510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K093295

B. Purpose for Submission:

Substantial equivalence determination for a new device

C. Measurand:

Norovirus, genogroup 1 and genogroup 2.

D. Type of Test:

Qualitative enzyme immunoassay

E. Applicant:

R-Biopharm AG
An der neuen Bergstraße 17
64297 Darmstadt
Germany

F. Proprietary and Established Names:

RIDASCREEN® Norovirus 3rd Generation EIA

G. Regulatory Information:

1. Regulation section: 21 CFR 866.3395

2. Classification: Class: II (de novo)

3. Product code: OUC (Norovirus serological reagent)

4. Panel: 83- Microbiology

H. Intended Use:

1. Intended use(s):

   The RIDASCREEN® Norovirus 3rd Generation test is a qualitative enzyme immunoassay (EIA) intended for the detection of selected genogroup I (GI.1, GI.2, GI.3, GI.4, GI.7) and genogroup II (GII.1, GII.2, GII.3, GII.4, GII.5, GII.6, GII.7, GII.8, GII.10, GII.12, GII.13, GII.14, GII.17) norovirus strains in human feces as an aid in investigating the cause of acute gastroenteritis outbreaks. The likelihood of detecting a norovirus outbreak by use of the RIDASCREEN®

Norovirus 3rd Generation test improves as the number of patients tested during an outbreak increases, as well as when quality of the specimens increases. Preliminary identification of norovirus as the cause of an acute gastroenteritis outbreak by RIDASCREEN® Norovirus 3rd Generation testing should be confirmed by reference methods as appropriate, particularly if only a limited number of positive samples are associated with a suspected outbreak. Additional testing of negative samples by other methods should be performed if norovirus is strongly suspected as the cause of an acute gastroenteritis outbreak.

2. Indication(s) for use:

   Identical to intended use

3. Special conditions for use statement(s):

   For prescription use

4. Special instrument requirements:

   None

I. Device Description:

RIDASCREEN® Norovirus 3rd Generation test is a solid phase sandwich-type EIA for the detection of genogroups GI and GII noroviruses in stool samples. Microwell strips are coated with a mixture of GI and GII norovirus specific monoclonal antibodies. An aliquot of fecal suspension is added to the microwell together with biotinylated monoclonal norovirus antibodies. After washing, streptavidin peroxidase conjugate is added. Norovirus antigens that are present in the stool sample are captured in a sandwich complex of the immobilized antibodies, the norovirus antigens and the monoclonal antibodies conjugated with the biotin-streptavidin-peroxidase complex. Unbound streptavidin peroxidase conjugate is removed by washing and a chromogenic colorless substrate solution (hydrogen peroxide/TMB) is added. The substrate is hydrolyzed by any bound peroxidase, changing the chromogen to a blue color. Stopping the reaction with acid converts the blue to a yellow color indicating the presence of norovirus antigens.

Test results are read photometrically; intensity significantly above background levels is indicative of the presence of Norovirus antigen in the specimen or control. The RIDASCREEN® Norovirus 3rd Generation test does not identify specific norovirus strains.

J. Substantial Equivalence Information:

1. Predicate device name(s): None
2. Predicate 510(k) numbers: None
3. Comparison with predicate: Not applicable

K. Standard/Guidance Document Referenced (if applicable):

A special control guidance document will be promulgated.

L. Test Principle:

Enzyme immunoassay

M. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

i. Inter-assay reproducibility:

Inter-assay precision was studied with 6 fecal samples run in triplicates on 10 consecutive days by three different operators using 3 separate test kits.

Inter-assay Reproducibility (Results reported are for Total Precision)

		Sample 1 High Positive	**Sample 2 Medium Positive**	**Sample 3 Medium Positive**	**Sample 4 Low Positive**	**Sample 5 Low Positive**	**Sample 6 Negative**
Min/max Standard [OD 450]		**1.303/2.420**	**0.811/1.506**	**0.568/1.088**	**0.499/0.927**	**0.392/0.728**	**0.000/0.200**
Kit 1		**1.533**	**1.108**	**0.882**	**0.616**	**0.460**	**0.085**
	SD	0.179	0.084	0.142	0.062	0.058	0.024
	CV	**11.66%**	**7.55%**	**16.09%**	**10.11%**	**12.54%**	**28.02%**
Kit 2		**1.690**	**1.181**	**0.920**	**0.705**	**0.506**	**0.092**
	SD	0.124	0.070	0.088	0.119	0.051	0.019
	CV	**7.33%**	**5.89%**	**9.53%**	**16.93%**	**10.04%**	**20.45%**
Kit 3		**1.716**	**1.213**	**0.924**	**0.666**	**0.492**	**0.084**
	SD	0.181	0.113	0.158	0.080	0.077	0.018
	CV	**10.53%**	**9.28%**	**17.10%**	**12.08%**	**15.66%**	**20.86%**

ii. Intra-assay reproducibility:

Intra-assay precision was determined by measuring 40 replicates of six fecal samples within one assay run. The accepted OD levels of the samples are listed below. Positive and negative controls were measured in triplicates each run, and three independent kit lots were tested. Mean values, standard deviations and coefficients of variance (CV) are summarized below.

Intra-assay Reproducibility (Results Reported are for Total Precision)

		Sample 1 High Positive	Sample 2 Medium Positive	Sample 3 Medium Positive	Sample 4 Low Positive	Sample 5 Low Positive	Sample 6 Negative
Min/max Standard [OD 450]		**1.172/ 2.176**	**1.001/ 1.859**	**0.737/ 1.369**	**0.484/ 0.900**	**0.284/ 0.527**	**0.000/ 0.200**
Kit 1		**1.653**	**1.205**	**1.014**	**0.563**	**0.309**	**0.051**
	SD	0.042	0.114	0.034	0.021	0.016	0.002
	CV	**2.54%**	**9.48%**	**3.39%**	**3.80%**	**5.16%**	**4.35%**
Kit 2		**1.643**	**1.198**	**1.012**	**0.561**	**0.308**	**0.051**
	SD	0.041	0.114	0.035	0.021	0.016	0.002
	CV	**2.50%**	**9.50%**	**3.42%**	**3.72%**	**5.14%**	**4.41%**
Kit 3		**1.481**	**0.953**	**1.006**	**0.559**	**0.307**	**0.051**
	SD	0.101	0.066	0.035	0.021	0.016	0.003
	CV	**6.81%**	**6.92%**	**3.43%**	**3.78%**	**5.19%**	**5.11%**

iii. Inter-lot reproducibility:

Inter-lot reproducibility was determined with three kits, each tested in triplicate over 10 days. As part of the inter-assay reproducibility study reported above. The following results are from the same data analyzed across kits:

Inter-lot Reproducibility (Results reported are for Total Precision)

	Sample 1 High Positive	Sample 2 Medium Positive	Sample 3 Medium Positive	Sample 4 Low Positive	Sample 5 Low Positive	Sample 6 Negative
No. Days	10	10	10	10	10	10
No. Results	90	90	90	90	90	90
No. Kit Lots	3	3	3	3	3	3
Total precision:						
Mean value [OD450]	**1.646**	**1.167**	**0.909**	**0.662**	**0.486**	**0.087**
SD	0.185	0.103	0.133	0.098	0.065	0.020
CV	**11.23%**	**8.82%**	**14.60%**	**14.84%**	**13.33%**	**23.26%**

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The sponsor conducted LoD validation of their Region B and region D conventional PCR assays using Norovirus positive samples of known viral concentrations. Serial dilution testing of GI.3b, GI,7, GII.3, and GII.4 containing stool samples using the sponsor's conventional Region B and Region D PCR assays estimated assay sensitivity at between 1 - 100 copies/µl for all strains, or approximately 250 – 3,500 mean copies/gm of stool. Additional strain reactivity studies examining Genogroup I: GI.2, GI.3b, GI.4, GI.6, GI.8, and Genogroup II: GII.1-4, GII.6, GII.7, GII.12, GII.14, GII.16, GII.17 samples found similar sensitivity using the Region D and region B assays, with the exception of GII.16 and GII.17, where sensitivity was approximately 1 – 2 logs less. Region B and region D conventional PCR sensitivities were similar (i.e., within 1 – 2 logs) for each strain tested with the exceptions of GII.14 and GII.17 which differed between the two assays by three logs; in both cases Region B conventional PCR was more sensitive than Region D testing. The sensitivity from combined use of the Region B and Region D assays was deemed acceptable for use as a reference standard for the clinical studies.

d. *Detection limit:*

The limit of detection was determined by conventional RT-PCR followed by bi-directional sequencing for norovirus regions B and Region D, and for electron microscopy. One GI.1 and one GII.4 isolate were studied. Results were reported as follows:

Limit of Detection

Genotype	Dilution	RIDASCREEN® Norovirus 3rd Generation ELISA	Conventional RT-PCR	Real-time RT-PCR	EM
		Result	Result	Genomic copies per g	Particles per g**
GI.1	$1x10^{0}$ *	**positive**	**positive**	**1.10E+08**	**2.9E+08**
	$1x10^{1}$	**positive**	**positive**	**5.78E+07**	**3.5E+07**
	$1x10^{2}$	**positive**	**positive**	**5.45E+06**	**2.4E+07**
	$1x10^{3}$	negative	**positive**	**6.70E+05**	negative
	$1x10^{4}$	negative	**positive**	**1.24E+05**	n/a
	$1x10^{5}$	negative	**positive**	negative	n/a
	$1x10^{6}$	negative	**positive**	negative	n/a
GII.4	$1x10^{0}$ *	**positive**	**positive**	**1.91E+09**	**4.7E+08**
	$1x10^{1}$	**positive**	**positive**	**7.35E+08**	**5.9E+07**

	$1x10^2$	**positive**	**positive**	**2.89E+07**	**1.6E+07**
	$1x10^3$	negative	**positive**	**6.65E+05**	negative
	$1x10^4$	negative	**positive**	negative	n/a
	$1x10^5$	negative	**positive**	negative	n/a
	$1x10^6$	negative	negative	negative	n/a

* $1x10^0$ equals 20% dilution

** Genomic copies per gram and number of viral particles in the native fecal samples were calculated back from each positive dilution and the mean values are presented here

e. Stability:

Stability was assessed by kit for 3 lots against a panel including positive and negative controls, and 4 samples with different norovirus OD readings. Analyte stability was tested after sample freeze/thawing, in suspension ('ready to use'), and transport (shipping of samples internationally). Overall, decrement in OD values for positive samples at 12 months was < 20%.

Stability Testing Over 12 Months

Sample	Mean OD 0 Months	Mean OD 12 Months	% Decrease
#1	1.686	1.382	-18.0
#2	1.416	1.093	-22.8
#3	1.113	0.926	-16.8
#4	0.719	0.555	-22.8
#5	0.602	0.487	-19.0
#6	0.333	0.294	-11.5

f. Analytical specificity:

i. Cross-reactivity:

Cross-reactivity was assessed for the following:

Bacteria*: Acinetobacter iwoffi, Aeromonas hydrophila aerogenes, Aeromonas hydrophila hydrophila, Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, Citrobacter freundii, Clostridium difficile, Clostridium perfringens, Clostridium sordellii, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, E. coli, E. coli (O157:H-), E. coli (O116:H21), E. coli (O111:H-), E. coli (O22:H8), E. coli (O26:H11), Escherichia hermannii, Helicobacter pylori, Lactococcus lactis, Listeria innocua, Morganella morganii, Proteus mirabilis, Proteus vulgaris. Providencia stuartii, Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas putida, Salmonella agona, Samonella choleraesuis, Salmonella enteritidis, Salmonella infantis, Salmonella Ohio, Salmonella typhimurium, Serratia proteamaculans (liquefaciens), Shigella flexneri, Shigella sonnei,*

Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis

Fungi: *Candida albicans*

Toxins*:* Shigatoxin STX, Shigatoxin STX2

Amoeba/Parasites: *Cryptosporidium parvum, Entamoeba histolytica, Giardia lamblia*

Viruses*: Astrovirus, Adenovirus, Rotavirus, Sapovirus*

No cross-reactivity was seen for any of the bacteria, viruses, toxins, for fungi tested.

ii. Interfering substances

Interference was checked with the following substances spiked into a negative and a positive specimen and then measured in triplicate.

Substances Tested for Interference

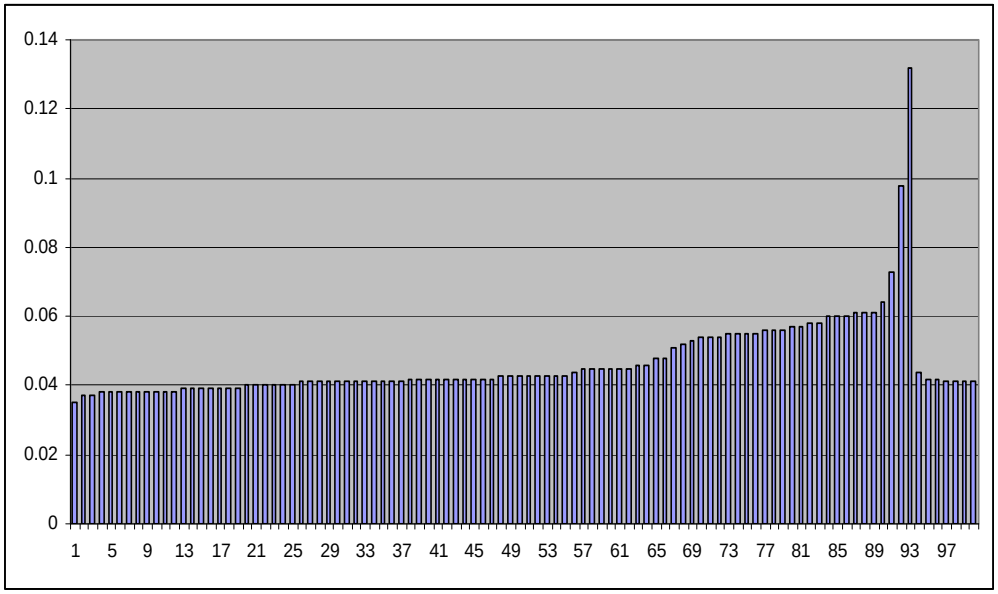
Substance	**Concentration**
Mucin	5 % (w/w)
Human blood	5 % (v/w)
Barium sulfate (contrast medium)	5 % (w/w)
Loperamide (anti-diarrhea drug)	5 % (w/w)
Pepto-Bismol (anti-diarrhea drug)	5 % (v/w)
Stearic acid / Palmitic acid (1:1) (fatty acids)	40 % (w/w)
Metronidazole (0.5) (antibiotic)	5 % (v/w)
Diclofenac (analgesic)	0.00263 % (v/w)
Cyclamate (artificial sweetener)	5 % (v/w)

No of interference was seen for any of the substances tested.

g. Assay cut-off:

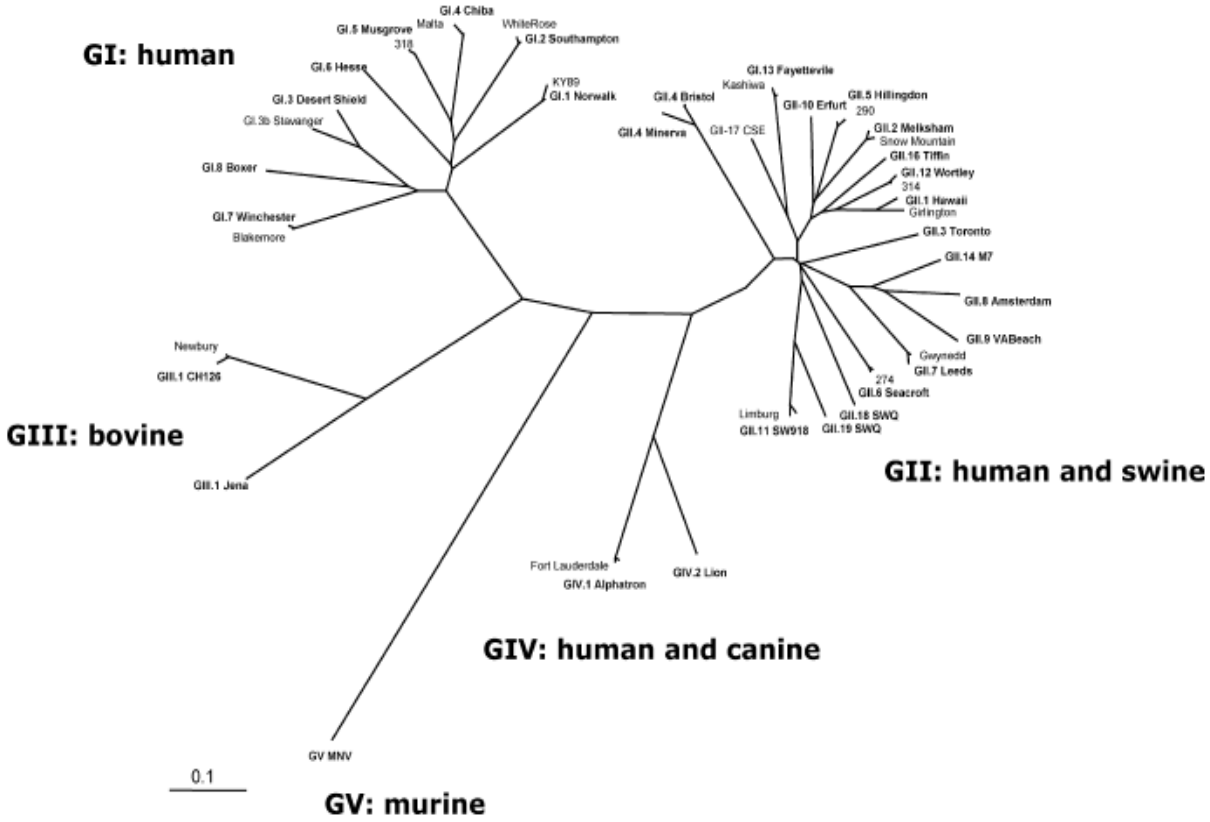
Assay cut-off is established by adding 0.15 OD units to negative controls for each run; the 0.15 addition is based on the mean OD for negative samples (Limit of Blank) plus 5 x the standard deviation for negative and low analyte samples. Samples are considered positive if the OD is > 10% above the cut-off and negative if > 10% below the cut-off. Values between these two limits are considered equivocal and the test repeated.

The following are the results for 100 negative stool isolates tested by the sponsor, sorted in ascending order: 98/100 were below <0.075, with one value at 0.098 and 0.132 each.



h. Strain-reactivity:

There are 5 norovirus Genogroups (GI – V); human disease has only been identified in groups I, II, and IV; Genogroup IV-associated disease is uncommon and has been only observed outside the US.



(Reproduced from Patel et al., Journal of Clinical Virology 44 (2009) 1–8.)

The sponsor studied 100 archived samples that included 80 samples across different genotypes from Genogroups I and II, 10 negative samples, and 10 other viruses. Results are summarized below:

Noroviruses Genotypes Samples for Strain Reactivity

Sample tested	Number	#positive/#tested
Genogroup I		
1	2	2/2
2	2	1/2
3	3	1/3
4	3	0/3
5	4	0/4
6	2	0/2
7	2	2/2
8	2	0/2
Genogroup II		
1	3	2/3
2	4	3/4
3	8	7/8
4	16	16/16
5	5	3/5
6	5	1/5
7	4	4/4
8	3	3/3
9	1	0/1
10	1	1/1
12	3	1/3
13	2	1/2
14	2	1/2
16	1	0/1
17	2	1/2
Other viruses		
Sapovirus	3	0/3
Rotavirus	3	0/3
Astrovirus	2	0/2
Enterovirus	2	0/2
Negative	10	0/10

2. Comparison studies:

a. Method comparison with predicate device:

There is no predicate device for Norovirus detection; following pre-IDE discussions with FDA/DMD, a composite reference endpoint of quantitative real time RT-PCR (qRT-PCR), conventional RT-PCR with bi-directional sequencing, with a subset of samples undergoing electron microscopy (EM), was agreed to. Specimens positive by either conventional RT-PCR (region D + sequencing) or Electron Microscopy were considered 'Clinical Diagnostic Truth' positive. The following table describes the original algorithm for establishing the reference standard:

Original Composite 'Clinical Diagnostic Truth' Algorithm

Real-time RT-PCR	**Conventional RT-PCR** (region D + sequencing)	**EM** (with and without)	**Clinical Diagnostic Truth**
positive	positive	positive	positive
		negative	positive
		-	positive
negative	positive	positive	positive
		negative	positive
		-	positive
positive	negative	positive	positive
		negative	Indeterminate*
		-	Indeterminate*
negative	negative	positive	positive
		negative	negative
		-	negative

* - For indeterminate results, an additional conventional RT-PCR of Region-B followed by bi-directional sequence analysis was performed; clinical diagnostic truth was then determined on the basis of this result.

However, due to concerns regarding the execution of the real-time reverse transcriptase assay at the sponsor's clinical site, it was agreed that the sponsor would perform Region B RT-PCR testing on all Region D RT-PCR negative samples and that a revised 'Clinical Diagnostic Truth' algorithm would be used: in the revised algorithm, samples that were positive by either Region D RT-PCR, Region B RT-PCR, or Electron Microscopy were classified as 'Clinical Diagnostic Truth' positive.

Revised 'Clinical Diagnostic Truth' Algorithm

Region D RT-PCR	Region B RT-PCR	EM (with and without)	Clinical Diagnostic Truth
positive	-	positive	positive
		negative	positive
		-	positive
positive	-	positive	positive
		negative	positive
		-	positive
negative	positive	positive	positive
		negative	positive
		-	positive
	negative	positive	positive
		negative	negative
		-	negative
negative	positive	positive	positive
		negative	positive
		-	positive
	negative	positive	positive
		negative	negative
		-	negative

b. Matrix comparison:

Not applicable.

3. Clinical studies:

a. Clinical Protocol

Clinical Trial Protocol 001-07: ELISA for Detection of Norovirus Antigens in Stool, Version 2.3.

i. Main Objectives

The protocol main objectives were listed as follows:

a. Primary objective:
To evaluate the sensitivity and specificity of the R-Biopharm Norovirus test for the detection of GI and GII viruses using stool samples from outbreaks or sporadic cases of gastroenteritis.

b. Secondary objectives:
- To evaluate predictive values
- To verify cut-off values

- Sampling of supportive clinical data (subject age, gender, geographic region, duration of illness before collection of stool sample, vomiting, diarrhea) and evaluate influence on diagnostic accuracy.

A minimum of 410 specimens were to be collected.

ii. Major Inclusion/Exclusion Criteria

Major inclusion criteria included:

- Stool specimen (non-bloody after visual inspection) submitted for gastroenteritis testing
- Patient information: age and gender, date of specimen collection if available symptoms and signs of gastroenteritis (e.g., fever, vomiting, diarrhea (presence or absence, frequency), nausea, abdominal cramps and other symptoms)

Major exclusion criteria included:

- No date of stool collection OR date of initial onset of symptoms provided
- Days from onset of symptoms to stool collection more then three days

b. Clinical Sensitivity and Specificity:

i. Overall Performance

The study was conducted at four sites (three in the United States and one in Canada). Samples were collected from patients with diarrhea and vomiting; subject selection was described as follows:

> Specimens were be from patients (inpatient/outpatient, nursing homes occupants, various party attendees, conference attendees, restaurant guests and children) with signs and symptoms of acute gastroenteritis who are having samples submitted for diagnostic testing and for which there is at least 2.0 ml or gram residual stool for performance of the ELISA and reference assays.

Clinical Study Sites

Site	Loc.	N	Norovirus ELISA	RT-PCR Sequencing	EM
1	Ohio Dept. of Health, Columbus, OH	200	Performed on site	Performed at Calif. Dept. Of Public Health (CPDH)	National Calicivirus Laboratory, Centers for Disease Control and Prevention (CDC)
2	California Dept. of Public Health, Richmond, CA	214	Performed on site	Performed at CPDH	Performed at CDC
3	Cincinnati Children's Hospital Medical Center, Cincinnati, OH	211	Performed on site	Performed at CPDH	Performed at CDC
4	St. Joseph's Healthcare, Hamilton, Ontario	55	Performed on site	Performed at CPDH	Performed at CDC
Total		**680**			

Of the 680 samples collected, 69 were reported as invalid: 54 for 'no result' (although this described as the sponsor as not qualified by weight or volume), 10 with a missing PCR result, and 5 with a PCR result but no ELISA. Two equivocal samples were excluded from analysis. Overall results were reported for these 609 samples.

Overall performance results as follows:[1]

Overall Clinical Trial Performance

		Presence of Norovirus ("Clinical Diagnostic Truth")		
		+	-	Total
RIDASCREEN Result	+	159	37	196
	-	85	328	413
Total		244	365	609

Positive agreement (95% CI)	65 % (59 – 71 %)
Negative agreement (95% CI)	90 % (86 – 93 %)

Ninety-seven samples were studied by EM; of n = 34 PCR (+) samples, 4 were EM positive and 24 ELISA positive. Of n = 59 disease negative samples, none were EM positive but 11/59 were ELISA (+).

[1] Of the two equivocal samples, one sample was Clinical Diagnostic Truth (+) and the other (-).

ii. Sensitivity for specific Norovirus strains (strain reactivity)

As noted earlier, the RIDASCREEN® Norovirus 3rd Generation assay shows differential sensitivity across norovirus genotypes. The sponsor reports that 167 'PCR positive' specimens were analyzed for genogroup/subtype with the following results:

Clinical Genotypes Detected

Genogroup I			**Genogroup II**		
Subtype	No. of PCR positive samples	No. of samples detected by ELISA	Subtype	No. of PCR positive samples	No. of samples detected by ELISA
2	2	1	1	1	1
3	10	2	3	3	3
3b	2	0	4	123	96
4	8	6	7	1	1
5	1	0	Unable to type	5	4
Unable to type	11	0			

The performance was better in the archived, higher inoculum well-characterized specimens described earlier relative to clinical specimens; several strains were not detected in the archived panel, e.g., GI.1, GI7, GI.8, and GII.9, and limited detection of others, e.g., only 1/5 GII.6 samples were ELISA positive. However, strains other than GII.4 and GI.3 were relatively uncommon in the clinical study, and literature supports GII.4 as the predominant strain in the US. Performance in the clinical study generally parallels that seen for archived specimens.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

Noroviruses are a frequent cause of gastroenteritis outbreaks in semi-closed settings that involve children and/or adults such as day-care centers, nurseries, hospitals, nursing homes, prisons, and cruise ships, or other settings that may facilitate person-person spread. Noroviruses account for ~50% of all acute gastroenteritis outbreaks worldwide, although the proportion of outbreaks expected to be positive and the rate of positive test results within an outbreak depend on a number of factors, including (among others), the prevalence of

norovirus within the population, the specific genotype(s) circulating, how rapidly specimens are taken, and the setting of the outbreak.

The expected value for RIDASCREEN® Norovirus 3rd Generation should be negative, although approximately 1/3 of infected patients may be asymptomatic. The design of the clinical study did not permit definitive analysis of the effects of age or gender on test performance, but exploratory analysis suggested that performance was similar across all age ranges and for both genders.

N. Proposed Labeling:

The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The petition for Evaluation of Automatic Class III Designation for this device is accepted. The device is classified as Class II under regulation 21 CFR 866.3395 with special controls. The special control guidance document "Class II Special Controls Guidance Document: Immunoassay or Antigen Detection-based *In Vitro* Diagnostic Devices for Norovirus Detection" will be available shortly.