BOSCH

vivalytic

Noro-, Rotavirus, C. diff

PCR Test for Norovirus, Rotavirus, C. difficile (tcdA/tcdB)

Instructions for use
Gebrauchsanweisung

Introduction

Clostridioides difficile (C. difficile) is a gram-positive, spore-forming anaerobic bacillus consisting of toxigenic and non-toxigenic strains. *C. difficile* is one of the most common pathogens of nosocomial diarrhea, especially in patients with antibiotic- associated diarrhea. In recent years, the number and severity of cases has increased in Europe and worldwide.1 The symptoms of *C. difficile* infection (CDI) ranges from mild diarrhea to severe life-threatening pseudomembranous colitis, although colonization of *C. difficile* does not necessarily lead to a symptomatic infection. The disruption of the balance of gut microorganisms e.g. due to antibiotically treatment may cause toxigenic *C. difficile* to establish, dominate colonization and might be the start of CDI.2

Factors involved in the pathogenesis of *C. difficile,* are the genes *tcdA* and *tcdB* that code for toxin A (enterotoxin) and toxin B (cytotoxin) which are located in a 19.6 kb chromosomal region called the pathogenicity locus (PaLoc). Most pathogenic strains are toxin A and B positive (A+B+), but some pathogenic variant isolates may be toxin A negative and B positive (A-B+). They are the cause of *C. difficile*-associated diseases.3 Some strains of toxigenic *C. difficile* also produce a toxin called *C. difficile* transferase (CDT), or binary toxin.4

Norovirus belongs to the virus class Caliciviridae and is known to be a seasonal disease. It is a highly contagious pathogen that is responsible for the majority of cases of acute gastroenteritis worldwide. Norovirus infections in humans are mainly caused by genogroup II and I, while genogroup II being the predominant cause of Norovirus outbreaks. Infections of the gastrointestinal tract by Norovirus are characterized by sudden onset symptoms such as nausea, vomiting, diarrhea, and abdominal pain. The virus is particularly prevalent in communal settings such as hospitals, schools, and nursing homes, where it can easily spread from person to person. The illness typically lasts only a few days but can cause more severe complications in immunocompromised individuals or the elderly.5,6,7

Rotavirus belongs to the virus class Reoviridae. It is a significant cause of acute gastroenteritis, particularly in infants and young children. Its viral infection of the gastrointestinal tract is characterized by symptoms such as severe diarrhea, vomiting, fever, and dehydration. Rotavirus-associated gastroenteritis can lead to severe complications, including hospitalization and even death, making it a major public health concern. Efforts to prevent and control rotavirus infections, such as through vaccination programs, have been crucial in reducing the burden of this disease. Acute gastroenteritis caused by both Norovirus and Rotavirus are self-limiting diseases although if untreated can cause a critical course of disease especially in the youngest or elderly. 7,8,9,10

Package Contents

15 Vivalytic Noro-, Rotavirus, C.diff test cartridges for the detection of *Clostridioides difficile* (tcdA/tcdB toxin genes), human Norovirus genogroup I/ II and Rotavirus type A.

Pathogen List		
Clostridioides difficile (tcdA/tcdB)	Norovirus genogroup I/II	Rotavirus Type A

Intended Use

The Vivalytic Noro-, Rotavirus, C.diff test is an automated qualitative in vitro diagnostic test based on real-time polymerase chain reaction (PCR) for the detection of nucleic acids from toxigenic *Clostridioides difficile* (toxin genes tcdA/tcdB), Human Norovirus genogroup I/ II and Human Rotavirus type A from swabs samples from liquid or soft human stool to aid in the diagnosis of acute gastrointestinal infections of symptomatic individuals.
Results should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Positive results do not exclude co-infection with other pathogens. The agent(s) detected may not be the definite cause of disease. Negative results do not exclude a *C. difficile*, Norovirus, Rotavirus infection or another gastrointestinal infection. Results must be clinically correlated with patient history, clinical observations and epidemiological information. Other diagnostic information is necessary to determine patient infection status. Intended for use with a Vivalytic one analyser by healthcare professionals only in laboratory settings.

Safety Information
These Instructions for Use contain test-specific information only. For additional warnings and instructions refer to the Instructions for Use provided with your Vivalytic *one* analyser (chapter device safety information). Only use Vivalytic cartridges and accessories approved for the Vivalytic *one* analyser. Take care to avoid any contamination when handling patient samples and cartridges. When sample was spilled on the cartridge, do not use the cartridge, and dispose it.

For *in vitro* diagnostic use by trained healthcare professional.

WARNING

- Always follow good laboratory practice to ensure the proper performance of this test.
- Make sure to wear appropriate personal protective equipment (PPE).
- Do not use a cartridge if the sealed pouch or the cartridge itself is visibly damaged.
- Do not touch or scratch the detection area of the cartridge.
- Do not reuse a cartridge.
- Do not use expired cartridges. The expiration date can be found on the packaging and the cartridge label.
- Do not wait longer than 15 minutes after opening the cartridge pouch to begin the test. This maintains hygiene and avoids performance loss due to humidity. Prolonged exposure to humidity has a negative impact on test performance.
- Do not shake a cartridge that contains a sample.
- Do not turn the cartridge upside down.
- Place the cartridge on a clean and flat surface only.
- Do not use sample types, media and volumes that are not approved for the test.
- Always follow good laboratory practice to ensure the proper performance of this test.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Handle potentially infectious patient samples and cartridges according to national laboratory standards and dispose samples and cartridges according to regional and laboratory standards.
- Be compliant with the national safety regulations and practices.

Note: Further information can be found in the safety data sheet (SDS) of the product. Please contact the customer support of your local distributor.

Additional Equipment & Consumables Required but not Provided

- Bosch Vivalytic *one* analyser (reference number F09G300115)
- Pipettor (100–1000 µl)
- Sterile filter pipette tips 100 –1000 µl
- Swab collection kits
  - Regular Flocked Swab FLOQSwabs® 552C (COPAN Italia s.p.a.)
- Transport medium
  - eNAT® 606C (COPAN Italia s.p.a.)
- Suitable protective clothing

Test Principle

Vivalytic Noro-, Rotavirus, C. diff is a qualitative real-time PCR based test.

Storage und Usage Conditions

Product is stable until the expiry date if stored at +15 °C to +25 °C. Storage and usage conditions can be taken either from the cartridge, pouch, or box label. Cartridge has to be used at +15 °C to +25 °C, relative humidity <65 %, within 15 min upon pouch opening. This maintains hygiene and avoids performance loss due to humidity. Prolonged exposure to humidity has a negative impact on test performance.

Reagents All reagents necessary for the sample processing are integrated into the cartridge. The processing includes cell lysis, nucleic acid extraction, DNA amplification and detection.

Reagents are PCR bead, binding buffer, washing buffer, and elution buffer. The PCR bead contains the DNA polymerase, primers, and probes. Binding buffer facilitates binding of nucleic acids during the purification process. Washing buffer is a formulation of different salts and solvents to remove impurities e. g. proteins during the extraction process. Elution Buffer is a low-salt buffer and contains the purified nucleic acids at the end of the extraction process.

Sample Type/Medium

The test is intended for use with stool swab samples in eNAT® medium (Regular Flocked Swab FLOQSwabs® 552C, eNAT® transport medium Ref. 606C, COPAN Italia s.p.a.).

Collect and store samples as indicated in the manufacturer's instructions.

In case the sample is not processed immediately after sample collection, nucleic acids will be preserved in eNAT® transport medium for up to 4 weeks at room temperature and at 4°C and up to 6 months at -20° C to -80° C (see Instructions for Use eNAT® transport medium11).

Sample Preparation

Use a Regular Flocked Swab FLOQSwabs® (COPAN Italia s.p.a.) to collect a small amount of stool by inserting the tip of the flocked swab into the stool sample and rotate it. Bloody, slimy, or watery area of stools should be selected and collected. After collection, examine the swab to make sure there is fecal material visible on the tip. In case it is not, again insert the flocked swab into the stool sample and rotate taking care that all the area of the swab tip is in contact with the sample. Make sure that the swab is just covered with stool and remove excess stool by gently rotating the swab against the rim of the sample. After collection transfer the swab into the 2 ml Copan eNAT® tube with eNAT® transport medium. Hold the swab shaft between thumb and finger, mash and mix the stool specimen against the side of the tube to evenly disperse and suspend the specimen in the preservation medium. Hold the swab shaft close to the rim of the tube, bend it at a 180 degrees angle to break it off at the marked breakpoint. Discard the broken upper part of the swab shaft and tighten the cap. Shake the sample tube containing the swab sample and eNAT® medium (COPAN Italia s.p.a.) thoroughly at least for 10 seconds for homogenization. Use a pipettor in order to fill 300 µl of homogenized patient sample in the sample input of the cartridge. Ensure to only pipet from the supernatant (top of the sample) to prevent the carryover of stool particles. In case of an excess amount of particles in the sample it is recommended to place the sample tube on a flat surface and let the particles sediment for 5 minutes. Do not use viscous samples that are difficult to pipette.

Test Result

After automatic processing of the sample with the Vivalytic *one* analyser the test result is shown on the screen of the Vivalytic *one* analyser. The time to result is about 58 min.

For high titer specimens results are available after less than 41 min and the test run can be terminated earlier (see Chapter Test Termination).

The sample is classified either as *C. difficile* positive, Rotavirus and *C. difficile* positive, Norovirus and *C. difficile* positive, Norovirus and Rotavirus and *C. difficile* positive, Rotavirus positive, Norovirus and Rotavirus positive, Norovirus positive, negative or invalid. In case of a positive detection of a pathogen, the test is considered valid even if the Human Control is negative.

Detection of the human cell based whole process control (Human Control) in negative samples shows a successful extraction procedure and excludes an inhibition of the PCR reaction. Interpretation of results is shown in the table below.

Norovirus genogroup I/II	*Rotavirus Type A*	*C. difficile* (tcdA / tcdB)	Human control	Validity	Result
-	-	+	+/–	valid	Sample is considered *C. difficile* positive.
-	+	+	+/–	valid	Sample is considered Rotavirus and *C. difficile* positive.

+	-	+	+/–	valid	Sample is considered Norovirus and *C. difficile* positive.
+	+	+	+/–	valid	Sample is considered Norovirus, Rotavirus and *C. difficile* positive.
-	+	-	+/–	valid	Sample is considered Rotavirus positive.
+	+	-	+/–	valid	Sample is considered Norovirus and Rotavirus positive.
+	-	-	+/–	valid	Sample is considered Norovirus positive.
-	-	-	+	valid	Sample is considered negative.
-	-	–	–	invalid	Not evaluable. [1]

[1] Retesting is recommended

PCR – Curve and Cq Value

Real-time PCR curves (software-modified) are shown and classified as positive or negative by the software. In case of positive curves, the respective Cq value is displayed. Inconclusive results are marked by the software (⚠). Retesting is advised.

Invalid or Failed Tests

A test is rated as invalid if neither target DNA nor Human Control is detected. Possible reasons for an invalid run might be poor sample quality due to a partial or complete absence of human cellular material in the sample. Results are displayed for an invalid test but are not allowed to be used for diagnostic interpretation. Pay attention to use the correct sample type, sample collection and storage of the sample and cartridges prior to the test run. If required, repeat the analysis with a new sample. In case of a failed test, first check for correct operating conditions of the Vivalytic *one* analyser (refer to Vivalytic *one* analyser's Instructions for Use). Restart the Vivalytic *one* analyser. If the problem persists, contact the customer support of your local distributor.

Test Termination

As soon as a valid, positive result is shown for one of the three pathogens on the screen, the user has the option to finish the test.

Test Report

In the printed test report, pathogen, results, control and information on user, patient and Vivalytic *one* analyser are listed with a signature field.

Notice to Users in EU

Any serious incident that has occurred in relation to the device, should be reported to the manufacturer and the competent authority of the Member State in which the user and/or patient is established.

Limitations

The results of the Vivalytic Noro-, Rotavirus, C.diff test must be interpreted by a trained healthcare professional only. The results of the Vivalytic Noro-, Rotavirus, C.diff test must not be used as the sole parameter for diagnosis.

- A negative result does not exclude pathogens being present in the sample at a level below assay sensitivity or other pathogens being present not covered by this assay.
- There is a risk of false negative or false positive results due to improperly collected, transported, or handled samples.
- In borderline cases atypical PCR characteristics (e. g. flat curve with low or high Cq-value) can occur. In case of atypical characteristics results are not allowed to be used for diagnostic interpretation. Inconclusive results are marked by the

software. Retesting is advised.
- Vivalytic Noro-, Rotavirus, C.diff is a qualitative real-time PCR test and does not provide a quantitative result.
- A positive result does not necessarily mean that viable pathogens are present.
- The test detects *C. difficile* carrying *tcdA/tcdB* genes, but does not differentiate between toxin producing and non-toxin producing strains
- A negative result does not preclude a Norovirus, Rotavirus, *C. difficile* or co-infection. It is recommended that negative tested samples are interpreted in the context with additional laboratory data
- An excess amount of stool may have inhibitory effects on the assay performance.
- A reduced performance was observed for tcdA+/tcdB- *C. difficile* strains
- An excess amount of Rotavirus A virus particles can lead to a reduced performance of the *C. difficile* PCR
- No exclusivity for Rotavirus Type C (porcine Rotavirus) could be achieved. Therefore a misidentification of Rotavirus Type C as Type A cannot be excluded.

Analytical Performance Evaluation

Analytical Sensitivity (Limit of Detection)
The limit of detection of the Vivalytic Noro-, Rotavirus, C. diff test was determined as the lowest concentration of analyte that can be consistently detected (≥95% of samples tested under routine laboratory conditions using a defined type of sample). (Table 1)

Inclusivity
To evaluate inclusivity, an *in silico* analysis (BLAST alignment) of the genomic sequence of various relevant Noroviruses, Rotaviruses and *Clostridioides difficile* strains against the sequence of the PCR primers and hydrolysis probe used in the Vivalytic Noro-, Rotavirus, C. diff test for amplification and detection of the respective pathogens was performed. Inclusivity could be shown for strains listed in Table 2.

Exclusivity/ Analytical Specificity
To exclude cross-reactivity (exclusivity), an *in silico* analysis (BLAST alignment) of the target region of Norovirus, Rotavirus and *Clostridioides difficile* against the genomic sequence of various other pathogens representing common gastrointestinal pathogens or closely related species was conducted. For the detection system of Rotavirus, sequence matches in the probe and primer area could be detected for porcine Rotavirus (group C), concluding a possible amplification. There was no evidence of an interference for the detection system of Norovirus and *Clostridioides difficile*. (Table 3).

Reproducibility
The reproducibility of the Vivalytic Noro-, Rotavirus, C. diff test was established using a panel with 3 different concentrations of Norovirus, Rotavirus and *Clostridioides difficile*. At 3 test sites, each mix was tested on the same set of Vivalytic instruments by the same operator with 3 LOTs in 4 replicates on 3 days, respectively. The obtained positivity rates for the different combinations were correlated to the expected positivity rate (Table 4a).

Repeatability
The repeatability of the Vivalytic Noro-, Rotavirus, C. diff test was established using a panel with 1 concentration (3x *c95*) of Norovirus, Rotavirus and *Clostridioides difficile*. At 1 test site, the mix was tested on the same set of Vivalytic instruments by the same operator with 3 LOTs in 20 replicates, respectively, yielding a total of 60 observations per target pathogen. The obtained positivity rates for the different combinations were correlated to the expected positivity rate (Table 4b).

Interferences
Interferences were evaluated for endogenous and exogenous substances, that are potentially present in the patient sample. Refer to Table 5 for substances that have the potential to interfere with the test.

Clinical Performance evaluation Sensitivity and Specificity
Sensitivity and specificity results derived from native liquid and soft human stool samples. Samples were collected in a clinical setting and compared with results of reference methods.
Samples for testing with Vivalytic Noro-, Rotavirus, C. diff cartridges were freshly used or frozen for storage and prepared as describe above in eNAT® (COPAN Italia

S.p.A.). In case of reference testing samples were prepared according to recommendations of used reference methods. In total, 159 samples were analysed. Sensitivity or Positive Percent Agreement (PPA) was calculated as 100 % x TP/ (TP+FN). Specificity or Negative Percent Agreement was calculated as 100 % x TN / (TN+FP). The results of the clinical performance evaluation are shown in **Table 6, Table 7 and Table 8**.

Technical Support

If you require any support, technical help or have additional questions, please contact your local distributor or visit the Bosch Vivalytic website at www.bosch-vivalytic.com.

References

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[9]Mas Marques A, Diedrich S, Schreier E: Group A Rotavirus Genotypes in Germany 2005/2006. Arch Virol 2007; 152:1743–1749

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[11]COPAN Instructions for Use of Copan eNAT™ Collection and Preservation System; Version EIFU002R01 Date 2021.06.

Symbols

Manufacturer	Serial number
Date of manufacture	Temperature limit
Expiry date	Do not use if package is damaged
Lot number	For single use only
Reference number	Consult Instructions for Use
Contains <n> tests	*in vitro* diagnostic medical device
CE mark 0123	

Pipette the indicated sample volume in the sample input of the cartridge as marked by the black triangle.

WARNING

Hazard component in cartridge
(F09G300919; binding buffer RB-BB-34a)
Guanidinium chloride; guanidine hydrochloride

Hazard statements

H302	Harmful if swallowed.
H315	Causes skin irritation.
H319	Causes serious eye irritation.

Precautionary statements

P264	Wash hands thoroughly after handling.
P280	Wear protective gloves and eye/face protection.
P302+P352	IF ON SKIN: Wash with plenty of water and soap.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice/attention.
P501	Dispose of contents/container in accordance with local/regional/ national/ international regulation

Table 1 Limit of Detection (LoD)		
Clostridioides difficile (*tcdA/tcdB*)*	10,000	copies/mL
Rotavirus group A**	10,000	copies/mL
Norovirus GII**	168,700	copies/mL

*Determined by using Vircell material (surrogate strain R20291 Ribotype 027 (toxin A + B positive) for a spiking approach in eNAT® (COPAN) supplemented with approximately 1000 Human Bronchial/Tracheal Epithelial Cells (hPBTEC) and 6% (v/v) Stool Matrix Negative Control (artificial simulated matrix). **Determined by using quantified clinical isolates (Rotavirus group A, Norovirus GII) for a

spiking approach in eNAT® (COPAN) supplemented with approximately 1000 Human Bronchial/Tracheal Epithelial Cells (hPBTEC) and 6% (v/v) Stool Matrix Negative Control (artificial simulated matrix). Norovirus GI was confirmed at the 3x LoD (as determined for Norovirus GII) using artificial nucleic acid constructs.

Table 2 Inclusivity
Clostridioides difficile toxin A gene (*tcdA*)
Clostridioides difficile toxin B gene (*tcdB*)
Rotavirus group A
Norovirus GI/GII

Strains validated for inclusivity via BLAST alignment

Table 3 – Exclusivity		
Abiotrophia defective	*Enterococcus faecium*	*Enterococcus*
Acinetobacter baumannii	*gallinarum*	*Enterococcus hirae*
Acinetobacter lwoffii	*Enterococcus raffinosus*	Enterovirus
Adenovirus	*Escherichia blattae*	
Aeromonas caviae	*Escherichia coli* (Shiga toxin producing) (STEC)	
Aeromonas hydrophila	*Escherichia coli* (Enteroaggregative) (EAEC)	
Alcaligenes faecalis	*Escherichia coli* (Enteroinvasive) (EIEC)	
	Escherichia coli (Enteropathogenic) (EPEC)	
Anaerococcus tetradius	*Escherichia coli* (Enterotoxigenic) (ETEC)	
Arcobacter butzleri	*Escherichia coli* (non-pathogenic) (K-12 MG1655)	
Arcobacter cryaerophilus	*Escherichia coli* (Enterohemorrhagic) (EHEC)	
Ascaris lumbricoides	*Escherichia fergusonii*	
Astrovirus	*Escherichia hermannii*	
Bacillus cereus	*Escherichia vulneris*	
Bacillus subtilis	*Eubacterium rectale*	
Bacteroides caccae	*Flavonifractor plautii*	
Bacteroides fragilis	*Fusobacterium varium*	
Bacteroides merdae	*Gardnerella vaginalis*	
Bacteroides stercoris	*Gemella morbillorum*	
Bacteroides thetaiotaomicron	*Giardia lamblia*	
Bifidobacterium adolescentis	*Giardia muris*	
Bifidobacterium bifidum	*Grimontia hollisae*	
Bifidobacterium longum	*Hafnia alvei*	
Blastocystis hominis	*Helicobacter fennelliae*	
Campylobacter coli	*Helicobacter pylori*	
Campylobacter concisus	*Helicobacter cinaedi*	
Campylobacter curvus	*Helicobacter hepaticus*	
Campylobacter fetus	Hepatitis A virus	
Campylobacter gracilis	Herpes Simplex Virus 2	
Campylobacter helveticus	*Hymenolepis nana*	
Campylobacter hominis	*Klebsiella oxytoca*	
Campylobacter hyointestinalis	*Klebsiella ozaenae*	
Campylobacter jejuni	*Klebsiella pneumoniae*	
Campylobacter lari	*Lactobacillus acidophilus*	
Campylobacter mucosalis	*Lactobacillus reuteri*	
Campylobacter rectus	*Lactococcus lactis*	
Campylobacter showae	*Leminorella grimontii*	
Campylobacter sputorum	*Listeria grayi*	
Campylobacter upsaliensis	*Listeria innocua*	
Candida albicans	*Listeria monocytogenes*	
Candida catenulate	*Megamonas hypermegale*	
Cedecea davisae		
Chilomastix mesnili		
Chlamydia trachomatis		

Table 3 – Exclusivity	
Citrobacter amalonaticus	*Morganella morganii*
Citrobacter freundii	Norovirus GIV
Citrobacter koseri	*Pentatrichomonas hominis*
Citrobacter sedlakii	*Peptoniphilus asaccharolyticus*
Clostridium beijerinckii	*Peptostreptococcus anaerobius*
Clostridium bifermentans	*Plesiomonas shigelloides*
Clostridium bolteae	*Porphyromonas asaccharolytica*
Clostridium botulinum	*Prevotella melaninogenica*
Clostridium butyricum	*Proteus mirabilis*
Clostridium chauvoei	*Proteus penneri*
Clostridium difficile (non-toxigenic)	*Proteus vulgaris*
Clostridium fallax	*Providencia alcalifaciens*
Clostridium haemolyticum	*Providencia rettgeri*
Clostridium histolyticum	*Providencia stuartii*
Clostridium innocuum	*Pseudomonas aeruginosa*
Clostridium methylpentosum	*Pseudomonas fluorescens*
Clostridium nexile	*Pseudomonas putida*
Clostridium novyi	Rotavirus B
Clostridium orbiscindens	Rotavirus C
Clostridium paraputrificum	Rotavirus D
Clostridium perfringens	Rotavirus F
Clostridium ramosum	Rotavirus G
Clostridium scindens	Rotavirus H
Clostridium septicum	Rotavirus I
Clostridium sordellii	*Ruminococcus bromii*
Clostridium sphenoides	*Saccharomyces boulardii*
Clostridium spiroforme	*Salmonella enterica*
Clostridium sporogenes	*Salmonella enterica* spp. *enterica* serovar Typhimurium
Clostridium symbiosum	*Salmonella enteritidis*
Clostridium tertium	*Salmonella subterranea*
Clostridium tetani	Sapovirus
Collinsella aerofaciens	*Serratia liquefaciens*
Corynebacterium genitalium	*Serratia marcescens*
Coxsackie virus	*Shigella boydii*
Cryptosporidium canis	*Shigella dysenteriae*
Cryptosporidium cuniculus	*Shigella flexneri*
Cryptosporidium felis	*Shigella sonnei*
Cryptosporidium fetus	*Staphylococcus aureus*
Cryptosporidium hominis	*Staphylococcus aureus* subsp. *aureus*
Cryptosporidium meleagridis	*Staphylococcus epidermidis*
Cryptosporidium muris	*Stenotrophomonas maltophilia*
Cryptosporidium parvum	*Streptococcus agalactiae*
Cytomegalovirus	*Streptococcus dysgalactiae*
Desulfovibrio piger	*Streptococcus dysgalactiae* subsp. *dysgalactiae*
Dientamoeba fragilis	*Streptococcus intermedius*
Diphyllobothrium latum	*Streptococcus pyogenes*
Dysgonomonas capnocytophagoides	*Streptococcus salivarius*
Echovirus	*Streptococcus uberis*
Edwardsiella tarda	*Taenia saginata*
Eggerthella lenta	*Trabulsiella guamensis*
Encephalitozoon intestinalis	*Veillonella parvula*
Endolimax nana	*Vibrio cholerae*
Entamoeba coli	*Vibrio mimicus*
Entamoeba hartmanni	*Vibrio parahaemolyticus*
Entamoeba histolytica	*Vibrio vulnificus*
Entamoeba moshkovskii	*Yersinia bercovieri*
Entamoeba polecki	*Yersinia enterocolitica*
Enterobacter aerogenes	*Yersinia enterocolitica* subsp. *enterocolitica*
Enterobacter cloacae	*Yersinia frederiksenii*
Enterococcus casseliflavus	*Yersinia intermedia*
Enterococcus cecorum	*Yersinia mollaretii*
Enterococcus dispar	*Yersinia pseudotuberculosis*
Enterococcus faecalis	*Yersinia rohdei*

Strains validated for exclusivity via BLAST alignment

Table 4a Reproducibility

Target	c	No of total tests	No of pos. tests	No of neg. tests	Proportion of positive/ negative results (%)	95 % Wilson-Score confidence interval (%)	95 % Pearson-Clopper confidence interval (%)
none	*blank*	108	0	108	100	96.6% - 100%	96.6% - 100%
Norovirus GII	*3x c95*	108	108	0	100	96.6% - 100%	96.6% - 100%
	<c100	108	108	0	100	96.6% - 100%	96.6% - 100%
Rotavirus A	*3x c95*	108	108	0	100	96.6% - 100%	96.6% - 100%
	c100	108	108	0	100	96.6% - 100%	96.6% - 100%
Clostridioides difficile	*3x c95*	108	106	2	98.1	93.5% - 100%	93.5% - 99.8%
	<c100	108	107	1	99.1	94.9% - 100%	95.0% - 100%

3x *c95* = 95 % predicted positive agreement
c100 = 100 % predicted positive agreement
< *c100* = < 100 % predicted positive agreement (Norovirus GII: verified with 3,33x106 copies/mL, *c100* = 3,8x107 copies/mL; *Clostridioides difficile*: verified with 1,83x105 copies/ml; *c100* = 5,48x105 copies/ml)

Table 4b Repeatability

Target	LOT	No of total tests	No of pos. tests (3x *c95*)	Proportion of positive/ negative results (%)	95 % Wilson-Score confidence interval (%)	95 % Pearson-Clopper confidence interval (%)
Norovirus GII	1	20	20	100	83.9% - 100%	83.2% - 100%
	2	20	20	100	83.9% - 100%	83.2% - 100%
	3	20	20	100	83.9% - 100%	83.2% - 100%
	Total	60	60	100	94% – 100%	94% – 100%
Rotavirus A	1	20	20	100	83.9% - 100%	83.2% - 100%
	2	20	20	100	83.9% - 100%	83.2% - 100%
	3	20	20	100	83.9% - 100%	83.2% - 100%
	Total	60	60	100	94% – 100%	94% – 100%
Clostridioides difficile	1	20	20	100	83.9% - 100%	83.2% - 100%
	2	20	20	100	83.9% - 100%	83.2% - 100%
	3	20	20	100	83.9% - 100%	83.2% - 100%
	Total	60	60	100	94% – 100%	94% – 100%

3x *c95* = 95 % predicted positive agreement

Table 5 – Tested Substances for Interference
No interference detected

Cholesterol; 0.5 % (v/v)
Mucus; 3 mg/mL
Palmitic acid; 2mg/mL
Stearic Acid; 2mg/mL
Triglyceride; 0.8 % (w/v)
Whole blood; 3.0 (v/v)
Barium Sulfate; 1.3 % (w/v)
GLYICILAX for adults; 1.0 % (w/v)
GRÜNWALDER Sennalax film-coated tablet; 0.1 mg/mL
Hemorrhoid ointment with witch hazel; 1 % (v/v)
LOPERAMID-ratiopharm acute 2 mg film-coated tablet; 0.2 % (v/v)
MAALOXAN 25 mVal Liquid; 0.1 mg/mL
METRONIDAZOL Aristo 400 mg tablet; 0.5 % (w/v)
Naproxen axicur® tablet; 0.5 % (w/v)
Nonoxynol-9; 1 **% (w/v)**
Nystatin STADA®; 1 % (w/v)
Postericort ointment; 0.5 % (v/v)
Claversal 4 g/60 ml clysms; 0.5 % (v/v)
Vancomycin; 1.4 mg/mL
Vaseline; 1 % (w/v)

Interference was experimentally verified at 3xLoD for Norovirus GII, Rotavirus A and *C. difficile* using a spiking approach in eNAT® (COPAN) plus 1000 hPBTEC and 6% (v/v) Stool Matrix Negative Control (artificial simulated matrix).

Table 6 – **Norovirus:** Clinical Sensitivity (PPA) [1] and Specificity (NPA) [2] for samples in eNAT® (95 % confidence interval, clinical study)

[1] 94.23% (84.05 – 98.79%)*
[2] 100.00% (96.48 – 100%)

*3 samples were detected as false negative for Norovirus.

Table 7 – **Rotavirus**: Clinical Sensitivity (PPA) [3] and Specificity (NPA) [4] for samples in eNAT® (95 % confidence interval, clinical study)

[3] 92.59% (81.11 – 97.94%)*
[4] 97.03% (91.56 – 99.38%)**

*4 samples were detected as false negative for Rotavirus.
**3 samples were detected as false positive for Rotavirus.

Table 8 – **C. difficile**: Clinical Sensitivity (PPA) [5] and Specificity (NPA) [6] for samples in eNAT® (95 % confidence interval, clinical study)

[5] 97.96% (89.15 – 99.95%)*
[6] 98.18% (93.59 – 99.78%)**

*1 sample was detected as false negative for *C. difficile*.
**2 samples were detected as false positive for *C. difficile*.

In total, 159 clinical samples were tested within the scope of the clinical study. In this data set, 52 samples were found positive for Norovirus, 54 samples were found positive for Rotavirus and 49 samples were found positive for *C. difficile*. 14 samples were found negative for all pathogens. All samples were tested with the two reference tests from Seegene (Allplex™ GI-Bacteria(I) and Allplex™ GI-Virus Assay) and Vivalytic Noro-, Rotavirus, C. diff to determine the clinical performance for Vivalytic. Discrepant results were verified using RIDAGENE RIDA®GENE HSP (R-Biopharm) (12 samples).

Table 7 – Document History

Revision 01	Initial document
Revision 02	Pre-release changes

Latest change 2024-06-19 – Rev. 2

For more information see
www.bosch-vivalytic.com

Bosch Healthcare Solutions GmbH
Stuttgarter Straße 130
71332 Waiblingen, Germany

F09G300896_02

REF F09G300891

IVD CE 0123