Modaplex *Clostridium difficile* Analysis Kit IFU - Instructions for Use

REF BTI-C004-F1-3-0050

50 reactions

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CE IVD

IVD

For use in diagnostic procedures

Intended for use with the Modaplex System

BIOTYPE

Contents

Intended Use

The Modaplex *Clostridium difficile* Analysis Kit is a PCR-based multiplex assay for the qualitative detection of the *Clostridium difficile* toxin B gene (*tcdB* gene) in liquid or soft human stool specimen from patients suspected of having *C. difficile* Associated Disease (CDAD). The assay is intended to aid in the CDAC diagnosis.

The Modaplex *Clostridium difficile* Analysis Kit must be used by qualified and trained personnel in a professional laboratory environment only. The results are intended for diagnostic use.

Summary and Explanation

Clostridium difficile infection is one of the most prevalent causes of healthcare associated infection and is rapidly expanding in healthcare communities. Clinical manifestations of *Clostridium difficile* infection can range from asymptomatic colonization to mild diarrhea and to more severe states including pseudomembranous colitis, a condition that could be life-threatening to the patients. *Clostridium difficile* infection most commonly develops after patient treatment with certain groups of antibiotics.

Clostridium difficile infection is a toxin-mediated intestinal disease. Toxin B is generally recognized as a major virulent factor associated with *Clostridium difficile* infection. This toxin is encoded by the *tcdB* gene. Some strains of *Clostridium difficile* are capable of producing additional toxins, including Toxin A and binary toxin, however the role of these two toxins in CDAD is less clear.

The Modaplex *Clostridium difficile* Analysis Kit is a molecular diagnostic test for the qualitative detection of toxigenic *Clostridium difficile* nucleic acids isolated and purified from liquid or soft stool specimens obtained from symptomatic patients. This test targets the *Clostridium difficile* toxin B encoding gene (*tcdB*).

Principle of the Procedure

The Modaplex *Clostridium difficile* Analysis Kit has been developed for use on the Modaplex System. This instrument platform integrates multiplex PCR-based amplification with capillary electrophoresis (CE) based detection of amplification products. Oligonucleotide primers are designed to produce PCR products with unique CE mobility, enabling simultaneous measurement of multiple targets and controls in a single reaction. Each individual test includes three target sites of the *tcdB* gene, Internal Control and Calibrators. Calibrators of three sizes are used for aligning and assigning CE peaks; this procedure is unique to the Modaplex System.

Kit format and components

The Modaplex *Clostridium difficile* Analysis Kit contains reagents that can be used to perform 50 tests.
It includes the following components:

25X Primers Mix
This tube contains the Oligonucleotide primers specific to the *tcdB* gene of *Clostridium difficile*, Internal Control and Calibration Controls. Each primer is either not labeled or has FAM or TYE-665 fluorescent label. The mix also contains salmon sperm DNA, BSA and Proclin.

2X PCR Buffer
This is a solution optimized to promote enzyme activity for the PCR in the Modaplex *Clostridium difficile* Analysis Kit. The buffer contains Tris buffer, KCl, dNTPs, MgCl2, betaine, BSA, DTT, glycerol and Proclin.

PCR Enzyme
Hot-start DNA Polymerase

25X Calibrators Mix
This is a liquid concentrate containing DNA template for Calibration Controls.

Internal Control
This non-infectious and non-contagious artificial DNA is used as internal control for this kit.

10X Injection Buffer
This liquid is used by diluting 1:10 to fill those wells in the PCR plate which are not being used for samples.

Positive Control
This tube contains a liquid concentrate containing DNA template for PCR Positive Controls.

Controls

The Modaplex *Clostridium difficile* Analysis Kit contains a control concept that comprises the controls listed below.

Calibration Control

A group of Modaplex specific elements is used to align electropherograms and assign identities of the target peaks. It also controls for the integrity of the kit reagents and the presence of PCR inhibitors in a given sample.

Internal Control

The Internal Control is a non-target nucleic acid that is co-extracted and co-amplified with the *tcdB* target. It controls for nucleic acid extraction efficiency, for the integrity of the reagents and for the presence of PCR inhibitors in a given sample. The Internal Control needs to be spiked into each sample before extraction.

Negative Control (NC)

The user needs to set up a negative control (no-template control) for each run. Substitute S.T.A.R. buffer for the clinical specimen and process normally through the extraction system and on the Modaplex Instrument. The negative control is used to assess any potential contamination while setting up the assay.

Positive Control (10x)

The user needs to set up a positive control for each run.

The Positive Control (10x) contains a plasmid of the *tcdB* gene. The results of the positive control are assessed to ensure that the kit performs within the stated acceptance criteria. The *tcdB* gene must be detected within acceptable ranges, which would confirm the proper functioning of the Primer Mix *Clostridium difficile*.

Prior to use, the Positive Control (10x concentrate) must be diluted 1:10 prior to use to ensure target concentration is at the appropriate level.

Known Positive Sample

It is also required to include previously characterized positive sample or simulated sample with every easyMAG extraction run and include it in the subsequent Modaplex Instrument run to verify the successful lysis.

Platform and software

The Modaplex *Clostridium difficile* Analysis Kit is designed to be used with the Modaplex instrument, software version 10.7. This platform is a fully automated, bench-top system for molecular diagnostic applications.

Materials Provided

Kit contents

The following reagents, which are needed to run the Modaplex *Clostridium difficile* Analysis Kit assay, are included in the Modaplex *Clostridium difficile* Analysis Kit. Table 1 summarizes the kit contents and indicates the storage conditions.

Table 1: Content of the Modaplex Clostridium difficile Analysis Kit

Component	Ingredients	Vials	Vol./Vial (µl)	Cap Color	Storage
2X PCR Buffer	a) Tris-HCl, KCl, dNTPs b) BSA Buffer c) Glycerol	1	1,500	Red	−20 °C ± 5 °C
PCR Enzyme	a) Taq Polymerase b) Glycerol	1	22	Orange	−20 °C ± 5 °C
25X Primers Mix	a) FAM or TYE labeled and unlabeled primer for *tcdB* gene of *Clostridium difficile* b) BSA Buffer	1	110	Purple	−20 °C ± 5 °C
25X Calibrators Mix	a) DNA b) BSA Buffer	1	110	Yellow	−20 °C ± 5 °C
Positive Control	a) Plasmid b) BSA Buffer	1	50	Green	−20 °C ± 5 °C
10X Injection Buffer	**a)** Capillary Electrophoresis Buffer DNA	2	1,500	Blue	−20 °C ± 5 °C
Internal Control	a) Plasmid b) BSA Buffer	1	500	Natural	−20 °C ± 5 °C

Package Inserts

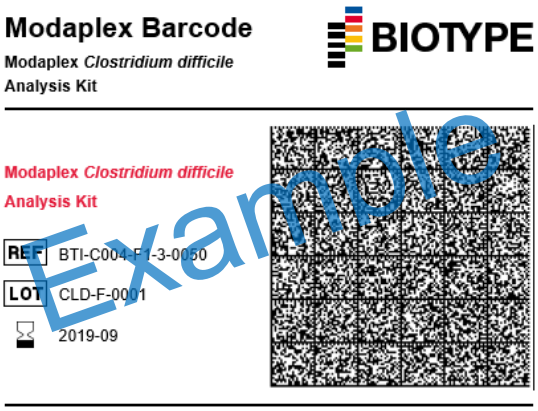
The Modaplex *Clostridium difficile* Analysis Kit is delivered with two packaging inserts—the Modaplex Barcodes and the IFU. Each kit must be used with the packaging inserts supplied therein. This guarantees that the test is set up according to the latest IFU and that the correct Modaplex assay definitions are used for analysis. Both inserts are described in the following subsections.

Modaplex Barcode

The package insert Modaplex Barcode *Clostridium difficile* contains the kit-specific Modaplex assay definition. Prior to the first use of a new Modaplex *Clostridium difficile* Analysis Kit, the barcode must be scanned. This automatically transfers the Modaplex *Clostridium difficile* Analysis Kit-related assay specifications, such as assay ID, expiration date, list of targets. In terms of traceability, the assay specifications are present in the software report once the barcode has been scanned and a Modaplex run has been performed with the new Modaplex *Clostridium difficile* Analysis Kit.

Note: The Modaplex barcode needs to be scanned only once, prior the first use of the lot.

Figure 1: Example of a Modaplex Barcode



Instructions for Use

The instructions for use for the Modaplex *Clostridium difficile* Analysis Kit are part of the kit. Each kit comes with the packaging insert IFU, in order to guarantee that the kit will be used according to the latest instructions for use.

Materials Required (Not Included in the Kit)

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the respective Safety Data Sheets (SDSs). In addition to the kit contents, the researcher will need the following:

Reagents and Consumables

- S.T.A.R. buffer (Roche Cat# 03 335 208 001)
- bioMérieux NucliSENS easyMAG™ System
- bioMérieux NucliSENS easyMAG reagents and disposables
- Vortexer
- Molecular grade water (Teknova Cat# W3350)
- 96-well plate sealer
- Control Sample – previously characterized positive sample or simulated sample
- Micropipettes with appropriate filtered nuclease free tips
- Disposable exam gloves
- 1.5 mL or 2mL Eppendorf tubes
- 15 mL Falcon tubes
- TE Buffer, pH 8.0 (Thermofisher Cat#: AM9849)
- PCR Microplates 96
- Mineral Oil
- Aluminum Sealing Film
- Modaplex Cartridge CE 48
- Modaplex Buffer
- Modaplex Decon
- Modaplex Wash
- Modaplex CE Gel
- Modaplex CE Plates

Equipment

- Modaplex System
- Micropipettes
- Bench-top centrifuge with rotor for 2-ml reaction tubes
- Bench-top centrifuge with plate adaptor
- Bench-top vortex

Warnings and Precautions

Intended for use with the Modaplex instrument.

Do not dispose of the sample and test waste in the sewage system. Discard sample and test waste according to local safety regulations.

Safety Information

General Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information about the Modaplex *Clostridium difficile* Kit components, please consult the corresponding SDSs. These are available upon request. For the safety information of the components not provided with the kit, please contact the respective supplier.

Modaplex Safety Information

Due to the high voltage required for CE separation, the failure to fill all wells is a general safety threat that may cause damage to the Modaplex instrument.

General Precautions

The user should always pay close attention to the following:

- Follow good laboratory practice guidelines.
- Only skilled laboratory technicians who are properly trained to perform reactions using PCR technology should be allowed to use this product.
- Clean and disinfect all surfaces according to the laboratory's standard operating procedure (SOP) guidelines.
- Use DNase-, RNase-, and DNA-free pipette tips with filters. Ensure that the pipettes have been calibrated according to the manufacturer's instructions.
- Change pipette tips after each pipetting step, in order to avoid sample mixing and cross-contamination.
- Do not reuse disposables.
- Open and close the reagent containers carefully.
- Follow the instructions for reagent storage and handling.
- Ensure that the reagents are not exposed to light during storage.
- Do not use reagents beyond their expiration dates.
- Do not substitute the reagents with the same from other manufacturers.
- Do not substitute the equipment listed in this document.
- Follow the instructions in the Modaplex System User Manual for the proper operation of the Modaplex System.

Note: Any wells in the PCR plate that are not being used for testing a sample must be filled with 50 µl of 1X Injection Buffer, included in the Modaplex *Clostridium difficile* Analysis Kit, and overlaid with mineral oil.

Note: Use extreme caution to prevent the contamination of the PCRs with synthetic control material. We recommend using separate, dedicated pipettes for setting up the reaction mixes and adding the DNA template. To avoid potential (cross-) contamination issues, separate the different procedure steps into at least two distinct working areas: one clean area for the preparation of the PCR master mixes and a second area for the addition of the DNA template samples to the PCR reactions and the operation of the Modaplex instrument.

Note: The reagents are validated for manual set-up. If an automated method is used, this may reduce the number of possible reactions due to the reagent required to fill "dead volumes" on these instruments.

Note: All reagents in the Modaplex *Clostridium difficile* Analysis Kit are formulated specifically for use with the stated test. All the reagents supplied in the kit are intended to be used solely with the other reagents in the same Modaplex *Clostridium difficile* Analysis Kit. The reagents in the kit must not be substituted if optimal performance is to be maintained.

Note: Use only the Taq DNA polymerase (PCR Enzyme) provided in the kit. Do not substitute with Taq DNA polymerase from other kits of the same or any other type, or with Taq DNA polymerases from another supplier.

Reagent Storage and Handling

The Modaplex *Clostridium difficile* Analysis Kit is shipped on dry ice. The assay should immediately be stored upon receipt at −15 °C to −25 °C in a constant-temperature freezer and protected from light. The fluorescent-labeled molecules must be protected from light to avoid photobleaching.

Repeated thawing and freezing should be avoided.

Do not use expired or incorrectly stored components.

It is recommended to store Internal Control and Positive Control outside of the clean area used to prepare PCR master mixes.

If stored under the recommended conditions, the Modaplex *Clostridium difficile* Analysis Kit will maintain performance through the indicated expiration date on the label.

All kit components are diluted optimally and no further treatment is necessary, except for the Positive Control and the Injection Buffer.

The Positive Control tube is delivered as a 10x concentrate. Prior to use, an appropriate amount must be diluted in a 1:10 ratio in a separate tube with a TE buffer (alternatively, with nuclease-free water), keeping in mind the number of positive control wells to be filled. The dilution should always be prepared fresh before setting up the Modaplex run.

The Injection Buffer tube is delivered as a 10x concentrate. Prior to use, an appropriate amount must be diluted in a 1:10 ratio in a separate tube with nuclease-free water, keeping in mind the number of empty wells to be filled. The dilution should always be prepared fresh before setting up the Modaplex run.

Note: Upon receipt, please check the Modaplex *Clostridium difficile* Analysis Kit immediately and contact Biotype GmbH's technical service if:

- A component is not properly frozen
- The kit labels are damaged
- The outside boxes have been opened
- The reagents are not present
- The packaging inserts (Modaplex Barcode and IFU) are missing

Procedure

Specimen collection and handling

Appropriate Specimen type for testing

This assay is intended for use with liquid or soft raw stool specimens. Do not use on well-formed stools or on other types of specimens.

Collecting the specimen

Standard stool collection and handling procedures are appropriate to obtain raw stool. Obtained sample must be placed in a sterile container that can be adequately sealed.

Storing Specimens

Samples should be tested as soon as possible. It is possible to store specimens refrigerated (2 - 8 ˚C) for up to 48 hours prior to testing. If sample cannot be processed within 48 hours, store frozen at -70 ˚C or below.

Note: Do not allow multiple freezing and thawing cycles for the samples. More than one freeze-thaw may produce a higher rate of invalid results.

Nucleic acid extraction

Preparation for the easyMAG

Determine the number of samples for the easyMAG run. Determine the total number (n) of Modaplex *Clostridium difficile* Analysis tests to be performed. Make sure to include the following controls:

- Negative Control
- Known Positive Sample
- Positive Control (Not processed through extraction)

Nucleic Acid Extraction Using Internal Control

1. Thaw, vortex and spin down Internal Control (natural cap), record lot number and expiration date.
2. Aliquot 400 µl of S.T.A.R. buffer to a fresh 1.5 mL or 2 mL tube(s).
3. Homogenize stool sample for testing by vortexing.
4. Using a wide-bore or pre-cut pipette tip, transfer 100 µl sample into the tube containing S.T.A.R. buffer.

   NOTE: For very viscous samples, the volume of S.T.A.R. buffer can be increased from 400 to 600 µl prior to centrifugation.
5. Include a Negative Control and Known Positive Sample in each extraction run.
6. Vortex and spin samples down at 13,000 g for 1 minute to obtain clarified lysate.
7. Add 10 µl of Internal Control to each sample or control port in the easyMAG extraction cartridge.
8. Transfer 80 µl of clarified lysate into the designated sample port in the easyMAG extraction cartridge.
9. Proceed with the nucleic acid extraction procedure according to the bioMérieux' NucliSENS easyMAG instructions. Elute in 60 µl.

   Note: Undiluted extracted DNA will be used with the Modaplex *Clostridium difficile* Analysis Kit.

Protocol: Preparing the Modaplex run

This protocol is for the preparation of the Modaplex instrument, prior to the set-up of the Modaplex *Clostridium difficile* Analysis run.

1. Determine the number of PCR reactions.

Before setting up the Modaplex instrument run, it is recommended that the number of PCR reactions is determined. Include the following controls in the calculation:

- Negative Control
- Known Positive Sample
- Positive Control (Not processed through extraction)

2. Determine which wells are to be filled with the 1x Injection Buffer.

This number is based on the number of capillaries on the cartridge, minus the total number of PCR reactions to be performed.

3. Add the Modaplex *Clostridium difficile* Analysis assay definition to the Modaplex instrument.

Read the Modaplex barcode for the Modaplex *Clostridium difficile* Analysis Kit included in the respective kit box. The assay definition will be automatically added to the Modaplex System. See the Modaplex System User Manual for further instructions.

Note: The Modaplex barcode needs to be scanned only once, prior to the first use of the lot.

4. Confirm the Modaplex settings for the Modaplex *Clostridium difficile* Analysis run.

Before setting up the Modaplex *Clostridium difficile* Analysis Kit, the following conditions regarding the consumables are to be satisfied for the planned Modaplex run:

- Sufficient number of remaining runs in the Modaplex cartridge
- Adequate quantities of the consumables

Note: To replace the Modaplex System cartridge or the consumables, please refer to the Modaplex System User Manual for further instructions.

5. Create a run definition and a plate map on the Modaplex instrument.

Note: Please refer to the Modaplex System User Manual for further instructions.

6. Register new lots of the Modaplex *Clostridium difficile* Analysis Kit.

Note: Please refer to the Modaplex System User Manual for further instructions.

7. Set up the run definition or run info.

Note: When setting up the run definition, deselect all quality controls on the Run Info tab.

Note: Please refer to the Modaplex System User Manual for further instructions.

8. Set up a sample plate map.

Note: When adding negative and positive controls to the plate map, use "_NC" and "_PC" as the sample names. Please refer to Point 12 of the section "Protocol: Setting up the Modaplex *Clostridium difficile* Analysis run", on Page 16, for the instructions for naming the samples.

Note: When assigning the single wells to the assay, always click "Set all" to ensure that all targets are detected correctly.

Note: Please refer to the Modaplex System User Manual for further instructions.

Protocol: Setting up the Modaplex *Clostridium difficile* Analysis run

This protocol is for the preparation of the reagents in the Modaplex *Clostridium difficile* Analysis Kit and the PCR plate for the Modaplex run.

1. Remove and thaw the following components from the Modaplex Clostridium difficile Analysis Kit.

- 2X PCR Buffer (red cap)
- PCR Enzyme (orange cap)
- 25X Primers Mix (purple cap)
- 25X Calibrators Mix (yellow cap)
- Positive Control (10x) (green cap)
- 10X Injection Buffer (blue cap)

Note: The PCR Enzyme is temperature-sensitive. Keep the enzyme at −20 °C ± 5 °C at all times.

2. Homogenize the thawed reagents by inverting the tubes, pipetting, or gently vortexing. Then, briefly centrifuge.

3. Dilute the Positive Control (10x).

Thaw, vortex and spin down Positive Control.

Add 72 µl molecular grade water to the 8 µl Positive Control aliquot tube. This will bring volume up to 80 µl. Diluted Positive Control should be discarded after reactions setup is complete.

Note: Aliquot the Positive Control for future use (if necessary). Thaw, vortex and spin down Positive Control (green cap), record lot number and expiration date. Make 5 aliquots 8 µl each, store at -20 °C or below until ready to use.

4. Preparing the Modaplex *Clostridium difficile* Analysis Master Mix.

In a dedicated clean area, prepare the PCR reagent Master Mix (MMx) in an appropriately sized microcentrifuge tube for the total number of samples to be tested. Use Table 2 to determine the volume of each reagent.

Table 2: Modaplex Clostridium difficile Analysis Master Mix (MMx): The volumes of the reagents needed for the master mix

Component	Volume of Reagents Calculated According to the # of Samples					
	# 1	# 2	# 3	# 4	# 5	# 10
2X PCR Buffer	25.0 µl	50.0 µl	75.0 µl	100.0 µl	125.0 µl	250.0 µl
PCR Enzyme	0.4 µl	0.8 µl	1.2 µl	1.6 µl	2.0 µl	4.0 µl
25X Primers Mix	2.0 µl	4.0 µl	6.0 µl	8.0 µl	10.0 µl	20.0 µl
Molecular Grade Water	10.6 µl	21.2 µl	31.8 µl	42.4 µl	53.0 µl	106.0 µl
25X Calibrators Mix [1]	2.0 µl	4.0 µl	6.0 µl	8.0 µl	10.0 µl	20.0 µl
Total Volume of Master Mix (MMx)	**40.0 µl**	**80.0 µl**	**120.0 µl**	**160.0 µl**	**200.0 µl**	**400.0 µl**

[1]**Note**: It is recommended to add the 25x calibrators mix in a PCR lab.

Note: As a rule of thumb, if you are testing fewer than 10 samples, use enough master mix for one extra sample. If you are testing 10 or more samples, use an excess reagent master mix volume of + 10 %.

5. **Mix gently** by inverting the tube or by pipetting. Briefly, spin down in a bench-top microcentrifuge.

6. **Aliquot 40 µl** of the Modaplex *Clostridium difficile* Analysis Master Mix to the designated wells in the PCR plate.

7. **Add 10 µl** each of the following:

   - Undiluted Extracted DNA (template), to be added to the corresponding sample well(s)
   - Known Positive Sample control to be added to the corresponding positive sample well
   - Negative Control, to be added to the negative control well
   - Diluted Positive Control, to be added to the positive control well

   Note: The total volume of the PCR reaction is 50 µl

8. **Add 50 µl of 1x Injection Buffer (1x IB)** to the remaining empty wells that are not used for PCR, NC, or PC.
   Refer to Figure 3 for an example of the sample plate layout.

   - Prepare a 1:10 dilution of the 10x Injection Buffer in water.
   - Add 50 µl of the 1x Injection Buffer to each of the empty wells. Please refer to the plate map set-up in the Modaplex System Instructions for Use for further explanation.

Due to the high voltage required for the CE separation, the failure to fill all wells is a general safety threat and may damage the Modaplex instrument.

Figure 2: Example of a sample plate layout for 30 samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 01 MMx	Sample 09 MMx	Sample 17 MMx	Sample 25 MMx	Empty 1x IB	Empty 1x IB						
B	Sample 02 MMx	Sample 10 MMx	Sample 18 MMx	Sample 26 MMx	Empty 1x IB	Empty 1x IB						
C	Sample 03 MMx	Sample 11 MMx	Sample 19 MMx	Sample 27 MMx	Empty 1x IB	Empty 1x IB						
D	Sample 04 MMx	Sample 12 MMx	Sample 20 MMx	Sample 28 MMx	Empty 1x IB	Empty 1x IB						
E	Sample 05 MMx	Sample 13 MMx	Sample 21 MMx	Sample 29 MMx	Empty 1x IB	Empty 1x IB						
F	Sample 06 MMx	Sample 14 MMx	Sample 22 MMx	C.diff. pos. MMx	Empty 1x IB	Empty 1x IB						
G	Sample 07 MMx	Sample 15 MMx	Sample 23 MMx	C.diff._PC MMx	Empty 1x IB	Empty 1x IB						
H	Sample 08 MMx	Sample 16 MMx	Sample 24 MMx	C.diff._NC MMx	Empty 1x IB	Empty 1x IB						

9. **Seal** the PCR plate with aluminum sealing film. Gently vortex and spin the PCR plate in a tabletop centrifuge.

10. **Remove** the seal and overlay all 48 wells on the PCR plate with one drop of mineral oil.
    Ensure that each reaction is fully covered by oil.

11. **Seal** the PCR plate again with aluminum sealing film. Spin the PCR plate and CE plate in a tabletop centrifuge.

The plate cover seals must be **removed** from the PCR plate and from the CE plate before they are placed on the Modaplex instrument.

12. **Run the PCR plate** in the Modaplex instrument.

For automatic result interpretation and data assignment, the software requires the following form of sample naming (if you would like to avoid the sample name, any number or code that identifies a sample could be used instead).

Positive Control:	*Sample Name_PC*
Negative Control:	Sample Name_NC
Sample Well:	*Sample Name*
Known Positive Sample	*C.diff. pos.*

13. **End of Run**

At the end of the run, seal the PCR and CE plates with aluminum sealing film before disposing of the plates. Decontaminate the Hold Down.

Note: Refer to the Modaplex System Instructions for Use for further instructions.

Interpretation of the Results

The Modaplex System software performs automatic processing of the raw data for each of the specimens and controls. In brief, electropherograms are analyzed and screened for CE quality and performance of internal controls. If a result for a given sample is not meeting quality requirements, the sample is marked as invalid and the cause is reported to the operator (See page 15 for description of invalidity codes and suggested actions to resolve).

Note: Detection of the Internal Control is not required for a positive result.

The Modaplex System provides the operator with the following test result for the *tcdB* target:

- **Positive** - Amplification of appropriate nucleic acid is detected.
- **Negative** - No amplification matching the expected target size is detected.
- **Invalid, with the invalidity code -** The system was unable to accept the data quality for a given sample. Please refer to Modaplex data analysis invalidity codes on page 15 and the Modaplex User Manual for further explanations, descriptions of invalidity codes and actions to resolve invalidity conditions.

Run validation

The first step in the results interpretation is to ensure that the run is valid. The steps in run validation are described in Table 3.

Table 3: Modaplex Instrument Run validation. *See also Duplicate Run Controls Section: If there were duplicates of a control per plate, special run validation provisions may apply.*

Control type	*tcdB* target	Internal Control	Result
A) Negative Control	Negative	Pass	Control valid, Proceed to B)
	Positive, Invalid or not run	NA	Run invalid
B) Positive Control and Known Positive Sample	Negative, Invalid or not run	NA	Run invalid
	Positive	NA	Control valid, Run valid

Note: NA – not applicable

Duplicate Run Controls

When duplicates of a run control are included with the run, the following would apply:

a) If both replicates of a given control are invalid or inappropriate then run is invalid.

b) If one of the replicates of a given control is determined to be invalid due to low CE quality or low amplification curve quality (see Table 5 for the list of invalidity codes), it can be excluded from consideration. One of the replicates is required to be determined as valid and provide appropriate result for the control type in order to validate the run according to Table 3.

c) If one of the replicates is invalid for any reason other than listed in (b) then the run should be considered invalid.

Invalid Run

- Negative Control is positive or invalid: repeat all extractions, including Negative Control and prepare new PCR reactions using re-extracted samples.
- Positive control is negative or invalid: prepare new reactions using the same extracts and a new aliquot of Positive Control.

Individual sample results

Once the Modaplex System run is validated, results for the individual specimens are determined according to Table 4.

Table 4: Individual sample result

tcdB target	Internal Control	Test result
Positive	NA	Toxigenic *Clostridium difficile* detected
Negative	Pass	Toxigenic *Clostridium difficile* not detected
	Fail or Invalid	Invalid
Invalid	NA	

Note: NA – not applicable

Troubleshooting

Modaplex data analysis invalidity codes and suggested course of action

Modaplex System software performs automatic control over the quality of the acquired data. If a result for a given sample is found not meeting quality requirements, the sample is marked as invalid and the cause is reported to the operator. Please refer to Table 5 for the description of invalidity codes and suggested actions to resolve**.**

Table 5: Modaplex Instrument data analysis invalidity codes and suggested course of action to resolve. *(See also user manual for additional information.)*

Message	Priority	Explanation	Potential cause and resolution
(A). Low CE quality	8	Capillary electrophoresis data did not have adequate quality to support the analysis of sample results	Errors during CE electrophoresis. Re-run extracted sample on Modaplex.
(B). Low Calibrator Control quality	7	Internal calibrators did not amplify or had inadequate quality	1. PCR inhibition in the sample. Repeat extraction and re-run on Modaplex.
			2. Compromised kit reagents. Re-run with reagents from a new kit.
(C). Low amplification curve quality	6	A target was amplified, but the quality of the amplification curve was inadequate for estimating the result for a target	PCR amplification or data analysis error. Re-run extracted sample on Modaplex.
(D). Sensitivity Control Failure	5	Not used as a component in this assay	If reported, treat as (E) – Extraction Control out of range
(E). Extraction Control out of range	4	Internal Control measurement was not within the specified range	Error during extraction or PCR inhibition. Repeat extraction and re-run.
(F). Positive result in Negative Control Well	3	*tcdB* target is detected in Negative Run Control well	1. Error in Negative Control setup. Re-extract Negative Control and re-run on a separate easyMAG and Modaplex instrument run before re-testing patient samples.
			2. Compromised kit reagents. Re-run Negative Control extract on a separate Modaplex instrument run with reagents from a new kit before re-testing patient samples.
			3. *tcdB* target contamination. Identify and eliminate contamination source. Re-extract Negative Control and re-run on a separate Modaplex instrument run before re-testing patient samples.
(G). Negative result in Positive Control or Known Positive Sample well	2	*tcdB* target is not detected in Positive Run Control well	1. PCR inhibition in the Known Positive Sample. Repeat extraction and re-run on a separate Modaplex Instrument run before re-testing patient samples.
			2. Error in Control reaction setup. Re-run Positive Control and Known Positive Sample on a separate Modaplex instrument run before re-testing patient samples.
			3. Compromised kit reagents. Re-run Positive Control and Known Positive Sample on a separate Modaplex instrument run with reagents from a new kit before re-testing patient samples.
(H). Potential High Positive	1	Modaplex instrument reports a sample control failure that could be caused by excessive concentration of *tcdB* target.	*tcdB* target concentration is potentially too high to be measured. Dilute the extracted sample 1:100 with molecular grade water and re-run in a different PCR reaction well on the Modaplex. If the sample is called positive upon re-run, report a positive. If the sample is called negative upon re-run, repeat extraction and re-run without dilution in a different PCR reaction well on Modaplex. Note: 1:100 dilution of the extract should not be repeated more than twice.

Limitation of the Test

- This test detects but does not differentiate the NAP1 (Ribotype 027) strain from other toxigenic strains of *Clostridium difficile*.
- The Modaplex *Clostridium difficile* Analysis Kit is a nucleic-acid based molecular diagnostic test. Results should be interpreted by a qualified healthcare professional in conjunction with information from patient clinical evaluation.
- The test detects presence of *Clostridium difficile tcdB* gene. It will not detect strains that do not have the *tcdB* gene.
- Due to toxin sequence similarity, Modaplex *Clostridium difficile* Analysis Assay exhibit cross-reactivity with *C. sordelii*. Typically, *C. sordelii* is a lung pathogen and is not found in stool samples.
- Testing samples high in fecal fat may result in elevated rate of invalid results.
- The detection of bacterial nucleic acid is dependent on proper specimen collection, handling and preparation. Failure to follow instructions for collection, handling and preparation may cause incorrect results.

Performance Characteristics

Clinical studies

Performance characteristics of the Modaplex *Clostridium difficile* Analysis Kit run on the Modaplex instrument and platform were established during a prospective study, conducted at three US clinical sites. The Study was designed to test leftover raw stool specimens that were submitted to clinical lab for *Clostridium difficile* testing. Reference testing was performed at a centralized reference laboratory with Toxigenic *Clostridium difficile* Culture Assay employed as a reference method. The study collected 1103 patient samples, of which 969 specimens were found fully compliant with study protocol and analyzed. Of the 969 samples analyzed during the course of the study, 901 were reported as resolved by Modaplex system, providing a 93 % first pass valid results rate. Upon retest of the 68 invalid samples, 17 samples remained invalid (1.8 % of all analyzed samples). 3 out of the 17 unresolved invalids were reported positive by the reference method. 952 study samples with valid test results were used in statistical data analysis. Clopper-Pearson exact method was used to calculate confidence intervals.

Demographics details of the study subject's population is presented in the following table:

Age Group (years)	n[%]
2 - 5	77 (8.1 %)
6 - 21	261 (27.4 %)
22 - 59	283 (29.7 %)
>= 60	331 (34.8 %)

In the study, Modaplex *Clostridium difficile* Analysis Kit demonstrated following performance characteristics:

		Toxigenic *Clostridium difficile* Culture Assay		
		Positive	Negative	Total
Modaplex Clostridium difficile Analysis Kit	Positive	153	20[a]	173
	Negative	17[b]	762	779
	Total	170	782	952

Sensitivity (95 % CI)	90.0 (84.5 - 94.1)
Specificity (95 % CI)	97.4 (96.1 - 98.4)
Accuracy (95 % CI)	96.1 (94.7 - 97.2)
Prevalence (95 % CI)	17.9 (15.5 - 20.4)
Positive Predictive Value (95 % CI)	88.4 (82.7 – 92.8)
Negative Predictive Value (95 % CI)	97.8 (96.5 - 98.7)
Kappa Statistic	0.868

Discordant testing was performed for samples where Modaplex *Clostridium difficile* Analysis Kit and reference Toxigenic *C. difficile* Culture Assay reported results in disagreement. Discordant analysis included microbiological isolation and PCR targeting of three appropriate regions of the toxin B gene (different recognition sites than the ones used in the Modaplex *Clostridium difficile* Analysis Assay) with bi-directional DNA sequencing.

[a] 6 samples reported positive by discordant analysis

[b] 14 samples reported positive by discordant analysis

Reproducibility

Reproducibility of the Modaplex *Clostridium difficile* Analysis Kit was evaluated at three independent laboratory sites. The reproducibility study panel included four simulated samples –moderate positive, low positive (near assay limit of detection, expected positive > 95 % of the time), negative (expected positive < 5 % of the time) and C20-80, expected positive 20 - 80 % of the time. The panel also included positive and negative controls. Panel samples were tested at each site for five days with two runs per day and three replicates of each panel member per run. Study results are summarized in table 6:

Table 6:Reproducibility - test results

	Site 1				Site 2				Site 3				Overall			
	Agreement		*tcdB* Ct		Agreement		*tcdB* Ct		Agreement		*tcdB* Ct		Agreement		*tcdB* Ct	
	n	%	Avg.	%CV	n	%	Avg.	%CV	n	%	Avg.	%CV	n	%	Avg.	%CV
C20-80 [1]	23/30	77	34.4	5.3	23/30	77	34.5	5.1	16/30	53	34.4	6.1	62/90	69	34.5	*5.1*
Moderate Positive	30/30	100	24.6	1.2	30/30	100	24.5	1.2	29/29	100	24	1.3	89/89	100	24.4	1.6
Low Positive	29/29	100	28.5	1.1	30/30	100	28.1	1.1	30/30	100	27.7	0.4	89/89	100	28.2	1.6
Negative [2]	30/30	100	26.2	1.3	30/30	100	25.7	0.9	30/30	100	25.2	0.8	90/90	100	25.7	1.9
Negative control [2]	20/20	100	25.9	1.7	20/20	100	25.4	1.1	20/20	100	25.1	1.1	60/60	100	25.5	1.9
Positive control	20/20	100	32.0	2.5	20/20	100	31.0	1.7	20/20	100	30.8	2	60/60	100	31.3	2.6

[1] For C20-80 samples % agreement is given as % positive results

[2] Ct value statistics are calculated using Internal Control

Limit of detection

Analytical sensitivity of the Modaplex *Clostridium difficile* Analysis Kit was determined using a twofold serial dilution of two *C. difficile* strains that were spiked into qualified negative stool and processed according to instructions for use. 20 replicates at each concentration level were tested using the Modaplex *Clostridium difficile* Analysis Kit, each of the replicates were further tested on three different Modaplex instruments. To determine CFU counts, another 20 replicates from the same serial dilution was plated and cultured for the number of colonies after 48h incubation. Analytical sensitivity of the assay was defined as the lowest concentration at which at least 95 % of all replicates were reported positive.

Table 7: Limit of detection – testing result

Strain	LOD (CFU/rxn)
Clostridium difficile ATCC strain 43255 (Strain VPI 10463, Toxinotype 0, A+B+)	8
Clostridium difficile ATCC strain BAA-1805 (BI/NAP1/027, Toxinotype III)	2

Analytical reactivity

To assess the analytical inclusivity of the Modaplex *Clostridium difficile* Analysis Kit, a set of 20 additional toxigenic strains of *C. difficile* were spiked into negative matrix (pool of negative clinical samples) at the level approximately three times above the assay LOD. Spiked samples were processed in accordance with the Instructions for Use.

Table 8: Analytical reactivity - results

Strain	Toxinotype	Result
BAA-1382 (630)	A+B+	Positive
BAA-1871 (4111)	0, A+B+ binary-, NAP5	Positive
9689 (90556-M6S)	0	Positive
700792 (14797-2)	A+B+	Positive
BAA-1875 (5325)	V, A+B+, NAP7	Positive
51695 (BDMS 18 AN)	A+B+	Positive
43598 (1470)	VIII, A-B+	Positive
43600 (2149)	A+B+	Positive
43599(2022)	A+B+	Positive
43597	A+B+	Positive
43594 (W1194)	A+B+	Positive
43596 (545)	I, A+B+	Positive
17858 (1253)	A+B+	Positive
17857 (870)	A+B+	Positive
BAA-1808	A+B+	Positive
BAA-1806	A+B+	Positive
BAA-1803	III A+B+, NAP1	Positive
BAA-1814	XXII	NA*
BAA-1870 (4118)	III, binary+, NAP1	Positive
BAA-1873 (5283)	0, A+, B+,binary-	Positive

*NA: not applicable because the strain was not viable

Interference

A chemical interference study was performed using a panel of samples, consisting of pooled negative clinical matrix and contrived samples produced by supplementing pooled negative clinical matrix with culture stock of two *C. difficile* strains, ATCC BAA-1805 and 43255, added to produce samples resulting in 9 and 21 CFU/rxn, respectively (approximately three times the assay LOD). Table 9 shows potential interfering substances used in this study.

Table 9: Potential interfering substances

Substance	Active Ingredient	Concentration
Anti-Fungal /Anti-Itch Vaginal	Nystatin	1 % (w/v)
Creams/Ointments/Suppositories	Hydrocortisone	1 % (w/v)
Anti-Hemorrhoid Creams/Ointments	Phenylephrine	1 % (w/v)
Antacids	Calcium Carbonate/ Aluminum Hydroxide/ Magnesium Hydroxide	10 % (w/v)
Enemas	Mesalazine/Mineral Oil	10 % (w/v)
Condoms with Spermicidal Lubricant	Nonoxynol-9	1 % (w/v)
Anti-Diarrheal Medication	Loperamide Hydrochloride/ Bismuth Subsalicylate	10 % (w/v)
Laxatives	Sennosides	1 % (w/v)
Antibiotic	Metronidazole	12.5 mg/mL
Antibiotic	Vancomycin	12.5 mg/mL
Non-Steroidal Anti-Inflammatory Medications	Naproxen Sodium	12.5 mg/mL
Moist Towelettes	Benzalkonium Chloride, Ethanol	0.1 % (v/v), 1 %(v/v)
Fecal Fat	Lipids, etc.	40 % w/v
Whole Blood	Glucose, Hormones, Enzymes, Ions, Iron, etc.	40 % v/v
Mucus	Mucin protein	3.5 % (w/v)

None of the substances interfered with detection of *C. difficile* from both tested strains or caused a false positive result in the negative samples. Results of the study revealed potential of producing elevated levels of invalid results in samples rich in fecal fat.

Cross reactivity

A microbial cross-reactivity study was performed using a panel of samples, consisting of pooled negative clinical matrix. Table 10 shows potential cross-reactive organisms used in this study and study results.

Table 10: Cross reactivity with Modaplex C. difficile assay

Pathogen/non-pathogen	Concentration	Modaplex *C. difficile* result Cross-reactivity
Abiotrophia defectiva	$1x10^7$ orgs/mL	Negative
Acinetobacter baumannii	$1x10^7$ orgs/mL	Negative
Aeromonas hydrophila	$1x10^7$ orgs/mL	Negative
Bacillus cereus	$1x10^7$ orgs/mL	Negative
Bacteroides fragilis	$1x10^7$ orgs/mL	Negative
Bifidobacterium adolescentis	$1x10^7$ orgs/mL	Negative
Campylobacter coli	$1x10^7$ orgs/mL	Negative
Campylobacter jejuni subsp. jejuni	$1x10^7$ orgs/mL	Negative
Candida albicans	$1x10^7$ orgs/mL	Negative
Citrobacter freundii	$1x10^7$ orgs/mL	Negative
Clostridium beijerinckii	$1x10^7$ orgs/mL	Negative
Clostridium bifermentans	$1x10^7$ orgs/mL	Negative
Clostridium chauvoei	$1x10^7$ orgs/mL	Negative
Clostridium difficile 43593	$1x10^7$ orgs/mL	Negative
Clostridium difficile 43601	$1x10^7$ orgs/mL	Negative [1]
Clostridium difficile 43602	$1x10^7$ orgs/mL	Negative
Clostridium difficile 700057	$1x10^7$ orgs/mL	Negative
Clostridium difficile BAA-1801	$1x10^7$ orgs/mL	Negative
Clostridium haemolyticum	$1x10^7$ orgs/mL	Negative
Clostridium histolyticum	$1x10^7$ orgs/mL	Negative
Clostridium novyi	$1x10^7$ orgs/mL	Negative
Clostridium orbiscindens	$1x10^7$ orgs/mL	Negative
Clostridium perfringens	$1x10^7$ orgs/mL	Negative
Clostridium scindens	$1x10^7$ orgs/mL	Negative
Clostridium septicum	$1x10^7$ orgs/mL	Negative
Clostridium sordellii [2]	$1x10^7$ orgs/mL	Positive [2]
Clostridium sporogenes	$1x10^7$ orgs/mL	Negative
Clostridium symbiosum	$1x10^7$ orgs/mL	Negative
Clostridium tetani	$1x10^7$ orgs/mL	Negative
Edwardsiella tarda	$1x10^7$ orgs/mL	Negative
Enterococcus dispar	$1x10^7$ orgs/mL	Negative
Enterobacter cloacae	$1x10^7$ orgs/mL	Negative
Entamoeba histolytica	$1x10^7$ orgs/mL	Negative
Enterococcus faecium vanA	$1x10^7$ orgs/mL	Negative
Enterococcus faecalis vanB	$1x10^7$ orgs/mL	Negative
Escherichia coli	$1x10^7$ orgs/mL	Negative
Escherichia coli (serotype O157:H7)	$1x10^7$ orgs/mL	Negative
Escherichia fergusonii	$1x10^7$ orgs/mL	Negative
Escherichia hermannii	$1x10^7$ orgs/mL	Negative
Helicobacter pylori	$1x10^7$ orgs/mL	Negative
Klebsiella pneumoniae	$1x10^7$ orgs/mL	Negative
Lactobacillus acidophilus	$1x10^7$ orgs/mL	Negative
Lactococcus lactis	$1x10^7$ orgs/mL	Negative
Listeria grayi	$1x10^7$ orgs/mL	Negative
Listeria monocytogenes	$1x10^7$ orgs/mL	Negative
Peptostreptococcus anaerobius	$1x10^7$ orgs/mL	Negative
Plesiomonas shigelloides	$1x10^7$ orgs/mL	Negative
Porphyromonas asaccharolytica	$1x10^7$ orgs/mL	Negative
Proteus mirabilis	$1x10^7$ orgs/mL	Negative
Providencia alcalifaciens	$1x10^7$ orgs/mL	Negative
Pseudomonas aeruginosa	$1x10^7$ orgs/mL	Negative
Salmonella enterica serovar Typhimurium	$1x10^7$ orgs/mL	Negative
Salmonella enterica subsp. arizonae	$1x10^7$ orgs/mL	Negative
Salmonella enterica subsp. enterica	$1x10^7$ orgs/mL	Negative
Serratia liquefaciens	$1x10^7$ orgs/mL	Negative
Serratia marcescens	$1x10^7$ orgs/mL	Negative
Shigella boydii	$1x10^7$ orgs/mL	Negative
Shigella dysenteriae	$1x10^7$ orgs/mL	Negative
Shigella sonnei	$1x10^7$ orgs/mL	Negative
Staphylococcus aureus	$1x10^7$ orgs/mL	Negative
Staphylococcus epidermidis	$1x10^7$ orgs/mL	Negative
Streptococcus agalactiae	$1x10^7$ orgs/mL	Negative
Vibrio cholerae	$1x10^7$ orgs/mL	Negative
Vibrio parahaemolyticus	$1x10^7$ orgs/mL	Negative
Yersinia enterocolitica	$1x10^7$ orgs/mL	Negative
Adenovirus	$1x10^6$ TCID50/mL	Negative
Rotavirus	$1x10^{5.5}$ TCID50/mL	Negative
Norovirus	$1x10^6$ TCID50/mL	Negative

Pathogen/non-pathogen	Concentration	Modaplex *C. difficile* result Cross-reactivity
Enterovirus	$1x10^6$ TCID50/mL	Negative
Echovirus	$1x10^6$ TCID50/mL	Negative
Coxsackie virus	$1x10^6$ TCID50/mL	Negative
Cytomegalovirus	$1x10^{5.5}$ TCID50/mL	Negative

Note[1]: One of three replicates for *Clostridium difficile* 43601 was reported positive at 14 cps/rxn, just above the assay cut-off set at 12 cps/rxn.

Note[2]: *Clostridium sordellii* is not typically found in gastrointestinal tract. Organism was included in cross-reactivity testing due to high toxin sequence homology identified during sequence database screen.

Inhibition by other microorganisms

Microbial interference study was performed using panel of samples produced by supplementing pooled negative clinical matrix with culture stock of two *C. difficile* strains, ATCC BAA-1805 and 43255, added to produce samples resulting in 9 and 21 CFU/rxn, respectively. Table 11 shows potential interfering organisms used in this study and testing results.

Table 11: Microbial interreference study results

Pathogen/non-pathogen	Concentration	Modaplex *C.difficile* result	
		BAA-1805	VPI 10463
Abiotrophia defectiva	$1x10^7$ orgs/mL	Positive	Positive
Acinetobacter baumannii	$1x10^7$ orgs/mL	Positive	Positive
Aeromonas hydrophila	$1x10^7$ orgs/mL	Positive	Positive
Bacillus cereus	$1x10^7$ orgs/mL	Positive	Positive
Bacteroides fragilis	$1x10^7$ orgs/mL	Positive	Positive
Bifidobacterium adolescentis	$1x10^7$ orgs/mL	Positive	Positive
Campylobacter coli	$1x10^7$ orgs/mL	Positive	Positive
Campylobacter jejuni subsp. jejuni	$1x10^7$ orgs/mL	Positive	Positive
Candida albicans	$1x10^7$ orgs/mL	Positive	Positive
Citrobacter freundii	$1x10^7$ orgs/mL	Positive	Positive
Clostridium beijerinckii	$1x10^7$ orgs/mL	Positive	Positive
Clostridium bifermentans	$1x10^7$ orgs/mL	Positive	Positive
Clostridium chauvoei	$1x10^7$ orgs/mL	Positive	Positive
Clostridium difficile 43593	$1x10^7$ orgs/mL	Positive	Positive
Clostridium difficile 43601	$1x10^7$ orgs/mL	Positive	Positive
Clostridium difficile 43602	$1x10^7$ orgs/mL	Positive	Positive
Clostridium difficile 700057	$1x10^7$ orgs/mL	Positive	Positive
Clostridium difficile BAA-1801	$1x10^7$ orgs/mL	Positive	Positive
Clostridium haemolyticum	$1x10^7$ orgs/mL	Positive	Positive
Clostridium histolyticum	$1x10^7$ orgs/mL	Positive	Positive
Clostridium novyi	$1x10^7$ orgs/mL	Positive	Positive
Clostridium orbiscindens	$1x10^7$ orgs/mL	Positive	Positive
Clostridium perfringens	$1x10^7$ orgs/mL	Positive	Positive
Clostridium scindens	$1x10^7$ orgs/mL	Positive	Positive
Clostridium septicum	$1x10^7$ orgs/mL	Positive	Positive
Clostridium sordellii	$1x10^7$ orgs/mL	Positive*	Positive*
Clostridium sporogenes	$1x10^7$ orgs/mL	Positive	Positive
Clostridium symbiosum	$1x10^7$ orgs/mL	Positive	Positive
Clostridium tetani	$1x10^7$ orgs/mL	Positive	Positive
Edwardsiella tarda	$1x10^7$ orgs/mL	Positive	Positive
Enterococcus dispar	$1x10^7$ orgs/mL	Positive	Positive
Enterobacter cloacae	$1x10^7$ orgs/mL	Positive	Positive
Entamoeba histolytica	$1x10^7$ orgs/mL	Positive	Positive
Enterococcus faecium vanA	$1x10^7$ orgs/mL	Positive	Positive
Enterococcus faecalis vanB	$1x10^7$ orgs/mL	Positive	Positive
Escherichia coli	$1x10^7$ orgs/mL	Positive	Positive
Escherichia coli (serotype O157:H7)	$1x10^7$ orgs/mL	Positive	Positive
Escherichia fergusonii	$1x10^7$ orgs/mL	Positive	Positive
Escherichia hermannii	$1x10^7$ orgs/mL	Positive	Positive
Helicobacter pylori	$1x10^7$ orgs/mL	Positive	Positive
Klebsiella pneumoniae	$1x10^7$ orgs/mL	Positive	Positive
Lactobacillus acidophilus	$1x10^7$ orgs/mL	Positive	Positive
Lactococcus lactis	$1x10^7$ orgs/mL	Positive	Positive
Listeria grayi	$1x10^7$ orgs/mL	Positive	Positive
Listeria monocytogenes	$1x10^7$ orgs/mL	Positive	Positive
Peptostreptococcus anaerobius	$1x10^7$ orgs/mL	Positive	Positive
Plesiomonas shigelloides	$1x10^7$ orgs/mL	Positive	Positive
Porphyromonas asaccharolytica	$1x10^7$ orgs/mL	Positive	Positive
Proteus mirabilis	$1x10^7$ orgs/mL	Positive	Positive

Pathogen/non-pathogen	Concentration	Modaplex *C.difficile* result	
		BAA-1805	VPI 10463
Providencia alcalifaciens	$1x10^7$ orgs/mL	Positive	Positive
Pseudomonas aeruginosa	$1x10^7$ orgs/mL	Positive	Positive
Salmonella enterica serovar Typhimurium	$1x10^7$ orgs/mL	Positive	Positive
Salmonella enterica subsp. arizonae	$1x10^7$ orgs/mL	Positive	Positive
Salmonella enterica subsp. enterica	$1x10^7$ orgs/mL	Positive	Positive
Serratia liquefaciens	$1x10^7$ orgs/mL	Positive	Positive
Serratia marcescens	$1x10^7$ orgs/mL	Positive	Positive
Shigella boydii	$1x10^7$ orgs/mL	Positive	Positive
Shigella dysenteriae	$1x10^7$ orgs/mL	Positive	Positive
Shigella sonnei	$1x10^7$ orgs/mL	Positive	Positive
Staphylococcus aureus	$1x10^7$ orgs/mL	Positive	Positive
Staphylococcus epidermidis	$1x10^7$ orgs/mL	Positive	Positive
Streptococcus agalactiae	$1x10^7$ orgs/mL	Positive	Positive
Vibrio cholerae	$1x10^7$ orgs/mL	Positive	Positive
Vibrio parahaemolyticus	$1x10^7$ orgs/mL	Positive	Positive
Yersinia enterocolitica	$1x10^7$ orgs/mL	Positive	Positive
Adenovirus	$1x10^6$ TCID50/mL	Positive	Positive
Rotavirus	$1x10^{5.5}$ TCID50/mL	Positive	Positive
Norovirus	$1x10^6$ TCID50/mL	Positive	Positive
Enterovirus	$1x10^6$ TCID50/mL	Positive	Positive
Echovirus	$1x10^6$ TCID50/mL	Positive	Positive
Coxsackie virus	$1x10^6$ TCID50/mL	Positive	Positive
Cytomegalovirus	$1x10^{5.5}$ TCID50/mL	Positive	Positive

Note*: Positive result may have contribution from *Clostridium sordellii* cross-reactivity

Carry-over and cross-contamination

Samples for carry-over and cross-contamination study were prepared by extraction of a series of high positive samples, with analyte concentration exceeding the concentration found in 95 % positive samples in the intended use population, alternating with negative samples. Location of positive and negative samples on the extraction instrument was altered run-to-run. On the Modaplex system, high positive and negative samples were run in a checkerboard fashion. In the consecutive run, the platemap was inverted to allow wells and capillaries that were running negative samples to run positive samples. The study included 6 runs, each run had 24 high positive and 24 negative samples. The study identified no contamination in the negative samples run in the course of the study, reporting FP rate at 0 % (0 false positives out of 141 valid negatives). These data allow for the conclusion there was no Well-to-well Cross Contamination or Run-to-run Carryover Contamination observed in the course of this study.

Limitations

All results obtained with the product must be interpreted within the context of all relevant laboratory findings. The kit is clinically validated and can be used in diagnostic procedures.

The product is to be used by specifically instructed personnel only. These personnel must be properly trained to use the Modaplex platform.

The product is intended for use with the Modaplex platform only.

For optimal results, strict compliance with the Modaplex *Clostridium difficile* Analysis Kit Instruction for Use (IFU) is required. The dilution of other reagents than these described in this IFU is not recommended and will result in a loss of performance.

Attention should be paid to expiration dates and storage conditions printed on the box and on the labels of all components. Do not use expired or incorrectly stored components.

Warranties and Disclaimer

This product is warranted to perform as described in this package insert when used in strict conformity with the instructions herein regarding its use. It is the user's responsibility to ensure that a given product is fit for a given application.

This product is warranted to perform as described when used in strict conformity with the instructions herein. The product has been designed for in vitro diagnostic use and is to be used solely by qualified professionals. It is the user's responsibility to ensure that a given product is suitable for a given application.

Biotype GmbH provides no other warranty, expressed or implied, and disclaims any implied warranty of merchantability or fitness for a particular purpose. Under no circumstances whatsoever shall Biotype be liable for any indirect, special, or consequential damages.

References

Modaplex System User Manual

Symbols

The following symbols may appear on the packaging and labeling:

Contains reagents sufficient for 50 reactions

Expiration date

Catalog number

Manufacturer

Temperature limitation

Protect from moisture

Follow instructions (IFU) for use

In vitro diagnostics

Batch number

Date of manufacturing

Protect from sunlight

Technical Assistance

For technical assistance or information, please call Biotype GmbH at +49-(0)351-8838 400.

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Ordering Information

Product	Contents	Cat. no.
Modaplex *Clostridium difficile* Analysis Kit	For 50 reactions: 25X Primers Mix, 2X PCR Buffer, PCR Enzyme, 25X Calibrators Mix, Positive Control, 10X Injection Buffer, Internal Control	BTI-C004-F1-3-0050
Modaplex and Consumables		
Modaplex System	Modaplex Instrument, Monitor, Barcode Reader Kit, PC Keyboard, PC Mouse, Printer, Cables, 1 Modaplex TC Hold Down Plate, 1 Modaplex Cartridge with 48 Capillaries	00-04901-0001
Modaplex TC Hold Down Plate	1 Modaplex TC Hold Down Plate	00-04X01-0001
Modaplex 48 Capillary Cartridge	1 Modaplex Cartridge with 48 Capillaries	00-14X02-0001
Modaplex Buffer	2 Bottles of Modaplex Buffer	00-14302-2000
Modaplex Decon	2 Bottles of Modaplex Decon	00-14303-2000
POP-7/ Modaplex CE Gel	1 Bottle of Modaplex CE Gel	00-04305-0028
Modaplex Wash	1 Bottle of Modaplex Wash	00-14304-0250
Modaplex CE Plates	1 Box of 20 Modaplex CE Plates	00-14306-0020
Mineral Oil	5 Bottles of Mineral Oil	00-04301-0025
PCR Microplates 96	1 Box of 25 96-well PCR Plates	00-14X03-0025
Aluminum Sealing Film	1 Box of 100 Aluminum Sealing Film	00-14X04-0100

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