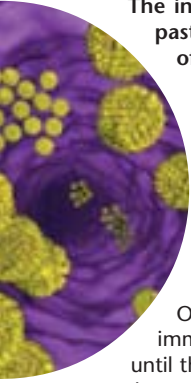


The Second Generation of RIDASCREEN® Norwalk-like Virus Enzyme Immunoassay.



The increasing occurrence in the past (2000-2003) of severe cases of gastroenteritis caused neither by bacteria nor parasites, but induced by Noroviruses – including the notorious outbreaks on cruise liners – have revealed the need for standardised diagnostic methods for viruses of the Caliciviridae family.

Owing to the fact that an enzyme immunoassay was not available until the end of the 1990s, PCR has to date played a predominant role in diagnosis, although there are as yet still no standardised test records worldwide. A Europe-wide study funded by the EU clearly exposed not only the difficulties resulting from the different primer pairs in use today, but also the problems relating to the reproducibility of these results. In five of the ten reference centres, the samples tested originated from individual samples which had already been defined as positive; most of the samples originated from the UK, Finland, the Netherlands and Germany. Each institute had used its own preferred combination of primers.

Only 84% of these samples could be identified by at least one of the five institutes. Overall sensitivity ranged from 52 to 73%, specifically ranging from 54 to 100% for genogroup (GG) I and from 58 to 85% for GG II.

This appears to be the root of the problem of non-comparability of the results. Owing to the absence of standardised and generally observed methods for sample preservation and storage, RNA isolation and timing of the tests/length of storage, different institutes will continue to produce discrepant and scientifically non-comparable results in the future. Moreover in view of the known genomic variability typical of RNA viruses, constant adaptation of the primers used will be necessary in order to maintain reliable diagnostic sensitivity of the method in the future.

The difficulty in evaluating the samples of healthy defecators in terms of hygiene management with such a highly sensitive method as PCR is also likely to cause further confusion.

The RIDASCREEN® Norwalk-like Virus ELISA, however, offers a good compromise between detection threshold on the one hand, and the stability of the target structure on the other. Possibly the major

advantage of this ELISA is that a large number of samples can be analysed inexpensively within a short time. Moreover, changes in the target antigens to be detected (capsid protein) are better tolerated than is the case with PCR detection of the target structures (base sequence of the genes), as individual point mutations can cause the primers not to bond with the defined target sequences. However, this does not inevitably lead to unwanted changes in the target epitopes in the virus capsid which are important for this ELISA, so that the antibodies used here specifically identify the antigen, whereas changes at genome level can lead to false negative PCR results.

Nevertheless, even a modern antigen ELISA is faced with the challenge – in the same way as PCR is faced with continuous adaptation of the primers – of ensuring detection of as wide as possible a spectrum of all Norovirus genotypes of both genogroups which are in circulation. This calls for controlled adaptation of specific antibodies to the changed capsid epitopes of the Noroviruses. The vital contact to experts worldwide working in this field enables us to identify new Norovirus types and, thanks to systematic ongoing development, to integrate these new types in our RIDASCREEN® Norwalk-like Virus ELISA.

This ELISA permits reliable Norovirus diagnosis within the currently possible and feasible scope. Other promising contacts with various institutions also enable us to maintain the quality of our RIDASCREEN® Norwalk-like Virus ELISA and simultaneously adapt it to the requirements of the different variants of the Norovirus types currently in circulation.

If you would like further information or have any questions about Norovirus diagnostics, please do not hesitate to contact our Project Manager Dr. Georgios Kiourkenidis at the following telephone number +49 (0)6151-8102-96 or by e-mail (g.kiourkenidis@r-biopharm.de).



About Our Products:

Convincing Study Results for our RIDASCREEN® Specific IgE and Total IgE Test Kit.

The transition period for transposition of IVD Directive 98/79/EC expired on 7 December 2003. In a harmonised Europe, the Directive prescribes quality standards which are to be observed by all manufacturers of in-vitro diagnostic products, insofar as these products are intended for sale in countries of the European Union.



As an outward sign of product conformity with the IVD Directive, products will bear the CE mark. The IVD Directive prescribes a number of steps which have to be satisfied before CE labelling is permissible.

In addition to risk management and proof that certain fundamental requirements of in-vitro diagnostic products have been fulfilled, as set forth in Annex I of the Directive, these steps also include performance assessment of the tests. The results of this performance assessment are highly convincing, thanks to continuous improvements to the quality of our products. Our production processes are also defined exactly

according to SOPs (Standard Operation Procedures), from selection of the raw allergens right through to charging the plates, to ensure a permanently high and constant quality standard.

The following tables indicate the reproducibility of our test for both specific and total IgE, both as interassay and intraassay. In the intraassay, each of the allergens listed was tested 32 times with different sera in the same batch: these results were then used to calculate the mean value and standard deviation, which served as the basis for calculating the coefficient of variation, CV. The CV can be used to scatter the values measured and thus obtain the mean value as a percentage. In the interassay, the allergens were tested in 8 batches with 4 repeats each; the results were then calculated in the same way.

Specific IgE:

Table 1: Specific IgE, Intraassay

Intra-Assay allergens tested	EAST class 1-2	EAST class 3-4	EAST class 5-6
	G6, T3, W6, D2, E1, M6, F2, F37, F4, F17, K82	G6, T3, W6, D2, E1, I3, M6, F37, F4, F17, F49, F85, SX1, K82	G6, T3, W6, D2, I3, F27, F4, F85, K82
Arithmetic mean of CVs	12.69%	3.86%	3.88%

Table 2: Specific IgE, Interassay

Interassay allergens tested	EAST class 1-2	EAST class 3-4	EAST class 5-6
	G6, T3, W6, D2, E1, M6	G6, T3, W6, D2, E1, I3, M6, F37, F4, F17, F49, F85, SX1, K82	G6, T3, W6, D2, I3, F27, F4, F85, K82
Arithmetic mean of CVs	11.7%	3.44%	3.65%

Total IgE:

Table 3: Total IgE , Intraassay

Serum	4528 (high)		4486 (high)		4478 (medium)		4530 (low)		4484 (low)	
	OD	IU/ml	OD	IU/ml	OD	IU/ml	OD	IU/ml	OD	IU/ml
CV	5.20	12.52	5.03	9.67	5.76	8.23	3.73	8.16	2.87	11.66

Table 4: Total IgE, Interassay

Serum	Kitlot	03163 Test 1		04333 Test 2		03433 Test 3		Test 1-3	
		OD	IU/ml	OD	IU/ml	OD	IU/ml	OD	IU/ml
4484 (low)	CV	4.07	25.63	7.66	53.85	10.19	34.79	30.5	31.6
4525 (low)	CV	6.35	20.64	7.22	14.49	8.68	14.98	18.2	26.2
4478 (medium)	CV	6.70	12.34	7.29	14.56	11.61	18.24	2.6	6.6
4486 (high)	CV	3.40	7.86	5.64	14.35	9.56	29.77	4.5	5.9
4571 (high)	CV	3.82	14.62	5.83	25.98	4.56	15.61	5.0	23.1

The IVD Directive also demands comparison with other competitive systems available on the market. In the field of allergy diagnostics, the UniCap System by Pharmacia is the most commonly used and thus the system we chose for comparison with our tests. We compared a total of 1024 individual tests. All samples which tested positive with the Pharmacia system and by R-Biopharm were valued as concurrent, as were all results tested negative with both systems. This resulted in the following comparative values:

Table 5: Comparison with Pharmacia:

Sensitivity	88.2%
Specificity	91.2%
Correlation	89.5%

Approx. 10 per cent of the tests were not concurrent. Despite the inherent variability of each system and the absence of standards in allergy diagnostics, the correlation factor is nevertheless extremely high. Both systems can therefore be rated equally suitable for in-vitro allergy diagnosis. As we charge the microtitre plates with the allergen slides according to your specifications, the risk of sample confusion or incorrect charging must be ruled out.

The use of a double light table and subsequent 4-stage control system ensures that all slides are placed properly, that no well was charged twice and that no well remained empty. This means you enjoy all possible benefits when using our RIDASCREEN® Specific IgE allergy system:

- Choice of more than 700 allergens/ allergen mixtures
- No deterioration of rare allergens
- No restriction to only 48 allergens per batch
- Comparable quality to Pharmacia
- Excellent value for money

If you have any questions about our company, our products or our quality management, please contact our Product Manager for Allergy Diagnostics, Mr. Joachim Zehender, at the following telephone number +49 (0)6151- 8102-45 or by e-mail: j.zehender@r-biopharm.de



RIDASCREEN® HSV Diagnostics – The Importance of Differential Antibody Detection

The most serious consequence of a herpes simplex virus infection is neonatal herpes in newborn infants. This happens when the virus is transferred from mother to child during delivery.

The risk of neonatal herpes is greatest if the mother does not have any specific protective antibodies which are transferred to the foetus via the placenta.

This is the case if the mother becomes infected only in the later stages of pregnancy. Type-specific antibodies are of great importance for protecting the infant. More than 80% of the entire population are infected with HSV-1.

However, HSV-1 antibodies do not afford protection against infection with HSV-2, the commonest cause of genital herpes. Information about the mother's immune status is therefore of primary importance in order to forecast the risk for the infant if the mother suffers from active genital herpes and to initiate appropriate action for the birth and post-natal period. For the diagnostic procedure, it is therefore important to have a test which can reliably differentiate between Type 1 and Type 2 antibodies. It must be ensured that the HSV-1 antibodies which are normally present in the serum do not

produce incorrect positive results in an HSV-2 test. This can only be achieved by using recombinant or highly purified glycoprotein G as antigen. In order to verify the high specificity of the glycoprotein G-based RIDASCREEN® ELISA, the test was validated by the German reference laboratory for HSV in Jena.

On the basis of defined serum panels, it demonstrated that both ELISAs have a specificity of 100% and differentiate clearly between HSV-1 and HSV-2 antibodies. Sensitivity in the test was 98.3% (HSV-1) and 99.3% (HSV-2). It could simultaneously be demonstrated that the test was able to rule out the common cross-reaction with VZV antibodies.

The results were presented on a poster produced in connection with the Infectiology Symposium held in Berlin in December 2003 in Berlin, where they met with great interest from the professional public. The test results are soon to be published in the "Clinical Laboratory".



RIDASCREEN® Campylobacter

This new enzyme immunoassay, which enables direct detection of the campylobacter-specific antigen from stool specimens, is the ideal addition to R-Biopharm's wide range of methods for the detection of gastroenteric pathogens responsible for diarrhoea. In addition to salmonella, campylobacter has meanwhile evolved into one of the major worldwide causes of bacterial gastroenteritis which is transferred by food.



In addition to the familiar and normally self-limiting enteritis, campylobacter is also responsible for extra-intestinal symptoms such as bacteraemia, endocarditis, meningitis, pancreatitis, haemolytic-uraemic syndrome as well as post-infectious late effects such as reactive arthritis and Guillain-Barré syndrome. The most commonly diagnosed species is *C. jejuni*, which accounts for more than 90% of all cases, followed by *C. coli*, *C. fetus* and *C. laridis*.

Until today, detection has been effected primarily by means of cultural enrichment of pathogens obtained from fresh stool specimens of the patients or of food samples using suitable nutrient media.

Isolation of the pathogen, which takes 2 - 5 days, succeeds only if the transport routes are short and the pathogen can be transported in suitable conditions.

Sole detection of the antigen thus means that not only can a great deal of time and effort be saved, but also that no living organisms which are capable of reproduction are required. The RIDASCREEN® Campylobacter Antigen Elisa thus offers an excellent alternative. It enables specific detection of *C. jejuni* and *C. coli* antigens within 2 hours.

Please refer to the following section for details of events at which we will present the new test, together with all other R-Biopharm antigen ELISA products.

Trade Fairs and Conferences

- | | |
|------------------------------------|---|
| 22 - 24 July 2004 | EHEC Workshop in Wildbad Kreuth, Germany, DGHM Gastrointestinal Infection Study Group |
| 25 - 29 July 2004 | Annual Meeting and Clinical Lab Exposition (AACC), Los Angeles, USA. CA Exposition July 27 - 29. |
| 19 - 22 August 2004 | II. Latin America Clinical Distributor Meeting/Training, Venezuela |
| 15 - 19 September 2004 | Joint Allergy Congress, Aachen 2004, Germany <ul style="list-style-type: none">• 28th Congress of the Association of German Allergologists• 23rd Conference of the German Association of Allergy and Clinical Immunology• 7th Annual Meeting for Paediatric Allergy and Environmental Medicine |
| 16 - 18 September 2004 | 7 th DGPI Infectiological Intensive Course in Greifswald, Germany |
| 24 - 29 September 2004 | First MENA Clinical and Food & Feed Distributor Meeting/ Training, Sharm El Sheik, Egypt |
| 26 - 29 September 2004 | 56 th DGHM Annual Meeting in Münster |
| 07 - 10 November 2004 | 72 nd Canadian Association for Clinical Microbiology and Infectious Diseases (CACMID), Delta Regina Hotel, Regina, Saskatchewan, Canada |
| 20. November 2004 | Gastro-symposium in Cologne, Germany |
| 24 - 27 November 2004 | MEDICA 2004 in Düsseldorf, Germany |
| 28 November 2004 - 2 December 2004 | Zdravochranenije, Russia |

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R-Biopharm AG
Landwehrstraße 54, 64293 Darmstadt
Telefon: (0 61 51) 81 02 - 0
Telefax: (0 61 51) 81 02 - 40

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