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Evaluation of the RIDA®GENE Clostridium difficile & Toxin A/B for molecular detection of toxigenic *Clostridium difficile* in diarrheal feces compared to cytotoxicity neutralization assay and BD GeneOhm™ Cdiff assay

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Background

Clostridium difficile is the primary cause of nosocomial diarrhea worldwide. Clinically symptomatic cases are caused by toxigenic C. diff strains that produce toxin A and B.
In recent years, the incidence and severity of C. diff infection increased noticeably worldwide. Health care costs associated with *C. difficile* infection (CDI) are estimated at around 3 billion euros per year in Europe and roughly 1.1 billion dollars per year in the USA.[1] A rapid and accurate diagnosis of CDI is important for patient management and also for timely infection control measures. A variety of laboratory test are available to diagnose CDI either by detection of the toxin (cytotoxicity assay or toxin enzyme immunoassay) or by detecting the organism (toxigenic culture, glutamate deyhdrogenase assay).[2] The gold standard for diagnosis of CDI is the cell cytotoxicity neutralization assay (CCNA) which is slow and requires cell culture expertise. Enzyme immunoassays for the detection of the toxins A/B are rapid but lack sensitivity. Toxigenic culture is an alternative more sensitive goldstandard but is tedious and too time consuming for clinical use, while recent studies show that GDH screening assay lacks sensitivity. So far none of these commercially available laboratory tests is sensitive, specific and rapid.[3] Real-time PCR is a rapid, highly sensitive and specific alternative for screening for CDI.
In this study we evaluated the performance of the RIDA®GENE Clostridium difficile & Toxin A/B LC assay against cell cytotoxicity neutralization assay (CCNA) and BD GeneOhm™ CDiff assay.

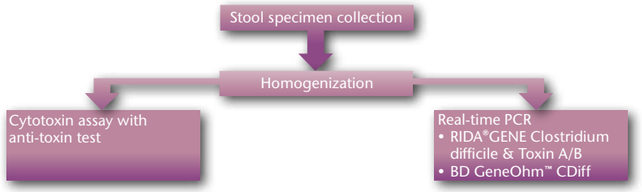
Picture 1: RIDA®GENE Clostridium difficile & Toxin A/B LC

Picture 2: Promega Maxwell®16

Methods

From April to August 2010 a total of 504 stool specimens from hospitalized patients suspected of having CDI were included in the study. All samples were homogenized and tested by CCNA while an aliquot for real-time PCR was frozen until extraction. RIDA®GENE Clostridium difficile Toxin A/B assays are available in different versions that can be run on commonly used real-time PCR instruments such as LightCycler®, SmartCycler®, ABI series or Rotor-Gene Q.
Real-time PCR was performed for RIDA®GENE Clostridium difficile & Toxin A/B LC assay on the Roche LightCycler® 480II and for BD GeneOhm™ CDiff assay on the Cepheid SmartCycler®.

Fig.1: Study design



The DNA extraction was performed with the automated Promega Maxwell®16 using the Tissue LEV Total RNA Purification Kit. Before extraction the stool samples were diluted 1:3 with water, intensely vortexed and centrifuged at 3,000 rpm for 30 sec. An appropriate volume of the supernatant was transferred into the Tissue LEV Total RNA Purification cartridge and placed into the Promega Maxwell®16.

Cell cytotoxicity neutralization assay was performed using the Techlab antitoxin kit.
Tests were performed according to manufacturer´s instructions after preparation of stool samples. Samples were incubated with and without antitoxin in Vero cell monolayers (micro well plates). Cells were incubated at 37 °C and checked for cytopathic effect (CPE) at 24 and 48 hours microscopically.

The presence of CPE in at least 50 % of the cell monolayer and no CPE in the control incubated with antitoxin were considered a positive result.

The RIDA®GENE Clostridium difficile & Toxin A/B assay is a commercial available real-time PCR for the direct, qualitative detection of Clostridium difficile (16S-rDNA) and Clostridium difficile toxin A (tcdA) and toxin B (tcdB) genes in human stool Samples. In each PCR reaction the internal amplification control that is included in the Reaction Mix is co-amplified and detected to determine possible PCR-inhibitions and to exclude false negative results. The BD GeneOhm™ CDiff assay was performed according to manufacturer´s instructions after DNA extraction.

Fig.2: RIDA®GENE Clostridium difficile & Toxin A/B LC Master-Mix preparation

Kit code	Master Mix components	Volume per reaction	10 reactions (10 % extra)
1 2	C. difficile Reaction Mix or Toxin A/B Reaction Mix	18.8 µl	206.8 µl
4	FDE (Fluorescence Detection Enhancer)	1.1 µl	12.1 µl
5	Taq Polymerase	0.1 µl	1.1 µl
	Total	**20 µl**	**220 µl**

Fig.3: Preparation of the PCR-Mix

Pipette 20 µl of the Master-Mix

Add 5 µl PCR Water to the pre-pipetted Master-Mix as negative control	Add 5 µl of the DNA-Extract to the pre-pipetted Master-Mix	Add 5 µl Positive Control to the pre-pipetted Master-Mix as positive control
NC	Sample	PC

Results

Of the 504 stool samples, 502 were evaluable by CCNA. 68 (14 %) stool samples were positive by CCNA. The overall agreement between the RIDA®GENE and CCNA was 87 % (436/502).
The sensitivity, specificity, PPV, NPV of RIDA®GENE were 96 %, 85 %, 51 % and 99 %, respectively.
Compared to a defined gold standard of CCNA and culture the agreement, sensitivity, specificity, PPV and NPV were 94 %, 100 %, 94 %, 68 % and 100 % respectively.

Fig.4: RIDA®GENE Clostridium difficile & Toxin A/B vs CCNA

		CCNA +	CCNA -	total
RIDA®GENE Clostridium difficile & Toxin A/B LC toxin A/B genes (tcdA/tcdB)	+	65	63	128
	-	3	371	374
	total	68	434	502

Sens: 96%
Spec: 85%
Prev: 14%
PPV: 51%
NPV: 99%

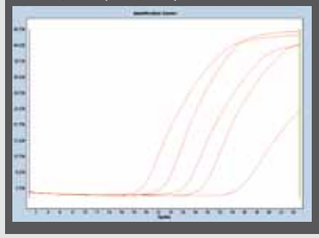
Compared to the BD GeneOhm™ CDiff assay, the RIDA®GENE Clostridium difficile & Toxin A/B LC had a positive agreement of 84 % and a negative agreement of 96 %.

Fig.5: RIDA®GENE Clostridium difficile & Toxin A/B LC vs BD GeneOhm™ CDiff

		BD GeneOhm™ CDiff +	BD GeneOhm™ CDiff -	total
RIDA®GENE Clostridium difficile & Toxin A/B LC toxin A/B genes (tcdA/tcdB)	+	116	13	129
	-	22	352	374
	total	138	365	503

Positive agreement 84%
Negative agreement 96%

Fig.6: Dilution series RIDA®GENE Clostridium difficile & Toxin A/B LC (tcdA/tcdB) on the LC 480II



The analytical sensitivity of the RIDA®GENE Clostridium difficile & Toxin A/B was determined with <5 DNA copies per reaction by dilution series of Clostridium difficile tcdA/tcdB, respectively.

No cross-reactivity with the nucleic acid preparations from major causes of gastroenteritias was observed.

Fig.7: Cross-reactivity testing

Listeria monocytogenes	-	Staphylococcus aureus	-
Yersinia enterocolitica	-	Salmonella	-
Campylobacter jejuni	-	Escherichia coli O157/H7	-
Escherichia coli	-	Shigella boydii	-

References
1. Kuijper et al., Clin Microbiol Infect 2006; 12(6): 2-18.
2. O´Donoghue and Kyne L. Current Opinion in Gastroenterolgy 2011; 27: 38-47.
3. Hookman B and Barkin JS. World J Gastroenterol 2009; 15(13): 1554-1580.

Conclusion

The RIDA®GENE Clostridium difficile & Toxin A/B assay is a rapid, sensitive and specific method for the detection of *C. difficile* directly from stool specimens.

Real-time PCR results are available in 30 min.

The assay can be included in a diagnostic algorithm to provide a rapid diagnosis of CDI, followed by appropriate patient management and timely infection control measures.

In a further study the performance of the RIDA®GENE Clostridium difficile & Toxin A/B assay needs to be compared to the more sensitive new “gold standard” toxigenic culture.

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