



ANCHOR
C. difficile PCR KIT

Instructions for Use
ANCHOR C. difficile PCR Kit



Qualitative


real-time PCR Kit

for *in vitro* diagnostic use

IVD For *in vitro* diagnostic use


REF A0200

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 -30°C to -15°C

 **ANCHOR** Diagnostics GmbH
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compatible with

QuantStudio 5 (Applied Biosystems)

LightCycler 480 II (Roche)

cobas z 480 Analyzer (Roche)

CFX96 (Bio-Rad)

Rotor-Gene Q (QIAGEN)

Mic qPCR (Bio Molecular Systems)

1	▶ Contents	
2	Intended Use.....	6
3	Product Description.....	6
4	Kit Components.....	7
5	Storage and Stability.....	8
6	Material Required but Not Provided.....	9
7	Limitations.....	9
8	Warnings and Precautions.....	10
9	Workflow.....	11
9.1	Sample Preparation.....	11
9.1.1	Sample Matrix.....	11
9.1.2	Sample Preparation.....	11
9.1.3	Internal Control.....	12
9.2	PCR Preparation.....	13
9.2.1	Master Mix Set-Up.....	13
9.2.2	PCR Reaction Set-Up.....	15
9.3	PCR Cycler Configuration.....	16
9.3.1	General PCR Cycler Settings.....	17
9.3.2	Specific PCR Cycler Settings.....	18
9.4	Data Analysis.....	20
9.4.1	Qualitative Analysis.....	22
10	Performance Data.....	23
10.1	Analytical Performance.....	23
10.1.1	Sensitivity.....	23
10.1.2	Specificity.....	23
10.1.3	Precision.....	25
10.2	Clinical Performance.....	26
11	Quality Control.....	26
12	Technical Assistance & Contact Information.....	26
13	Literature.....	27
14	Symbols.....	28

2 ▶ Intended Use

The ANCHOR C. difficile PCR Kit is an *in vitro* nucleic acid amplification test based on real-time PCR technology for the qualitative detection of toxigenic *Clostridioides difficile* DNA, isolated from human stool or stool suspensions. The product is intended to be used by professional users, such as laboratory technicians and physicians who are trained in molecular biological techniques.

3 ▶ Product Description

The ANCHOR C. difficile PCR Kit is a real-time PCR technology-based test for the amplification and detection of toxigenic *C. difficile* specific nucleic acids. The Master Mixes contain reagents for the amplification and differentiation of Toxin A (*tcdA*) and Toxin B (*tcdB*) specific DNA of *C. difficile*.

In addition, a heterologous amplification system (Internal Control) is included to supervise the success of the sample extraction procedure and to identify possible inhibition of the amplification reaction.

The *C. difficile* Toxin A (*tcdA*) / Toxin B (*tcdB*) specific nucleic acids and the Internal Control are parallel detected in two corresponding detector channels of the real-time PCR instrument using fluorescently labeled probes with distinguishable fluorescent dyes.

A combined *C. difficile* Toxin A and Toxin B Positive Control and a Negative Control are monitoring the integrity of the analyte-specific reagents of the kit and the proper performance of the reaction.

4 ▶ Kit Components

The ANCHOR C. difficile PCR Kit is a ready-to-use system.

Master A and Master B reagents contain all necessary components to allow PCR-mediated amplification and target detection of *C. difficile* of Toxin A and Toxin B specific DNA and Internal Control in one reaction setup.

PC (Positive Control) *C. difficile* Tox A/B and NC (Negative Control) DNA are supplied with the IC (Internal Control) DNA already incorporated (see also section 9.2.1 Master Mix Set-Up).

The reagents provided with the kit allow the preparation of 100 reactions.

Kit component	Mat. no.	No. of vials	Quantity	Ingredients
Master A C. difficile	A0201	4 vials	4x 125 µL	Buffer, bovine serum albumin, polymerase
Master B C. difficile	A0202	4 vials	4x 125 µL	Buffer, salt, nucleotides, target- and IC-specific oligonucleotides
IC DNA	AD00020	1 vial	1000 µL	Buffer, IC-specific synthetic polynucleotide
PC C. difficile Tox A/B*	A0203	1 vial	200 µL	Buffer, target- and IC-specific synthetic polynucleotide
NC DNA*	AD00021	1 vial	200 µL	Buffer, IC-specific synthetic polynucleotide

* INTERNAL CONTROL INSIDE !

5 ▶ Storage and Stability

- The ANCHOR *C. difficile* Kit is shipped on dry ice and should be stored at -30 to -15°C upon receipt.
- The components are stable until the expiration date stated on the label.
- Do not use components of the kit that have passed their expiration date.
- Store *C. difficile* DNA-positive and/or potentially positive material separated from the kit.
- Repeated thawing and freezing of the Master reagents of > 3x should be avoided, as this may reduce the assay performance.
- Due to the components used it might be possible that Master vials do not always freeze completely after initial thawing. This is not a matter of concern and does not influence the stability or performance of the assay.
- If the reagents are to be used only intermittently, they should be frozen in aliquots. Label aliquots clear and unambiguously to avoid a mix-up of reagents.
- During PCR set up the reagents should be kept cooled at +2 to +8°C – use cooling block.
- Do not store Master A and Master B *C. difficile* more than 3 h at +2 to +8°C.
- Protect all reagents from extensive light exposure.

6 ▶ Material Required but Not Provided

- Nucleic acid purification system
- Real-time PCR instrument
- Appropriate PCR reaction vessels and related accessories
- Cooling block (for reaction setup)
- Benchtop centrifuge (rotor holding 2 mL reaction tubes)
- Vortex mixer
- Pipettes (variable volume)
- Single-use pipette filter tips
- 1.5 mL or 2 mL reaction tubes (for Master mix set-up)
- Single-use gloves (powder-free)

Use all materials and equipment according to the manufacturer's instructions. Maintain and calibrate the equipment as recommended.

7 ▶ Limitations

- Strict compliance with the user manual is required for optimal PCR results.
- Any diagnostic result generated must be interpreted in conjunction with other clinical and/or laboratory findings.
- The presence of PCR inhibitors may cause invalid results.
- Occurrence of mutations within the target region might result in a reduced sensitivity or a complete detection failure.
- Following good laboratory practices is crucial for the successful usage of the product.
- Appropriate handling of the reagents is essential to avoid contaminations or impurities.

8 ► Warnings and Precautions

- For *in vitro* diagnostic use.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Specimens should always be treated as potentially infectious and/or biohazardous material in accordance with safe laboratory procedures.
- The ANCHOR Master A C. difficile contains a bovine-sourced potentially infectious component (albumin). The bovine plasma is sourced from New Zealand or USA, which are recognized by the world organization for animal health Office International des Epizooties (OIE, Paris) as having a negligible BSE risk.
- Wear protective single-use gloves, a laboratory coat and eye protection when handling specimens or kit components.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- Always use DNase/RNase-free single-use pipette tips with aerosol barriers.
- Use separated working areas for (1) specimen preparation, (2) PCR reaction set-up and (3) amplification/detection activities.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Discard sample and assay waste according to your local safety regulations.

9 ► Workflow

9.1 ► Sample Preparation

9.1.1 ► Sample Matrix

The recommended patient sample matrices for sample preparation input are:

- human stool
- stool suspensions

Storage recommendations:



Storage recommendations for stool are dependent on the downstream testing algorithm. For nucleic acid amplification test-based detection systems, the storage of samples for 48 hours at +15 to +30°C following 5 days at +2 to +8°C did not show a negative impact on the detectability of the *C. difficile* DNA or the reproducibility of results obtained from fresh samples. This was confirmed for samples stored for additional 32 days at -20°C or 5 months at -70°C.

9.1.2 ► Sample Preparation

Purified DNA is the sample input material for the ANCHOR C. difficile PCR Kit. It has to be ensured that the chosen nucleic acid purification method is compatible with real-time PCR technology. The extraction has to be executed according to the manufacturer's instructions. The diagnostic applicability of the ANCHOR C. difficile PCR Kit has been shown using the following sample preparation platforms:

Sample Preparation Platforms
NucliSENS® easyMag® System (bioMérieux)
EMAG® (bioMérieux)
EZ1 Advanced XL / EZ2 Connect (QIAGEN)
QIAcube Connect (QIAGEN)
QIASymphony® SP (QIAGEN)
MagNA Pure 96 System (Roche)
MagNA Pure Compact (Roche)
Maxwell® 16 / RSC Instrument (Promega)
KingFisher Systems (Thermo Fisher Scientific)
SEEPREP32™ (Seegene)
GenoXtract® (Hain Lifescience)


PLEASE NOTE

-  If sample eluates are not directly used for PCR analysis, store eluates at -30 to -15°C. In case of using eluates repeatedly, avoid frequent thaw/freeze cycles (no more than 3 cycles).
-  Eluates should be labeled clearly and unambiguously to avoid a mix-up of samples.

9.1.3 ▶ Internal Control

The Internal Control DNA provided with the ANCHOR C. difficile PCR Kit should be co-purified with the nucleic acid of interest to monitor sample preparation efficiency and quality and PCR inhibition.

PLEASE NOTE

-  The Internal Control DNA **MUST NOT** be added directly to the clinical sample.

Always add the Internal Control DNA after lysis buffer has been added to the sample.


The required volume of Internal Control DNA per sample purification is defined by the chosen elution buffer volume.

Ten percent of the elution buffer volume used should be added to the sample/lysis mixture.

Examples:

- Elution buffer per sample: 200 µL → IC DNA volume: 20 µL
- Elution buffer per sample: 60 µL → IC DNA volume: 6 µL


PLEASE NOTE

-  Secure the elimination of residual ethanol before elution of nucleic acids. Ethanol may inhibit the amplification process.

If no co-purification of the Internal Control is planned, the IC DNA is used only as an inhibition control of the PCR reaction, but not as a control for efficient sample preparation. For this, either the amount of IC related to the used elution volume could be added to each eluate or 1.5 µL of the IC DNA / per reaction should be added to the master mix (see section 9.2.1 Master Mix Set-Up).

9.2 ▶ PCR Preparation
9.2.1 ▶ Master Mix Set-Up

PLEASE NOTE

-  Consider configuring the run settings of the PCR cycler software to have the instrument ready before starting the PCR reaction preparation (Refer to section 9.3 PCR Cycler Configuration).

Prepare the Master Mix step by step:

- Thoroughly thaw Master components A and B.
- Mix Master A and B by gentle pipetting or short pulse-vortexing.
- Spin Master A and B shortly with a benchtop centrifuge to remove residual droplets from tube lids.
- According to your preferred workflow follow one of the pipette schemes below to mix Master A and B using a 1.5 mL or 2 mL reaction tube:

IC DNA present in sample eluates – NO IC DNA added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A C. difficile	5 µL (X)	55 µL	Y µL
Master B C. difficile	5 µL (X)	55 µL	Y µL
Volume Master Mix	10 µL	110 µL	Z µL

*10 reactions + 10%

** See formula next page


IC DNA to be used as PCR inhibition control only – IC DNA added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A C. difficile	5 µL (X)	55 µL	Y µL
Master B C. difficile	5 µL (X)	55 µL	Y µL
IC DNA	1.5 µL (X)	16.5 µL	Y µL
Volume Master Mix	11.5 µL	126.5 µL	Z µL

*10 reactions + 10%

** See formula next page

PLEASE NOTE

 We recommend calculating for an additional volume of at least 10% to compensate potential loss during pipetting. The needed volume will be calculated by using the following formula:

$$** N \times X \mu L \times 1,1 = Y$$


N = Number of reactions


X = Volume of component per reaction

Y = Total volume of component

Z = Total volume of Master Mix

- Mix prepared Master Mix by gentle and short pulse-vortexing.
- Spin Master Mix shortly with a benchtop centrifuge to remove residual droplets from tube lids.


 It is recommended to test the Positive Control and the Negative Control at least once in each PCR run.

 Positive Control C. difficile Tox A/B and the Negative Control DNA already contain the IC DNA in a ready-to-use concentration. No addition of IC necessary!

If you want to use a Master Mix preparation with added IC DNA (as inhibition control) in combination with PC and NC DNA, be aware that the IC signal of the controls will slightly shift towards a lower CT value in comparison to the IC signal of the controls using a Master mix without additional IC.

9.2.2 ▶ PCR Reaction Set-Up


PLEASE NOTE


 Always use a cooling block for the preparation of the PCR reaction mix.

Prepare the Reaction Mix step by step:

- If previously stored frozen, thaw eluates containing nucleic acid (and IC DNA) thoroughly.
- Mix eluates by gentle pipetting or brief pulse-vortexing.
- Spin eluates shortly with a benchtop centrifuge to remove residual droplets from tube lids.
- Pipette **10 µL of Master Mix** (see section 9.2.1 Master Mix Set-Up) into suitable reaction vessels for PCR analysis. This is also valid for Master Mix spiked with IC DNA.
- Add **15 µL of eluate** or control (Positive Control C. difficile Tox A/B or Negative Control DNA). **Mix well by repeated up and down pipetting.**
- Close reaction vessels securely with the appropriate sealing system.
- Immediately transfer closed and ready-to-use reaction vessels to the real-time PCR instrument. Avoid any delays!

PLEASE NOTE

 Carefully handle reaction vessels during transfer to avoid mixing up samples.

 Complete mixing of Master Mix reagents with a sample or control during reaction set up should be unconditionally secured by repeated up and down pipetting!

This is essential for achieving optimal amplification curve performance.

Master Mix	Eluate / Control	Reaction Mix
10 µL	15 µL	25 µL

9.3 ▶ PCR Cycler Configuration

The ANCHOR C. difficile PCR Kit has been evaluated in combination with the following different PCR Cycler platforms:

PCR Cycler Platform	Run Time
QuantStudio 5 (Applied Biosystems)	≈ 28 min.
LightCycler 480 II (Roche)	≈ 30 min.
cobas z 480 (Roche)	≈ 30 min.
CFX96 (Bio-Rad)	≈ 33 min.
Rotor-Gene Q (QIAGEN)	≈ 39 min.
Mic qPCR Cycler (Bio Molecular Systems)	≈ 33 min.

The listed run times for the different instruments are effectively measured durations and can differ from what is displayed on the graphical user interface of the individual instrument software. For basic information concerning set-up and programming of the respective real-time PCR instrument, refer to the instrument-specific manual.

9.3.1 ▶ General PCR Cycler Settings

Temperature cycling profile for **QuantStudio 5, LightCycler 480 II, cobas z 480 Analyzer, CFX96 and Rotor-Gene Q:**

Cycling	95°C	1 sec	x 40
	65°C *	2 sec	
	72°C	1 sec	

* Fluorescence acquisition for toxigenic *C. difficile* and IC

Temperature cycling profile for **Mic qPCR:**

Cycling	95°C	1 sec	x 40
	63°C *	2 sec	
	72°C	1 sec	

* Fluorescence acquisition for toxigenic *C. difficile* and IC

Reaction Volume: 25 µL

9.3.2 ▶ Specific PCR Cycler Settings

The following table contains PCR cycler-specific recommendations for the basic configuration of the run settings.

For additional information regarding the cycler settings recommended plastics, color compensation, gain optimization settings, etc. do not hesitate to contact us directly (see section 12 Technical Assistance & Contact Information).

QuantStudio™ 5		
Target	Tox A/B	IC
Detection	FAM	HEX
Run Settings <ul style="list-style-type: none"> Block Type: 96-Well 0.1-mL Block Experiment Type: Standard Curve Chemistry: TaqMan® Reagents Run Mode: Fast Plate attributes: Passive Reference - None Consumables: <ul style="list-style-type: none"> 96-Well Fast Thermal Cycling Plates (Life Technologies Mat.No. 4346907) MicroAmp™ Optical Adhesive Film (Life Technologies Mat. No. 4311971) or <ul style="list-style-type: none"> 96-Well-PCR-Plate, Skirted, „Low Profile“, white (Starlab Mat. No. E1403-5209) Xtra-Clear Advanced Polyolefin StarSeal (qPCR) (Starlab Mat. No. E2796-9795) 		

LightCycler® 480 II and cobas z 480 Analyzer		
Target	Tox A/B	IC
Detection	465/510	LightCycler 480 II: 533/580 cobas z 480 Analyzer: 540/580
Run Settings: <ul style="list-style-type: none"> Block size: 96 If clear plates are used, the sensor of the LightCycler® has to be disabled by selecting the Clear Plates option in the software before the run is started. Consumables: <ul style="list-style-type: none"> LC480 Multiwell Plate 96, white (Roche Mat. No. 04729692001) LC480 Multiwell Plate 96, clear (Roche Mat. No. 05102413001) LC480 Sealing Foil (Roche Mat. No. 04729757001) 		

Bio-Rad CFX96		
Target	Tox A/B	IC
Detection	FAM	HEX
Consumables: <ul style="list-style-type: none"> Hard Shell 96-well PCR Plate, white (Mat. No. HSP9655) Optical flat 8 Cap Strip for 0.2mL (Mat. No. TCS0803) 0.2 mL 8-Tube PCR Strips without Caps, low profile, white (Bio-Rad Mat. No. TLS 0851) 8-strip optical clear flat caps (Sarstedt Mat. No. 65.1998.400) 		

Rotor-Gene Q		
Target	Tox A/B	IC
Detection	Green	Yellow
Run Settings <ul style="list-style-type: none"> Use 72-Well Rotor Perform Auto-Gain optimization before 1st acquisition. Consumables: <ul style="list-style-type: none"> Strip Tubes and Caps, 0.1 mL (QIAGEN Mat. No. 981103) 		

Mic qPCR		
Target	Tox A/B	IC
Detection	Green	Yellow
Run Settings <ul style="list-style-type: none"> Temperature Control: Standard TAQ Consumables: <ul style="list-style-type: none"> Mic Tubes and Caps (Mat. No. 68MIC-60653) or similar product. 		

9.4 ▶ Data Analysis

The following table contains cycler-specific references for the configuration of analysis settings. They could serve as an initial orientation. Depending on local cycler- and workflow-related differences adaptations might be necessary.

For additional information concerning data analysis, refer to the instrument-specific manual of the respective real-time PCR instrument or contact us (see section 12 Technical Assistance & Contact Information).

QuantStudio™ 5
Analysis Settings (all channels): <ul style="list-style-type: none"> ▪ Plot Type: ΔR_n vs Cycle ▪ Graph Type: Linear ▪ Baseline Start/End: 3/15 ▪ Threshold: <ul style="list-style-type: none"> - FAM 600,000 - HEX 100,000

LightCycler® 480 II and cobas z 480 Analyzer
Analysis Settings: <ul style="list-style-type: none"> ▪ Abs Quant/2nd Derivative Max ▪ Color Comp (off) ▪ Mean ▪ High Confidence

Bio-Rad CFX96
Analysis Settings (all channels): <ul style="list-style-type: none"> ▪ Baseline Subtracted Curve Fit ▪ C(t) Determination Mode: Single Threshold ▪ Baseline Threshold: <ul style="list-style-type: none"> - Baseline Cycles: Auto Calculated - Single Threshold <ul style="list-style-type: none"> - FAM: 1,000 - HEX: 250

Rotor-Gene Q
Analysis Settings (all channels): <ul style="list-style-type: none"> ▪ Quantitation ▪ Linear Scale ▪ Dynamic Tube ON ▪ Threshold: <ul style="list-style-type: none"> - Green: 0.04 - Yellow: 0.08

Mic qPCR
Analysis Settings (all channels): <ul style="list-style-type: none"> ▪ Graph Type: Linear ▪ Method: Dynamic ▪ Ignore Cycles Before: 3 ▪ Threshold Start: 1 ▪ Exclusion: None ▪ Threshold Level: <ul style="list-style-type: none"> - Green: 0.12 - Yellow: 1.5

9.4.1 ► Qualitative Analysis

For a valid run and as a prerequisite for the interpretation of the individual clinical sample results, the following requirements have to be met by the included kit controls:

Channel/Target	Tox A/B	IC
PC <i>C.difficile</i> Tox A/B	+	+
NC DNA	-	+

If one of the conditions has failed, result interpretation of clinical sample results might be flawed. In case of kit control failure, it is recommended to repeat the PCR run.

In case of a valid run, the following result interpretation can be made:

Qual. result	Tox A/B	IC
Toxigenic <i>C. difficile</i> DNA positive	+	+/-
Toxigenic <i>C. difficile</i> DNA negative	-	+
Invalid	-	-

A positive result in the target-specific channel does not necessarily require a positive signal for the IC since high concentrations of the respective target nucleic acid can result in a competitive inhibition of the IC amplification.

An invalid result for a clinical sample can be due to PCR inhibition or a failure during the nucleic acid isolation procedure. In such cases, it is recommended to dilute the nucleic acid extract 1:10 (recommended to be done in elution buffer, if possible) for a PCR retest or to repeat the nucleic acid isolation procedure. Note that the dilution of the nucleic acid extract might also lead to a reduction of the target nucleic acid concentration below the limit of detection of the ANCHOR *C. difficile* PCR Kit.

10 ► Performance Data

10.1 ► Analytical Performance

10.1.1 ► Sensitivity

The LOD for the ANCHOR *C. difficile* PCR Kit was determined by undertaking a probit analysis on the Rotor-Gene Q platform. A dilution series of different concentration levels for *C. difficile* strain 630 DNA (ATCC / LGC Mat # 1382DQ) was used. Each dilution level was tested with overall 24 replicates using 3 different PCR reagent lots across 3 different days, executed by 2 different persons on 2 different instruments.

The LOD value determined on the Rotor-Gene Q was then confirmed or re-evaluated on the other 5 instruments.

Instrument	LOD	Unit
QuantStudio 5	0.6	copies/μL
LightCycler 480 II	0.3	copies/μL
cobas z 480 Analyzer	0.3	copies/μL
CFX96	0.3	copies/μL
Rotor-Gene Q	0.3	copies/μL
Mic qPCR	0.3	copies/μL

10.1.2 ► Specificity

Triplicates of different clinical isolates which have been independently characterized with regards to their ribotype affiliation, were tested at a concentration near the 3x LOD of the ANCHOR *C. difficile* PCR Kit.

C. difficile Ribotype	C. difficile Tox A/B
Ribotype 001	+
Ribotype 002	+
Ribotype 005	+
Ribotype 014	+
Ribotype 015	+
Ribotype 017	+
Ribotype 020	+
Ribotype 027	+
Ribotype 078	+

Nucleic acid of selected pathogens with a concentration of $\approx 5.00E+03$ copies/ μ L (alternative units CFU/ μ L or TCID50/ μ L) was added to the PCR reaction and tested in triplicates in the absence or presence of C. difficile DNA at its 3x LOD and 3x LOQ concentration on the Rotor-Gene Q.

Pathogen	- C.difficile	3x LOD C. difficile
C. difficile Ribotype 010*	-	Not tested
C. difficile Ribotype 140*	-	Not tested
Clostridium sordellii	-	+
Clostridium perfringens	-	+
Norovirus GI.P4_GI4	-	+
Norovirus GII.P16_GII4_2012	-	+
Escherichia coli	-	+
Human Rotavirus	-	+
Salmonella enterica	-	+
Campylobacter coli	-	+
Entamoeba histolytica	-	+
Giardia intestinalis	-	+
Listeria monocytogenes	-	+
Candida albicans	-	+
Enterococcus faecium	-	+
Human Hepatitis A Virus	-	+

* concentration of non-toxicogenic ribotypes was unknown, undiluted DNA was tested as provided

10.13 ▶ Precision

Precision testing was initially performed on the Rotor-Gene Q instrument. For intra-run variability, 3-6 replicates of each sample dilution were tested within one run using one instrument and reagent lot by one operator. For inter-run variability, 3-6 replicates of each sample dilution were tested within overall four runs using two instruments and one reagent lot by two operators across days. For inter-batch variability, 3-5 replicates of each sample dilution were tested within one run using one instrument and three reagent lots by one operator.

PC C. difficile Tox A/B			
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	27.31	0.14	0.53
Inter-Run	27.74	0.37	1.32
Inter-Batch	27.32	0.15	0.55
Total	27.61	0.37	1.34
IC DNA			
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	24.59	0.13	0.52
Inter-Run	25.00	0.30	1.20
Inter-Batch	24.86	0.28	1.14
Total	25.00	0.28	1.12
ATCC C. difficile 3xLOD			
Variability	AVE (CT)	SD (CT)	CV (%)
Intra-Run	35.73	0.32	0.89
Inter-Run	36.07	0.60	1.68
Inter-Batch	36.07	0.60	1.68
Total	36.12	0.62	1.71

Precision of the ANCHOR C. difficile PCR Kit in combination with the other instruments was evaluated for intra- and inter-run variability.

10.2. ▶ Clinical Performance

The clinical performance of the ANCHOR C. difficile PCR Kit for the qualitative detection of toxigenic *C. difficile* DNA in human stool samples was evaluated comparatively at 2 different study sites against an established *C. difficile* routine diagnostic workflow using a CE-marked PCR assay as reference standard.

Prospectively collected and banked specimens were analyzed with the ANCHOR C. difficile PCR Kit and with the comparator assays to determine their positive percent agreement (PPA) and negative percent agreement (NPA), respectively. Testing was done using the LightCycler 480 II, Bio-Rad CFX96, and Rotor-Gene Q Cycler.

ANCHOR C. difficile PCR Kit	Σ 671	Comparators	
		POS	NEG
POS		281	22
NEG		2	366

PPA: 99.3 % NPA: 94.3 %

11 ▶ Quality Control

In accordance with the implemented ISO 13485-certified Quality Management System, each lot of the ANCHOR C. difficile PCR Kit is tested against predetermined specifications to ensure consistent product quality.

12 ▶ Technical Assistance & Contact Information

For any questions, a need for technical assistance or if you identify difficulties using our products do not hesitate to contact us:















phone: +49 40 52 471 62 0

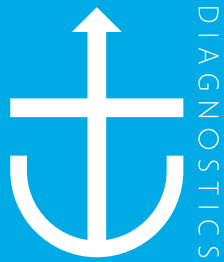
email: support@anchor-diagnostics.com

13 ▶ Literature

- (1) Chen S et al., Rapid detection of Clostridium difficile toxins and laboratory diagnosis of Clostridium difficile infections. *Infection*. 2017 Jun;45(3):255-262
- (2) Crobach MJ et al., European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for Clostridium difficile infection. *Clin Microbiol Infect*. 2016 Aug;22 Suppl 4:S63-81
- (3) Debast SB et al., European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for Clostridium difficile infection. *Clin Microbiol Infect*. 2014 Mar;20 Suppl 2:1-26
- (4) Fehér C et al., A Comparison of Current Guidelines of Five International Societies on Clostridium difficile Infection Management *Infect Dis Ther*. 2016 Sep;5(3):207-30
- (5) Gilligan PH, Optimizing the Laboratory Diagnosis of Clostridium difficile Infection. *Clin Lab Med*. 2015 Jun;35(2):299-312
- (6) Martin JS et al., Clostridium difficile infection: epidemiology, diagnosis and understanding transmission. *Nat Rev Gastroenterol Hepatol*. 2016 Apr;13(4):206-16
- (7) Planche T et al., Diagnostic pitfalls in Clostridium difficile infection. *Infect Dis Clin North Am*. 2015 Mar;29(1):63-82
- (8) Smits WK et al., Clostridium difficile infection. *Nat Rev Dis Primers*. 2016 Apr 7;2:16020
- (9) McDonald et al., Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clinical Infectious Diseases* 2018 Mar;66(7):e1–e48
- (10) Alfa et al., Fecal specimens for Clostridium difficile Diagnostic Testing are Stable for up to 72h at 4 °C. *J Med Microb Diagn*. 2014 Jun;3(2):1-3
- (11) Peterson et al., Evaluation of the cobas Cdiff Test for Detection of Toxigenic *Clostridium difficile* in Stool Samples. *J Clin Microbiol*. 2017 Dec;55(12):3426-3436

14 ▶ Symbols

-  Component in Kit
-  Volume per vial
-  For *in vitro* diagnostic use
-  Batch code
-  Number of vials
-  Quick Guide - Catalog number and version
-  Product - Catalog number
-  Unique Device Identifier
-  Catalog number and version
Consult Instructions for Use
-  Important Note
-  Use by
-  Contains sufficient reagents for <N> tests
-  Temperature limits for storage
-  Manufacturer



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