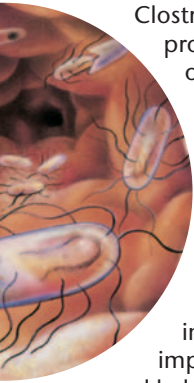


## Detection of *Clostridium difficile* Toxin A/B

### New Version of the RIDASCREEN® Assay

#### Pathogen and Disease



*Clostridium difficile* has occupied a prominent position in the group of most important nosocomial pathogens from the time it was first identified as a harmless intestinal commensal in 1935 until today. In fact, it is the most important cause of antibiotic-associated diarrhea (AAD). Chemotherapeutic agents and other multifactorial influences are also capable of impairing and damaging the natural balance of the intestinal flora, thus paving the way for successful colonization by *C. difficile*.

Whereas as many as 65 % of neonates and infants can be colonized with toxigenic and atoxigenic *C. difficile* strains in the first year of life without developing any symptoms and signs of disease, the rate of clinically asymptomatic *C. difficile* carriers in the adult population is less than 3 %. The high rate of colonization in the first year of life, which falls sharply by the end of the second year, is enhanced by the absence of protective intestinal flora, which is still undeveloped in early childhood.

The main reason why infants do not develop *C. difficile*-associated diarrhea (CDAD) at this early stage of life is that their undeveloped intestinal tract does not yet have receptors for the active *C. difficile* toxins. In addition, the larger quantities of mucous secreted by the intestinal mucosa at this early age also have a protective effect.

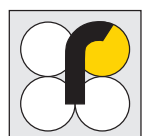
*Clostridium difficile* is characterized by the formation of two toxins with molecular weight of 308 kDa and 270 kDa, making them two of the largest known bacterial toxins to date. This is the main reason why *C. difficile* is such a significant pathogen. Both *C. difficile* toxins have cytotoxic effects, whereby toxin B is several times more potent than toxin A. Both types infiltrate to the interior of the cell by means of a receptor-mediated transport process similar to endocytosis. The

resulting toxin-induced damage to the tissues leads to the secretion of fluid into the intestinal lumen and, ultimately, to clinically apparent diarrhea. Pseudomembranous colitis is a severe form of the disease that can be lethal.

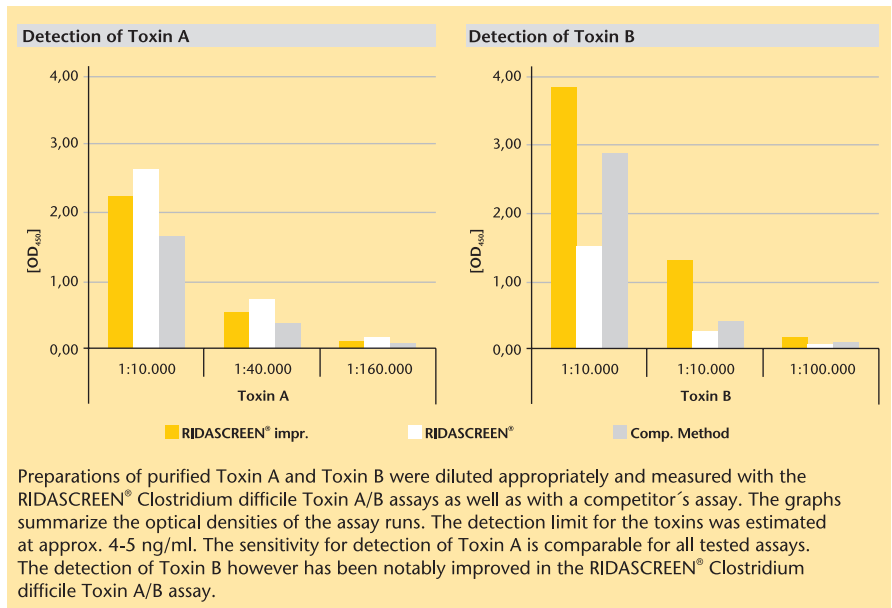
The virulence of the different *C. difficile* strains can vary greatly depending on the amount and type of toxins formed. Whereas the most toxigenic strains produce both toxins, certain strains produce only either toxin A or B. Among the single-toxin producers, those that produce only toxin B clearly predominate.

#### Diagnosis

In awareness of the differential modes of *C. difficile* toxin formation, test systems that are capable of identifying both toxin types have always been preferentially used. However, some ELISA manufacturers initially marketed tests capable of detecting only one type (toxin A), and then later redeveloped their products for proper detection of both toxin A and toxin B. The RIDASCREEN® *C. difficile* A/B assay, on the other hand, was marked in the proper and necessary format right from the beginning. Thanks to its diagnostic reliability, it has become a widely used and accepted *C. difficile* detection method since its introduction in 1993. Because it uses two conjugates in a biotin-streptavidin-triggered system to ensure a high level of sensitivity, the test initially took 2.5 hours to run, which was often criticized as a handling disadvantage. R-Biopharm therefore developed a new and highly sensitive anti-toxin B antibody to resolve this problem. This antibody makes it possible to dispense with the biotin-streptavidin enhancer system in favor of direct labelling with the peroxidase enzyme. This not only shortened the test duration by nearly an hour, but also significantly increased the sensitivity of the assay for detection of toxin B.



**Table 1**



As is impressively demonstrated in **Table 1**, signal strength in ELISA nearly doubles with the purified toxin.

The improved RIDASCREEN® C. difficile A/B assay was validated by testing stool samples in comparison to the results obtained using a competitor's assay that is highly rated and widely used in the USA and Canada (**Table 2**).

Both tables were taken from a poster presented at the 73rd Annual Conference of the Canadian Association for Clinical

Microbiology and Infectious Diseases (CACMID). The poster, which received great attention and commendation at the meeting, can be ordered as a PDF file.

The new ELISA version of the RIDASCREEN® C. difficile A/B assay is scheduled to replace the previous version in November and – according to present data – will surpass its predecessor.

**For further information, please contact Helmut Leidinger [h.leidinger@r-biopharm.de](mailto:h.leidinger@r-biopharm.de)**

**Table 2**

Sensitivity and Specificity			
A		Standard	
RIDASCREEN® Impr.	pos	neg	
pos	30	0	
neg	1	48	
	96,8%	100,0%	
	Sensitivity	Specificity	

B		Standard	
Comp. Method	pos	neg	
pos	30	2	
neg	1	46	
	96,8%	95,8%	
	Sensitivity	Specificity	

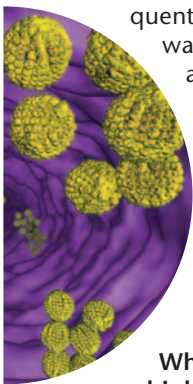
The table summarizes the calculation for sensitivity and specificity of the improved RIDASCREEN® Clostridium difficile Toxin A/B assay. A total of 79 previously characterized clinical samples (48 negative; 31 positive) were included in the study. The sensitivity for the improved RIDASCREEN® assay was calculated with 96.8 %, specificity was determined with 100 % in this study. The results are comparable to a commercially available competitor's assay as seen from table B.

## Noroviruses

**Is the increased detection of noroviruses a result of enhanced applications for more sophisticated diagnostic tools, or is there evidence suggesting that noroviruses are on the rise?**

Numerous reports have shown that periodic surges in outbreaks of norovirus-induced gastroenteritis can emerge both globally and nationally. Surges of norovirus genogroup II/cluster 4 (GG II/4) have been particularly prominent. Whether this strain is more virulent and/or more transmissible than other

members of the norovirus family, or whether cross-immunity to GG II/4 among the population is merely lower is still unclear. This type of periodic emergence presumably has also been a frequent phenomenon in the past. Still, we do not know whether the incidence of norovirus infection is more fre-



quent now than in 1929, when it was first described by Zahorsky as “winter vomiting disease”. Although there is not enough reliable retrospective data to find a definitive answer to this question, various factors support the assumption that the prevalence of norovirus infections is indeed much higher today.

### What evidence suggests a higher prevalence?

First, one can observe that the relative contribution of bacterial foodborne diseases to the overall number of reported cases of gastroenteritis is becoming smaller and smaller today. Improvements in the cold chain for food products have certainly helped. The contamination of food can also be avoided by good hygiene management and the use of suitable disinfectants. However, most of these measures are not effective in preventing or controlling norovirus infection. Noroviruses are highly resistant to many disinfectants and survive exposure to temperatures significantly

below 0°C. They remain on surfaces for extremely long periods of time, and only 10-100 virion particles are needed to trigger an infection.

Second, certain changes in society have contributed to an increasing number of elderly individuals spending the latter part of their lives in nursing homes; as a result, the risk of infection has increased tremendously. In the USA, for example, the number of beds in nursing homes has risen by over 75% since 1972.

Another factor to consider is that we now have a higher standard of living that allows people to eat out more often than they could 30 years ago.

In addition, eating habits have changed significantly in the last 30 years. For example, we now consume 20% more raw vegetables and fresh fruit.

Last but not least, the increasing intensity of travel in today’s globalized world has increased the risk of contracting a norovirus infection due to increased or more frequent exposure to the pathogens in hotels, airplanes and cruise ships.

## Norovirus Update

### Outbreak of norovirus gastroenteritis at a youth hostel demonstrates the difficulty in detecting norovirus by PCR.

An outbreak of norovirus-induced gastroenteritis at a youth hostel near Rosenheim, Germany was confirmed in early June 2005. Interestingly, the norovirus genome could only be identified by a special laboratory at the Robert Koch Institute (RKI), and nested PCR analysis was required for positive identification (the routine RT-PCR was almost completely useless). In supplementary sequencing tests, genogroup II / genotype 2 (GG II/2, Melksham) was identified as the causative agent (Epidemiologisches Bulletin, Vol. 25).

The RKI’s special laboratory likewise confirmed that a second outbreak in Prien on Lake Chiemsee was also caused by the same agent. The initial PCR analysis had also failed in this case (Epidemiologisches Bulletin, Vol. 24).

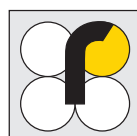
A sample obtained from one of the affected school children, who happened to be related to one of our employees and could therefore be tested using the RIDASCREEN® Norovirus ELISA, was clearly positive.

We have often written about this problem in the past, attempting to point out the many problems related to identification of the causative agent at genome level (PCR) while also highlighting the advantages of identifying the pathogen at the protein level (ELISA) – especially in the routine diagnostic setting.

Both of the aforementioned cases emphasize the fact that, even now, there is still no absolutely reliable method for identification of the causative agent. An isolated norovirus-positive test result must be interpreted very critically. If the typical clinical features of norovirus infection are lacking, a different diagnostic test should be performed to clarify the diagnosis. Our more than two years of experience in the field of norovirus diagnosis have repeatedly shown us that test discrepancies must always be reckoned with, even in the future.

**If you would like further information or if you have any questions concerning norovirus diagnosis, please feel free to contact our project manager, Dr. Georgios Kiourkenidis by phone Tel. +49 6151 / 8102-96 or by email [g.kiourkenidis@r-biopharm.de](mailto:g.kiourkenidis@r-biopharm.de)**

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# New RF Absorbent by R-Biopharm for Serological Enzyme Immunoassays

When performing serological tests for IgM antibodies, there are principally two sources of error that can lead to false test results.

- 1) IgG antibodies are present in excess in the course of an infection, whereas their IgM counterparts are not. As a result, IgG antibodies may obstruct the detection of IgM antibodies by blocking their specific binding sites, thus resulting in a false-negative test for IgM.
- 2) Rheumatoid factors, on the other hand, may cause an IgM test to be false-positive. Many rheumatoid factors are antibodies of the IgM class (IgM-RF) that are directed against the constant fragment (Fc) of IgM antibodies. IgM rheumatoid factors bind to the Fc region of IgG antibodies after specific binding of the antibodies to the test antigen. Added anti-human IgM conjugate then binds with the rheumatoid factors, thus simulating an IgM-positive result.

For this reason, IgG absorption is generally recommended for correct detection of IgM antibodies, but the technique has

often been complicated up to now. First, the serum had to be mixed with the absorbent at a specific ratio and then incubated. Only afterwards could the serum be diluted to the level needed to run the test. Hence, multiple pipetting steps were required. The new RIDA® RF absorbent simplifies the procedure. The user can prepare a ready-to-use IgM absorption buffer by adding RIDASCREEN® Sero EIA (SeroPP). The buffer can be stored for up to one week at 2 to 8°C without losing its activity. With the IgM absorption buffer, it is possible to perform sample dilution and absorption in a one-step pipetting procedure (e.g., 10 µl sample + 990 µl absorption buffer to yield a 1:100 dilution). This is especially advantageous when diluting samples and running tests on ELISA systems because some machines do not allow multiple pipetting steps in the sample absorption procedure. The combined method of one-step dilution and absorption therefore allows for optimal utilization of such machines.

## Trade Fairs and Conferences



24. – 28.07.2005	AACC 2005 – International Congress of Clinical Chemistry Orlando, Florida, U.S.
10. – 14.09.2005	WCOG 2005 – 13th World Congress of Gastroenterology Montreal/QC, Canada
11. – 15.09.2005	10th International Conference of Lyme Borreliosis and the other Tick-Borne Diseases, Vienna, Austria
25. – 28.09.2005	57. Jahrestagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM), Göttingen, Germany
13. – 14.10.2005	XVIII. Annual Workshop of the European Helicobacter Study Group (EHSG), Copenhagen, Denmark
04. – 09.11.2005	Annual Convention of the American College of Allergy, Asthma and Immunology (ACAAI), Anaheim/CA, U.S.
12.11.2005	R-Biopharm AG Gastro-Symposium, Berlin, Germany
16. – 19.11.2005	MEDICA 2005 – 37. World Forum of Medicine – International Trade Fair with MEDICA Congress, Düsseldorf, Germany
28.11. – 02.12.2005	Zdravochranenje, Russia

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