button become available. If desired, select "Sample Preparation results" button to review the results then select "Unload" to unload the plate carriers. Alternatively, select "Unload" to unload the plate carrier without reviewing the results. See the appropriate **cobas**® 4800 System Operator's Manual for **cobas**® Cdiff Test.
9. Follow the instructions in the appropriate **cobas**® 4800 System Operator's Manual for **cobas**® Cdiff Test to seal the microwell plate, transport the plate to the **cobas z** 480 analyzer and start the amplification and detection run.

Note: The cobas® 4800 System has an internal clock to monitor the length of time after addition of the prepared samples to activated master mix. Amplification and detection should be started as soon as possible but no later than 90 minutes after the end of the cobas x 480 instrument run. A countdown timer is displayed on the Workplace Tab. The system will abort the run if the timer has expired.

10. When the amplification and detection run is completed, unload the microwell plate from the **cobas z** 480 analyzer.
11. Follow the instructions in the appropriate **cobas**® 4800 System Operator's Manual for **cobas**® Cdiff Test to review and accept results.

Results

Quality control and validity of results

The user is responsible for performing quality control testing according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

One set of **cobas**® Cdiff Test Positive and Negative Controls are included in each run. For any run, valid results must be obtained for both the Positive and Negative Control for the **cobas**® 4800 Software to display the reportable **cobas**® Cdiff Test results from that run.

Positive control

The Cdiff (+) Control contains non-infectious DNA plasmids of *C. difficile*. The Cdiff (+) Control monitors nucleic acid extraction, amplification, and detection steps in a given run of the test. The Cdiff (+) Control result must be 'Valid'. If the Cdiff (+) Control results are consistently invalid, contact your local Roche office for technical assistance.

Negative control

The (–) Control result must be 'Valid'. If the (–) Control results are consistently invalid, contact your local Roche office for technical assistance.

Internal control

The Internal Control is a recombinant bacteriophage lambda that contains randomized sequences and targets for internal control-specific primers and probe. The Internal Control is added to all specimens and the Positive and Negative Controls during sample preparation on the **cobas x** 480 instrument. The Internal Control monitors nucleic acid extraction, amplification, and detection steps for a given specimen. The Internal Control is also required for validation of the run controls.

Interpretation of results

Note: All assay and run validation is determined by the cobas® 4800 Software.

Note: A valid run may include both valid and invalid specimen results.

For a valid run, specimen results are interpreted as shown in Table 1.

Table 1: Interpretation of results of the cobas® Cdiff Test

cobas® Cdiff Test Result	**Result Interpretation**
POS Cdiff	**Cdiff Positive** Specimen is positive for the presence of toxigenic *C. difficile* DNA.
NEG Cdiff	**Cdiff Negative*** Toxigenic *C. difficile* DNA, if present, could not be detected.
Invalid	**Invalid** Result is Invalid. The original specimen should be re-tested to obtain valid result. Place a new cap on the tube containing the stool suspension which had the invalid result and vortex for a minimum of 5 seconds. Add 0.5 mL of the vortexed stool suspension to a new **cobas**® PCR Media tube containing media. Cap the diluted tube and vortex for a minimum of 5 seconds. Uncap and place diluted tube(s) on 24-position sample carrier for processing.
Failed	**No Result for Specimen** Consult the appropriate **cobas**® 4800 System Operator's Manual for **cobas**® Cdiff Test for instructions to review run flags and recommended actions. In rare cases when pipetting error (e.g. clot or other obstruction) occurs, the original stool specimen suspension tube should be closed with a new cap and placed into a centrifuge. Accelerate to 1800 RCF (or 1800 x g) then stop. Ensure the vial is not shaken or mixed after centrifuge. Uncap and place tube on 24-position sample carrier for processing.

*A negative result does not preclude the presence of toxigenic *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 inhibitory substances that prevent nucleic acid target extraction and/or amplification and detection. See "Procedural limitations" for known interference substances.

Note: The minimum volume of stool suspension necessary for the **cobas**® Cdiff Test is 3 mL.

Procedural limitations

1. The **cobas**® Cdiff Test has only been validated for use with unformed stool specimens that have been transferred into the **cobas**® PCR Media according to this Instructions-For-Use document.

2. Reliable results are dependent on adequate specimen collection, transport, storage, and processing. Follow the procedures in this Instructions-For-Use document (also referred to as a Package Insert) and the appropriate **cobas**® 4800 System Operator's Manual for **cobas**® Cdiff Test.
3. Detection of toxigenic *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 the **cobas**® Cdiff Test 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 25% (w/v) mucin may generate false negative results.
5. A positive result is indicative of the presence of toxigenic *C. difficile* DNA and not necessarily viable organisms. Therefore, a positive result does not necessarily mean eradication treatment failure.
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 the **cobas**® Cdiff Test.
7. The predictive value of an assay depends on the prevalence of the disease in any particular population.
8. The addition of AmpErase enzyme into the **cobas**® 4800 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.
9. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
10. Only the **cobas x** 480 instrument and **cobas z** 480 analyzer have been validated for use with this product. No other sample preparation instrument or PCR System can be used with this product.
11. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to identify technology differences, and verify the new procedure.
12. Cross-contamination can cause false positive results. The **cobas**® 4800 System is an automated real-time PCR instrument designed to minimize the risk of cross-contamination during specimen processing, nucleic acid extraction, amplification and detection. To challenge the robustness of the system, a simulated, non-clinical checkerboard study was performed on a panel of alternating high positive and negative contrived samples, to assess a theoretical cross-contamination rate of the system. The high positive samples had Ct values earlier than would be observed in 95% of infected patients in the intended use population. The cross-contamination rate in this checkerboard study was determined to be 0.24% (1/423). Cross contamination rates in clinical settings depend on the proportion of high positive samples and prevalence of disease. Routine clinical cross-contamination rates are expected to be dramatically lower than what was observed in this study and need to be assessed in user's settings.

Non-clinical performance evaluation

Analytical sensitivity

The analytical sensitivity (Limit of Detection or LOD) for the **cobas**® Cdiff Test 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 using the **cobas**® Cdiff Test across three unique lots of **cobas**® Cdiff Test reagents. At least 21 replicates per reagent lot were tested at each level. 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 seven *C. difficile* strains tested in the analytical sensitivity study are shown in Table 2.

Table 2: cobas® Cdiff Test LOD (Limit of Detection)

Strain ID	Toxinotype	REA* Type	PFG† Type	Ribotype	Phenotype	LOD (CFU/swab)	
						By Positive Rate	By Probit Analysis (95% CI)
ATCC 43255 (VPI 10463)	0	N/A	N/A	087	A+B+CDT-	113	90 (66 – 311)
ATCC BAA-1382 (630)	0	R 23	N/A	012	A+B+CDT-	81	83 (62 – 145)
CDC 204118	III	BI 8	NAP1	027	A+B+CDT+	54	42 (30 – 129)
R12087 (CD196)	III	BI	NAP1	027	A+B+CDT+	54	54 (39 -126)
2748-06	V	N/A	N/A	078	N/A	54	45 (33-113)
ATCC 43598 (1470)	VIII	N/A	N/A	017	A-B+	225	130 (96 - 228)
F15	XII	N/A	N/A	N/A	N/A	54	59 (43 – 117)

* Restriction endonuclease analysis; † Pulse Field Gel

Detection of *C. difficile* genotypes

The limit of detection (LOD) of the **cobas**® Cdiff Test on 28 toxigenic strains representing additional toxinotypes were verified by testing 40 replicates per level at multiple levels. Dilutions and testing samples were prepared in a similar fashion as in the Limit of Detection study described above. The lowest level that had at least 95% observed hit rate are summarized in Table 3.

All 28 toxigenic strains (Table 3) were detected as positive in ≥ 95% of the replicates tested at concentrations ranging from 77.9 CFU/swab to 460 CFU/swab.

Table 3: Summary of toxigenic *C. difficile* verification results

Strain	Toxinotype	Ribotype	Conc. (CFU/swab)	Positive Rate
EX 623	I	102	77.9	95.0%
AC 008	II	103	77.9	95.0%
SE 844	IIIa	080	234	100.0%
55767	IV	023	77.9	100.0%
SE 881	V	045	234	100.0%
51377	VI	N/A	234	100.0%
57267	VII	063	77.9	97.5%
51680	IX	019	77.9	100.0%
8864	X	036	77.9	97.5%
R 9367	XIII	070	77.9	97.5%
R 10870	XIV	111	234	100.0%
R 9385	XV	122	234	100.0%
SUC36	XVI	078	234	100.0%
J9965	XVII	N/A	460	97.5%
K095	XVIII	014	234	95.0%
TR13	XIX	N/A	234	97.5%
TR14	XX	N/A	77.9	100.0%
CH6223	XXI	N/A	234	100.0%
CD07-468	XXII	N/A	234	100.0%
8785	XXIII	N/A	234	95.0%
597B	XXIV	131	234	97.5%
7325	XXV	027	234	100.0%
7459	XXVI	N/A	234	95.0%
KK2443-2006	XXVII	N/A	234	100.0%
CD08-070	XXVIII	126	234	97.5%
CD07-140	XXIX	056	234	97.5%
ES 130	XXX	N/A	234	100.0%
WA 151	XXXI	N/A	460	100.0%

Precision

In-house precision study was conducted using a panel composed of *C. difficile* cultures diluted into negative stool suspension in **cobas**® PCR Media to concentration levels below Limit of Detection (LOD), near LOD and above LOD of the **cobas**® Cdiff Test. A negative level composed of only the stool suspension in **cobas**® PCR Media was also tested. The study used three unique lots of **cobas**® Cdiff Test reagents and three instruments for a total of 36 runs over 12 days. A description of the precision panels and the study summary is shown in Table 4. Analysis of the variance components (Table 5) suggested that most variability of target Ct values is attributed to within run (random) and lot to lot factors (60.0% and 25.3%, respectively) for concentration level at or around LOD. For concentration level above LOD, most of the Ct value variability is attributed to within run (random) and instrument to instrument factors (72.5% and

24.7%, respectively). Results (Table 6) show that the target Ct values had overall CV (%) of 1.5% for concentration level at LOD and 1.1% for concentration level above LOD.

Table 4: In-house precision study positive rate analysis

Panel Member	N Tested	N Positive	Positive Rate	95% CL	
				Lower	Upper
Negative	72	0	0.0%	0.0%	5.0%
< 1 x LOD	72	21	29.2%	19.0%	41.1%
~ 1 x LOD	72	72	100.0%	95.0%	100.0%
~ 3 x LOD	72	72	100.0%	95.0%	100.0%

LOD = Limit of Detection

Table 5: Variance components analysis for precision panel members

Level	Mean Ct	Variance Components by Factor/Percent Contribution to Total					Total
		Lot	Instrument	Kit Size	Day	Within-Run	
~ 1 x LOD	38.5	0.0789	0.0189	0.0001	0.0270	0.1875	0.3123
		25.3%	6.0%	0.0%	8.6%	60.0%	100.0%
~ 3 x LOD	37.5	0.0047	0.0404	0.0000	0.0000	0.1188	0.1638
		2.8%	24.7%	0.00%	0.00%	72.5%	100.0%

LOD = Limit of Detection

Table 6: Standard deviations and coefficients of variation (%) analysis for precision panel members

Level	Mean Ct	SD by Factor/Percent CV					Total
		Lot	Instrument	Kit Size	Day	Within-Run	
~ 1 x LOD	38.5	0.28	0.14	0.01	0.16	0.43	0.56
		0.7%	0.4%	0.0%	0.4%	1.1%	1.5%
~ 3 x LOD	37.5	0.07	0.20	0.00	0.00	0.34	0.40
		0.2%	0.5%	0.0%	0.0%	0.9%	1.1%

LOD = Limit of Detection

Analytical specificity

To assess the analytical specificity of the **cobas**® Cdiff Test, the following organism panels were tested: 1) 103 bacteria, fungi and viruses that may be found in stool specimens and one human cell (Table 7); 2) 28 *Clostridium* genus organisms, including non-toxigenic *C. difficile* (Table 8).

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, except for Adenovirus Type 40, Cytomegalovirus (HHV5), and Human Rotavirus, which were spiked to lower concentrations due to stock concentration limitations. Testing was performed with the organisms alone or with 2 *C. difficile* isolates present individually at 3 x Limit of Detection (LOD) of the **cobas**® Cdiff Test. Results indicated that none of these organisms interfered with detection of the intended *C. difficile* targets. None produced false positive results when there was no intended *C. difficile* target present.

In silico analysis using BLAST and Fuzznuc programs against GenBank nucleotide sequence database to mimic PCR amplicon generation and probe detection steps indicated that *Clostridium botulinum* does not cross-react with the **cobas**® Cdiff Test.

*Bacteria were quantified as colony forming units (CFU)/mL, human cells were quantified as cells/mL, and viruses were quantified as plaque forming units (PFU)/mL, except the following microorganisms: *Chlamydia trachomatis* was quantified as elementary body (EB)/mL. Cytomegalovirus, Human Echovirus, and Human Enterovirus were quantified as IU/mL.

Table 7: Microorganisms and human cells

Abiotrophia defectiva	*Acinetobacter baumannii*	*Acinetobacter lwoffii*
Aeromonas hydrophila	*Alcaligenes faecalis* subsp. *Faecalis*	*Anaerococcus tetradius*
Bacillus cereus	*Bacillus cereus*	*Bacteroides caccae*
Bacteroides merdae	*Bacteroides stercoris*	*Bifidobacterium adolescentis*
Bifidobacterium longum	*Campylobacter coli*	*Campylobacter jejuni*
Candida albicans	*Candida catenulata*	*Cedecea davisae*
Chlamydia Trachomatis Serovar L2	*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 faecalis	*Enterococcus faecium*	*Enterococcus gallinarum*
Enterococcus hirae	*Enterococcus raffinosus*	*Escherichia coli*
Escherichia coli	*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	*Mitsuokella multacida*	*Mobiluncus curtisii*
Moellerella wisconsensis	*Morganella morganii*	*Neisseria gonorrhoeae*
Peptoniphilus asaccharolyticus	*Peptostreptococcus anaerobius*	*Plesiomonas shigelloides*
Porphyromonas asaccharolytica	*Prevotella melaninogenica*	*Proteus mirabilis*
Proteus penneri	*Providencia alcalifaciens*	*Providencia rettgeri*
Providencia stuartii	*Pseudomonas aeruginosa*	*Pseudomonas putida*
Ruminococcus bromii	*Salmonella choleraesuis* subsp. *choleraesuis*	*Salmonella enterica* subsp. *arizonae* (f.k.a. *Salmonella choleraesuis* ssp. *arizonae*)
Salmonella enterica subsp. *enterica* serovar Choleraesuis	*Serratia liquefaciens*	*Serratia marcescens*
Shigella boydii	*Shigella dysenteriae*	*Shigella sonnei*
Staphylococcus aureus	*Staphylococcus epidermidis*	*Stenotrophomonas maltophilia*
Streptococcus agalactiae	*Streptococcus dysgalactiae*	*Streptococcus intermedius*
Streptococcus uberis	*Trabulsiella guamensis*	*Veillonella parvula*
Vibrio cholerae	*Vibrio parahaemolyticus*	*Yersinia bercovieri*
Yersinia rohdei	Cytomegalovirus (HHV5)*	Human Adenovirus 40*
Human Coxsackievirus A 10	Human Echovirus 11	Human Enterovirus 71
Human Rotavirus*	Norovirus GII	-

* Cytomegalovirus (HHV5) at 2.0 x 10^3 IU/mL, Human Adenovirus Type 40 was spiked at 2.2 x 10^3 PFU/mL, and Human Rotavirus at 9.8 x 10^3 PFU/mL for testing.

Table 8: *Clostridium* genus organisms, including non-toxigenic *C. difficile*

Clostridium beijerinckii	*Clostridium bifermentans*	*Clostridium bolteae*
*Clostridium botulinum**	*Clostridium butyricum*	*Clostridium chauvoei*
Clostridium difficile Serogroup B (non-toxigenic)	*Clostridium difficile* Serogroup I (non-toxigenic)	*Clostridium fallax*
Clostridium haemolyticum	*Clostridium histolyticum*	*Clostridium innocuum*
Clostridium methylpentosum	*Clostridium nexile*	*Clostridium novyi*
Clostridium orbiscindens (re-named Flavonifractor plautii)	*Clostridium paraputrificum*	*Clostridium perfringens*
Clostridium ramosum	*Clostridium scindens*	*Clostridium septicum*
Clostridium sordellii	*Clostridium sphenoides*	*Clostridium spiroforme*
Clostridium sporogenes	*Clostridium symbiosum*	*Clostridium tertium*
Clostridium tetani	-	-

* Based on BLAST program.

Interference

Twenty-six commonly used medications, as well as fecal fat, whole blood, and mucin, were tested for potential interference effects with the **cobas**® Cdiff Test. 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 the concentration in the primary stool specimen. Two toxigenic *C. difficile* isolates were spiked to 3 x Limit of Detection (LOD) of the **cobas**® Cdiff Test and used as targets in the tests. No interference was observed for exogenous substances. For fecal fat, no interference was observed up to 28%, for whole blood, no interference was observed up to 50%, and for mucin, no interference was observed up to 25%. Mucin at 50% interfered with the detection of the toxigenic *C. difficile* isolates. These results are summarized in Table 9.

Table 9: Results from interference substances testing

Substance	Concentration	Results
Fecal Fat	4 ~ 28 % (w/v)	No interference
Whole blood	25, 50 % (v/v)	No interference
Mucin	25, 50* % (w/v)	No interference up to 25% (w/v)
Tums	10% (w/v)	No interference
Vancomycin	1% (w/v)	No interference
Metronidazole	10% (w/v)	No interference
Imodium AD®	10% (w/v)	No interference
Stool Softener	10% (w/v)	No interference
Pepto-Bismol® (Procter & Gamble)	10% (v/v)	No interference
Nystatin Ointment USP	10% (w/v)	No interference
Preparation H® with Bio-Dyne® Cream (Wyeth)	10% (w/v)	No interference
GYNOL II	10% (w/v)	No interference
Vagisil® Anti-itch cream	10% (w/v)	No interference
Anusol® Plus	10% (w/v)	No interference
Sunscreen	1% (w/v)	No interference
Monistat® 7	10% (w/v)	No interference
Vaseline™	10% (w/v)	No interference
SAB-Dimenhydrinate® Suppositories (SABEX®)	10% (w/v)	No interference
Mineral Oil	10% (v/v)	No interference
Equate Natural Vegetable Laxative	10% (w/v)	No interference
Dulcolax®	10% (w/v)	No interference
Fleet® (CB Fleet Company)	10% (w/v)	No interference
K-Y Jelly/Gelée® (McNeil-PPC)	1% (w/v)	No interference
Afrin Original Nasal Spray	10% (v/v)	No interference
Witch hazel	Liquid from 1 wipe/swab	No interference
E-Z-HD™ High Density Barium Sulfate for suspension (E-Z-EM Canada)	20% (w/v)	No interference
Palmitic acid	10% (w/v)	No interference
Stearic acid	10% (w/v)	No interference
Aleve	10% (w/v)	No interference

* Mucin at 50% (v/v) concentration interfered with the detection of toxigenic *C. difficile* isolates.

Clinical performance evaluation

Reproducibility

The reproducibility of the **cobas**® Cdiff Test on the **cobas**® 4800 System was established in a multi-site investigation using simulated clinical samples evaluated across lot, site/instrument, operator, day and within-run.

Reproducibility test panels of 4 specimens, with 3 replicates each, were prepared at varying concentrations of *C. difficile* strain ATCC 43255 (Negative, Below LOD, 1 x LOD, and 3 x LOD) into pooled, *C. difficile*-negative, unformed stool in **cobas**® PCR Media and tested at 3 sites by 2 operators/day for 5 days/lot over 2 lots for a total of 720 tests or 180 tests/panel member or 90 tests/panel member/lot (4 specimens x 3 replicates x 3 sites x 2 operators/site x 5 days/lot x 2 lots). The results are summarized in Table 10 and Table 11.

Results

Overall, 60 runs were performed; all were valid. Of the 720 test performed across 4 panel members (Negative, Below LOD, 1 x LOD, 3 x LOD), there were 712 (98.9%) valid results; 7 failed results were due to clot detection or pipetting errors, and 1 invalid result was due to IC dropout. All valid test results were included in percent agreement analyses.

Table 10 summarizes the Ct values and the overall percent agreement (two-sided 95% exact CI) by panel member and the percent agreement by lot, site/instrument, operator and day. The SD and CV (%) for Ct values across positive panel members ranged from 0.64 to 0.71 and 1.7 to 1.9%, respectively. The positive percent agreements for the *C. difficile* positive panel members “Below LOD,” “1 x LOD,” and “3 x LOD” were 66.1% (95% CI: 58.7% to 73.0%), 100.0% (95% CI: 98.0% to 100.0%), and 100.0% (95% CI: 97.9% to 100.0%), respectively. The negative percent agreement for negative panel members was 100.0% (95% CI: 97.9% to 100.0%).

Table 10: Summary of reproducibility results: Ct values and percent agreement by site and panel member

		Negative	Below LOD	1 x LOD	3 x LOD
Number of Valid Test Results		174	180	180	178
Ct	Mean	N/A	39.7	37.6	36.6
	SD	N/A	0.71	0.64	0.70
	CV (%)	N/A	1.8	1.7	1.9
Overall Hit Rate	Agreement (n/N)*	100.0% (174/174)	66.1% (119/180)	100.0% (180/180)	100.0% (178/178)
	95% CI	(97.9%, 100.0%)	(58.7%, 73.0%)	(98.0%, 100.0%)	(97.9%, 100.0%)
Lot	1	100.0% (85/85)	65.6% (59/90)	100.0% (90/90)	100.0% (90/90)
	2	100.0% (89/89)	66.7% (60/90)	100.0% (90/90)	100.0% (88/88)
Site/ Instrument	1	100.0% (60/60)	71.7% (43/60)	100.0% (60/60)	100.0% (60/60)
	2	100.0% (60/60)	68.3% (41/60)	100.0% (60/60)	100.0% (60/60)
	3	100.0% (54/54)	58.3% (35/60)	100.0% (60/60)	100.0% (58/58)
Operator	1	100.0% (30/30)	76.7% (23/30)	100.0% (30/30)	100.0% (30/30)
	2	100.0% (30/30)	66.7% (20/30)	100.0% (30/30)	100.0% (30/30)
	3	100.0% (30/30)	66.7% (20/30)	100.0% (30/30)	100.0% (30/30)
	4	100.0% (30/30)	70.0% (21/30)	100.0% (30/30)	100.0% (30/30)
	5	100.0% (24/24)	53.3% (16/30)	100.0% (30/30)	100.0% (29/29)
	6	100.0% (30/30)	63.3% (19/30)	100.0% (30/30)	100.0% (29/29)
Day	1	100.0% (35/35)	69.4% (25/36)	100.0% (36/36)	100.0% (35/35)
	2	100.0% (35/35)	61.1% (22/36)	100.0% (36/36)	100.0% (36/36)
	3	100.0% (34/34)	58.3% (21/36)	100.0% (36/36)	100.0% (36/36)
	4	100.0% (35/35)	63.9% (23/36)	100.0% (36/36)	100.0% (35/35)
	5	100.0% (35/35)	77.8% (28/36)	100.0% (36/36)	100.0% (36/36)

* For the negative panel member, percent agreement = (number of negative results/total valid results) x 100; for the positive panel members, percent agreement = (number of positive results/total valid results) x 100.
CI = confidence interval; Ct = cycle threshold; CV = coefficient of variation; LOD = limit of detection; N/A = not applicable; SD = standard deviation.

Table 11 presents the SD and CV (%) of Ct values for positive panel members overall and attributable to lot, site/instrument, operator, day, and within-run.

Table 11: Overall mean, standard deviations, and coefficient of variation (%) for Ct values from valid results for positive panel members

			Standard Deviation and Coefficient of Variation (%)											
			Lot		Site/Inst.		Operator		Day		Within-Run		Total	
Panel Member	**N**	**Mean Ct**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Below LOD	119	39.7	0.33	0.8%	0.00	0.0%	0.12	0.3%	0.21	0.5%	0.58	1.5%	0.71	1.8%
1 x LOD	180	37.6	0.54	1.4%	0.08	0.2%	0.00	0.0%	0.06	0.1%	0.33	0.9%	0.64	1.7%
3 x LOD	178	36.6	0.60	1.7%	0.13	0.4%	0.10	0.3%	0.09	0.3%	0.29	0.8%	0.70	1.9%

Ct = cycle threshold; CV = coefficient of variation; Inst. = instrument; LOD = limit of detection; SD = standard deviation.

Clinical performance

The clinical performance of the **cobas**® Cdiff Test was established in an IRB-approved, prospective, multi-site, investigation comparing the results with toxigenic culture using leftover, de-identified, unformed stool samples from subjects suspected of having CDI. Specimens were collected at five geographically diverse sites across the US from symptomatic eligible male and female subjects. The toxigenic culture was performed at a single reference laboratory and the **cobas**® Cdiff Test was performed at one of three designated sites. The toxigenic culture included direct and repeat direct and enrichment culture of stool followed by cytotoxicity testing. The direct culture included the transfer of sample to pre-reduced selective anaerobic media, cycloserine-cefoxitin-fructose agar with horse blood and taurocholate (CCFA-HT), followed by cytotoxicity testing on *C. difficile* recovered from stool. Briefly, suspected colonies obtained from direct cultures were identified as *C. difficile* by Gram stain, aerotolerance test, and by the Pro Disk test (Hardy Diagnostics, Santa Maria, CA) and then inoculated into anaerobic chopped meat broth and incubated for 5 to 7 days at 35°C for cytotoxicity testing. Supernatants obtained from anaerobic chopped meat broth were then processed for the detection of *C. difficile* toxin B using cell culture cytotoxicity testing (*C. DIFFICILE TOX-B TEST,* TECHLAB®). Enriched toxigenic culture included culture using cycloserine-cefoxitin-manitol broth with taurocholate, lysozyme and cysteine (CCMB-TAL), followed by subculture on *Brucella* agar plates, and with identification and cytotoxicity testing of *C. difficile* recovered from enrichment culture as described. A specimen was considered positive for toxigenic *C. difficile* if *C. difficile* was recovered from stool either by direct or enriched toxigenic culture and if isolates recovered tested positive by cytotoxicity testing (any positive rule). If *C. difficile* was isolated from the direct culture and the isolate tested positive by cell cytotoxicity assay, the enrichment culture was not further analyzed. Specimens were classified as negative for toxigenic *C. difficile* only if they tested negative by both direct, and repeat direct and enrichment culture. The sensitivity, specificity, and PPV and NPV values were calculated by comparing **cobas**® Cdiff Test results with the combined results of direct and enrichment toxigenic culture. Discrepant analysis was performed on all samples with discordant results, and a random subset of specimens with concordant results, between the **cobas**® Cdiff Test and toxigenic culture, using a second FDA-cleared nucleic acid amplification test (NAAT). In addition, the positive percent agreement (PPA) and negative percent agreement (NPA) was determined comparing the **cobas**® Cdiff Test with the initial direct culture results.

Results

Specimens were collected from 683 subjects; 306 males (44.8%) and 377 females (55.2%) with a mean age of 56 years (range 3 to 99). Specimens from all 683 subjects had valid results for both direct toxigenic culture and the **cobas**® Cdiff Test but one sample lacked sufficient volume for repeat direct and enrichment culture and was not included in the statistical analysis. Of the 683 specimens, 113 were positive for toxigenic *C. difficile* during the initial direct toxigenic culture and 141 of 682 were positive for toxigenic *C. difficile* using the combined results from the initial direct and repeat direct and enrichment toxigenic culture, for a prevalence rate of 20.7% for the study.

Comparison with combined direct and enrichment culture

The clinical performance of the **cobas**® Cdiff Test compared with the combined results of initial direct and repeat direct and enriched toxigenic culture are shown in Table 12. The sensitivity and specificity of the **cobas**® Cdiff Test was 92.9% (131/141; 95% CI: 87.4% to 96.1%) and 98.7% (534/541; 95% CI: 97.4% to 99.4%), respectively; and the PPV and NPV was 94.9% (95% CI: 89.9% to 97.5%) and 98.2% (95% CI: 96.6% to 99.0%), respectively. Of the 10 specimens with false-negative **cobas**® Cdiff Test results relative to combined direct culture and enrichment culture, all 10 were negative by a second NAAT method. Of the 7 specimens with false-positive **cobas**® Cdiff Test results relative to combined direct and enrichment culture, 3 were positive and 4 were negative by that second NAAT method.

Table 12: Comparison of cobas® Cdiff Test with combined direct culture and enrichment culture

		Combined Direct and Enrichment Culture[a]		
		Positive	Negative	Total
cobas® Cdiff Test	Positive	131	7[c]	138
	Negative	10[b]	534	544
	Total	141	541	682
Sensitivity:		92.9% (131/141; 95% CI = 87.4% to 96.1%)		
Specificity:		98.7% (534/541; 95% CI = 97.4% to 99.4%)		
PPV:		94.9% (95% CI = 89.9% to 97.5%)		
NPV:		98.2% (95% CI = 96.6% to 99.0%)		

[a] Includes combined results from an initial direct culture and a repeat direct and enrichment culture performed on all initial direct culture-negative samples. One specimen with an initial direct culture-negative result had insufficient specimen volume to perform repeat direct culture and enrichment culture and was excluded from the analysis. Thirty-six (36) specimens with initial direct culture-negative results had their combined direct and enrichment culture results based on repeat culture that used three culture plate media (CCFA, CCFA-HB, CCFA-VA) in combination with enrichment culture. Of these 36 specimens, 21 were culture positive.

[b] Of the 10 specimens with false-negative **cobas**® Cdiff Test results relative to combined direct and enrichment culture, all 10 were negative by a second NAAT method

[c] Of the 7 specimens with false-positive **cobas**® Cdiff Test results relative to combined direct and enrichment culture, 3 were positive and 4 were negative by that second NAAT method.

Comparison with direct culture

The performance of the **cobas**® Cdiff Test compared to initial direct culture is shown in Table 13. The PPA and NPA of the **cobas**® Cdiff Test compared to the initial direct culture for all 683 subjects was 97.3% (110/113) and 94.9% (541/570), respectively. Of the 3 specimens with false-negative **cobas**® Cdiff Test results relative to direct culture, all 3 were negative by a second NAAT method. Of the 29 specimens with false-positive **cobas**® Cdiff Test results relative to direct culture, 15 were positive and 13 were negative by that second NAAT method; 1 sample was not tested because of insufficient specimen volume.

Table 13: Comparison of cobas® Cdiff Test with direct culture

		Direct Culture		
		Positive	Negative	Total
cobas® Cdiff Test	Positive	110	29[b]	139
	Negative	3[a]	541	544
	Total	113	570	683
Positive Percent Agreement:		97.3% (110/113; 95% CI = 92.5% to 99.1%)		
Negative Percent Agreement:		94.9% (541/570; 95% CI = 92.8% to 96.4%)		

[a] Of the 3 specimens with false negative **cobas**® Cdiff Test results relative to direct culture, all 3 were negative by a second NAAT method.

[b] Of the 29 specimens with false positive **cobas**® Cdiff Test results relative to direct culture, 15 were positive, 13 were negative by that second NAAT method, and 1 sample was not tested because of insufficient specimen volume.

Expected values

Prevalence

The prevalence of *C. difficile* infection (CDI) depends on a variety of factors including predisposition for infection due to prior therapy with broad spectrum antibiotics, the presence of symptoms and test method. In this prospective clinical study, specimens were collected from 683 subjects suspected of CDI, with 306 males (44.8%) and 377 females (55.2%) and a mean age of 56 years (range 3 to 99). Of the 682 evaluable subjects, 141 were positive based on the combined results of direct and enrichment toxigenic culture for an observed prevalence of 20.7% The percentage of positive results observed with the **cobas**® Cdiff Test in this population was 20.4%.

The overall sensitivity and specificity of the **cobas**® Cdiff Test compared to combined direct and enrichment toxigenic culture was 92.9% and 98.7%, respectively. The hypothetical positive and negative predictive values (PPV & NPV) derived from disease prevalence of 5 to 30% for the **cobas**® Cdiff Test are shown in Table 14.

Table 14: Hypothetical positive and negative predicative values derived from disease prevalence

Prevalence (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
5	92.9	98.7	79.1	99.6
10	92.9	98.7	88.9	99.2
15	92.9	98.7	92.7	98.7
20	92.9	98.7	94.7	98.2
25	92.9	98.7	96.0	97.7
30	92.9	98.7	96.9	97.0

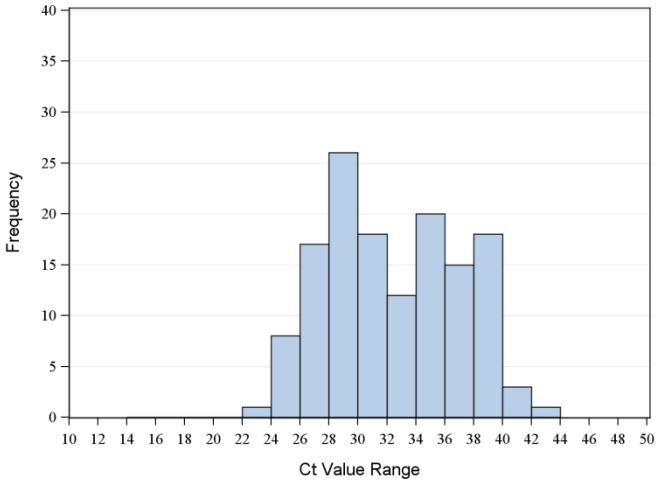
Sensitivity and specificity were established by comparing the **cobas**® Cdiff Test results to toxigenic culture on isolates recovered from unformed stool samples from patients suspected of having *C. difficile* infection (CDI). A positive toxigenic culture indicates the presence of toxigenic *C. difficile*.

Note: PPV = positive predictive value; NPV = negative predictive value.

Ct frequency distribution

The distribution of Ct values for positive **cobas**® Cdiff Test results for specimens with both true positive and false positive results relative to toxigenic culture is shown in Figure 2.

Figure 2: Frequency distribution of cycle threshold values for the cobas® Cdiff Positive Test results



Additional information

Key assay features

Sample type	Unformed stool specimens
Amount of sample required	4.3 mL of **cobas**® PCR media in the primary vial, a minimum of 3 mL is required for a **cobas**® Cdiff Test.
Test duration	Results are available within 2.5 hours after loading the specimen on the system.
Analytical sensitivity	From 54 to 460 CFU/swab depending on isolate.
Specificity	No cross-reactivity with 125 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 15: Symbols Used in Labeling for Roche PCR Diagnostic Products

	Ancillary Software		*In Vitro* Diagnostic Medical Device
	Authorized Representative in the European community		Lower Limit of Assigned Range
	Barcode Data Sheet		Manufacturer
	Batch code		Store in the dark
	Biological Risks		Contains Sufficient for <*n*> tests
	Catalogue number		Temperature Limit
	Consult instructions for use		Test Definition File
	Contents of kit		Upper Limit of Assigned Range
	Distributed by		Use-by date
	For IVD Performance Evaluation Only		Global Trade Item Number

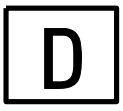
This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 16: Manufacturer and distributors

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



Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800-526-1247)

Trademarks and patents

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

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Document revision

Document Revision Information	
Doc. Rev. 3.0 11/2016	Add Uni swab collection kit (MN 07958030190). Added Roche web address www.roche.com. Please contact your local Roche Representative if you have any questions.
Doc. Rev. 2.0 08/2016	Updated hazard warnings for Updated hazard warnings for **cobas**® 4800 System Sample Preparation Kit and **cobas**® 4800 System Lysis Kit 1 Revised **Optional Materials** section: Added “Roche P/N 07958056190; for recapping post-run specimens in 13 mL Round Base tubes.” Omitted Sarstedt 65.176.026. Updated descriptions of the harmonized symbol page at the end of the package insert. Please contact your local Roche Representative if you have any questions.