PREMIER® ROTACLONE®

EIA for the detection of Rotavirus Antigen in Human Fecal Samples

REF **696004** IVD **Rx Only**

INTENDED USE
PREMIER Rotaclone is an Enzyme Immunoassay (EIA) intended for the detection of rotavirus antigen in human fecal specimens.

SUMMARY AND EXPLANATION OF THE TEST
Rotaviruses are the most important cause of early childhood non-bacterial gastroenteritis,[1-5] with the illness also being observed in older children and adults.[6-8] Rotaviral gastroenteritis may result in mortality for populations at risk such as infants, the elderly, and immunocompromised patients.[6-9] Nosocomial transmission of rotavirus is often a costly and difficult problem to resolve; therefore, the use of the monoclonal antibody based PREMIER Rotaclone test, which provides rapid accurate detection of rotavirus antigens, may lead to better patient management.

Transmission electron microscopy (EM) was the method initially used to detect virus in fecal and intestinal biopsy samples, and remains the standard to which rotavirus diagnostic tests are compared.[1, 2] Rotaviruses are generally very difficult to detect *in vitro*; therefore, cell culture is not routinely used for detection and diagnosis.[1, 2] The enzyme immunoassay (EIA) is a simple, highly sensitive method for the detection of rotavirus antigen, and is well suited for analysis of large numbers of samples.

BIOLOGICAL PRINCIPLES
PREMIER Rotaclone utilizes monoclonal antibodies in a solid phase sandwich type EIA. Plastic microtiter wells are coated with a monoclonal antibody directed against the product of the sixth viral gene (VP6), which is the group specific antigen for all known human rotaviruses.[12] An aliquot of fecal suspension is added to the well and incubated simultaneously with an anti-rotavirus monoclonal antibody conjugated to horseradish peroxidase, resulting in the rotavirus antigen being sandwiched between the solid phase and enzyme-linked antibodies. After 60 minutes incubation at room temperature, the sample well is washed in order to remove unbound enzyme labeled antibodies. Enzyme substrate A (urea peroxide) and substrate B (TMB) are added to the wells and incubated for 10 minutes at room temperature. The enzyme bound in the wells converts the colorless substrate to a blue color. The intensity of the blue color is directly proportional to the concentration of rotavirus antigen in the sample.

REAGENTS/MATERIALS PROVIDED
The maximum number of tests obtained from this test kit is listed on the outer box.
1. Monoclonal Antibody coated microtiter wells
2. PREMIER Rotaclone Conjugate: Horseradish peroxidase conjugated to anti-rotavirus monoclonal antibody in a buffered protein solution with gentamicin and 0.02% thimerosal as preservatives
3. Positive Control: Inactivated simian rotavirus SA-11 in buffered saline with 0.02% thimerosal as a preservative
4. Sample Diluent: Buffered saline with 0.02% thimerosal as preservative
5. Part A Substrate Buffer: Contains urea peroxide
6. Part B Substrate Solution: Contains tetramethylbenzidine (TMB)
7. Stop Solution: Contains 1N H_2S0_4
8. Transfer pipettes
9. Microtiter well holder

MATERIALS NOT PROVIDED
1. 12 x 75 mm test tubes, test tube rack
2. Deionized or distilled water
3. Absorbent paper
4. Precision micropipette tips to deliver 100 µL and 1000 µL (optional)
5. Waste container with a 1:10 dilution of household bleach. For autoclaving, use an iodophor disinfectant.
6. Microwell plate reader capable of reading absorbance at 450 nm (optional).
7. Device for dispensing wash solution such as multi-channel pipette, syringe with manifold, wash bottle, etc.
8. Timer (minimum 1 hour)

PRECAUTIONS
1. All reagents are for in vitro diagnostic use only.
2. Do not mouth pipette samples or reagents. Avoid contact with broken skin or mucous membranes.
3. Do not smoke, eat or drink in areas where specimens or kit reagents are handled.
4. Wear disposable gloves while handling samples and wash hands after assay is complete. Patient specimens, assay controls and all materials coming into contact with them should be handled at Biosafety Level 2 as recommended in the CDC/NIH manual "Biosafety in Microbiology and Biomedical Laboratories".
5. Avoid skin contact with stop solution (1N sulfuric acid). It may cause irritation and burns. Flush with water if contact occurs.
6. Dispose of all materials used to perform the test by autoclaving at 121 C for at least one hour. Liquid waste may be disposed by mixing with a 1:10 dilution of household bleach for a minimum of 30 minutes. **CAUTION:** Liquid waste containing stop solution must be neutralized before addition of household bleach.
7. Avoid splashing or generation of aerosols.
8. Do not use PREMIER Rotaclone reagents beyond the kit expiration date. Each reagent has been optimized for maximum performance. Dilution or adulteration of these reagents may result in a loss of sensitivity. Incubation time and temperatures other than those specified may give erroneous results.
9. Do not interchange or mix different lots of PREMIER Rotaclone reagents.
10. Avoid microbial contamination of reagents or incorrect results may occur. Contamination of samples could cause erroneous results.
11. Use separate pipettes or pipette tips for each sample, control and reagent.
12. DO NOT REUSE MICROWELLS.

HAZARD and PRECAUTIONARY STATEMENTS

 Premier Stop Solution	**Signal Word** Danger **Hazard Statements** H330 - Fatal if inhaled Contains Sulfuric acid **Precautionary Statements - EU (§28, 1272/2008)** P310 - Immediately call a POISON CENTER or doctor/ physician P304 + P340 - IF INHALED: Remove to fresh air and keep at rest in a position comfortable for breathing P260 - Do not breathe dust/fume/gas/mist/vapors/spray P403 + P233 - Store in a well-ventilated place. Keep container tightly closed
 Premier Substrate B	**Signal Word** Danger **Hazard Statements** H301 - Toxic if swallowed H311 - Toxic in contact with skin H330 - Fatal if inhaled H370 - Causes damage to organs Contains Methyl alcohol **Precautionary Statements - EU (§28, 1272/2008)** P301 + P310 - IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician P280 - Wear protective gloves/ protective clothing/ eye protection/ face protection P310 - Immediately call a POISON CENTER or doctor/ physician P304 + P340 - IF INHALED: Remove to fresh air and keep at rest in a position comfortable for breathing P403 + P233 - Store in a well-ventilated place. Keep container tightly closed P307 + P311 - IF exposed: Call a POISON CENTER or doctor/ physician P260 - Do not breathe dust/fume/gas/mist/vapors/spray P309 - IF exposed or if you feel unwell: P370 + P378 - In case of fire: Use dry sand, dry chemical or alcohol-resistant foam for extinction P210 - Keep away from heat/sparks/open flames/hot surfaces. - No smoking

SHELF LIFE AND STORAGE
Store kit reagents at 2-8 C. Bring kit reagents to room temperature before use and promptly return to 2-8 C after use. Return all unused microtiter wells to their original foil pouch.

INDICATION OF INSTABILITY OR DETERIORATION
The following conditions may indicate reagent deterioration:
1. Any evidence of microbial contamination or heavy precipitation.
2. Any blue color in the substrate solutions before addition to microwells.
3. A negative control value greater than 0.150 absorbance units at 450 nm may indicate deterioration of reagents.
4. A positive control value of less than 0.3 absorbance units may indicate deterioration of reagents.

If any of the above conditions are observed, contact our Technical Support Center at 1-800-343-3858.

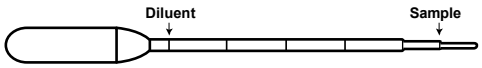
REAGENT PREPARATION
1. Bring all reagents to room temperature (20-30 C) before use.
2. Return all reagents to 2-8 C immediately after use.
3. Do not allow wells to dry between steps.
4. Prepare decontaminating vessel for discarding reagents and materials.
5. Reproducibility in any EIA assay is largely dependent upon the consistency with which the microwells are washed. Carefully follow the recommended washing sequence as outlined in the EIA test procedure.

SPECIMEN COLLECTION AND PREPARATION
Stool specimens should be collected as soon after onset of symptoms as possible. Peak viral counts are reported to occur on days 3-5 after onset of symptoms. Samples collected eight days or more after onset of symptoms may not contain enough rotavirus antigen to produce a positive reaction. Do not collect specimens in containers having media, preservatives, animal serum or detergent, as any of these may interfere with the PREMIER Rotaclone assay.

Diluted samples may be stored at 2-8 C for three days without interference with the assay performance. For long term storage of undiluted specimens, -20 C or colder is recommended. Repeated freezing and thawing of samples is not recommended and may cause erroneous results. Do not store in self-defrosting freezers.

SPECIMEN PREPARATION
Add 1 mL of sample diluent to properly marked tube, using a transfer pipette or precision pipette. Add samples by one of the following:
1. Solid stool – press sample into transfer pipette to first mark.
2. Liquid stool – aspirate sample into transfer pipette to first mark.
3. Rectal swab – swirl swab in one ml of sample diluent to release fecal material. Firmly press swab against side of tube to remove liquid. Mix thoroughly. *Do not dilute further.*

Resuspend sample in 1 mL of Sample Diluent.



TEST PROCEDURE
1. Snap off sufficient number of wells for samples and the controls and insert into the microtiter well holder. Record sample position.
2. Add 2 drops (100 µL) each of diluted fecal sample, positive control and negative control (sample diluent) to the bottom of separate wells.
3. Add 2 drops (100 µL) of enzyme conjugate to each well. Mix by gently swirling on tabletop.
4. Incubate at room temperature for 60 ± 5 minutes.
5. Pour the liquid out of the wells into a discard vessel. Tap the microtiter well holder upside down vigorously against absorbent paper to ensure complete removal of liquid from the wells.
6. Fill all the wells to overflowing with deionized water and pour the liquid out as in Step 5.
7. Repeat the washing procedure (Steps 5 & 6) four more times (for a total of 5 washes).
8. Add 2 drops (100 µL) of substrate A solution to each well.
9. Add 2 drops (100 µL) of substrate B solution to each well.
10. Incubate 10 minutes at room temperature.

Visual determinations can be made after 10 minutes incubation in step 10. Samples with blue color greater than the negative control are positive. Samples showing equal or less color than the negative control are negative.

OPTIONAL – Spectrophotometric Procedure
Spectrophotometric determinations can be made by adding 2 drops (100 µL) of Stop Solution (sulfuric acid) to each well after the incubation in Step 10. Read the absorbance of each well at 450 nm using a > 600 nm reference filter (optional) against an air blank within 60 minutes.

INTERPRETATION OF RESULTS
1. **Positive Results by Visual Determination.** Any **sample with blue** color **more** intense than that of the negative is considered positive. Any sample with color equal to or less intense than the negative control is considered negative.
2. **Positive Results by Spectrophotometric Determination.** Specimens with absorbance units (A_{450}) greater than 0.150 are considered positive. Specimens with absorbance equal to or less than 0.150 are considered negative. Occasionally, a discrepancy may occur between visual and spectrophotometric determinations with samples containing low amounts of antigen. Spectrophotometric determination, being an objective method, is slightly more accurate.

NOTE: A precipitate may form in high positive samples. This will not affect the results.

QUALITY CONTROL
This test should be performed per applicable local, state, or federal regulations or accrediting agencies.
PREMIER Rotaclone contains a Positive Control and a Negative Control (Sample Diluent) which should be run with each assay to ensure that kit reagents are functioning correctly and that proper assay procedures have been followed. If a visual evaluation is performed, the positive control should be deep blue and easily distinguished from the negative control. The negative control should be colorless or faint blue. If spectrophotometric determination is used, remove all bubbles and check the optical surface for spots or condensation; wipe with soft tissue if necessary. If the absorbance of the positive control does not equal or exceed 0.3 absorbance units, the assay is considered invalid and should be repeated. If the expected control reactions are not observed, repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor.

EXPECTED VALUES
Rotavirus infection is seasonal and is the most frequent cause of gastroenteritis in children six months to three years of age. Among children hospitalized for gastroenteritis, it can be expected that up to 50% of the patient specimens will give positive rotavirus test results.[12] The rate of positive test results may vary due to age, weather, seasonal factors, geographic location, and the general health environment for the group under study.[13]

LIMITATIONS OF THE PROCEDURE
PREMIER Rotaclone is highly specific and sensitive for rotavirus antigen. This does not preclude the presence of other pathogenic organisms. While the relationship between rotavirus and gastroenteritis is well established, co-infection with bacterial pathogens is possible. Bacterial testing should be performed in parallel with PREMIER Rotaclone to rule out bacterial etiology of the illness.

Clinical testing of neonatal stools with PREMIER Rotaclone indicates good specificity and the absence of false positives;[13] however, the results must be interpreted with caution. A negative result does not exclude the possibility of rotavirus infection, as too small a quantity of virus or inadequate or improper sampling may cause a false negative result.

SPECIFIC PERFORMANCE CHARACTERISTICS
PREMIER Rotaclone was tested in collaboration with two independent laboratories in the northeastern and southwestern USA on a total of 121 stools from children with gastroenteritis and compared with results obtained by EM. Discrepant results were resolved by use of a confirmatory blocking EIA.[14] RNA analysis or serology. Results are shown in Table 1.

Table 1.

		EM		EM/RNA		Kallestad	
		+	-	+	-	+	-
PREMIER Rotaclone	+	36	3	38	1	29	0
	-	0	35	0	35	7	49
Sensitivity		100%		100%		81%	
Specificity		92%		97%		100%	
Agreement		96%		99%		92%	

LIMITS OF DETECTION
Serial dilutions of human rotavirus of a known particle count determined by EM were tested by PREMIER Rotaclone. The results are shown in Table 2.

Table 2.

EM	PREMIER Rotaclone
(Particles/mL)	(A_{450})
1.1×10^9	> 2.40
3.7×10^8	> 2.40
1.2×10^8	> 2.40
4.1×10^7	> 2.40
1.4×10^7	> 2.40
4.5×10^6	2.40
1.5×10^6	.60
*5.0×10^5	.19
1.7×10^5	.09

A stool sample containing 3.3×10^{10} rotavirus particles/mL was serially diluted with a sample diluent.
* Sensitivity limits

REPRODUCIBILITY
1. Intra-Assay Variation
Three different concentrations of SA-11 rotavirus samples were assayed 21 times in duplicate (n=21). The results were as follows:

Intra-Assay Variation	Sample I (Negative)	Sample II (Low Pos)	Sample III (High Pos)
Mean Absorbance	0.059	0.184	0.561
Standard deviation	0.007	0.011	0.045
C.V. (%)	11.9	5.9	8.0

2. Inter-Assay Variation
Three different concentrations of SA-11 rotavirus samples were assayed 23 times using the same kit lot. The results were as follows:

Inter-Assay Variation	Sample I (Negative)	Sample II (Low Pos)	Sample III (High Pos)
Mean Absorbance	0.073	0.204	0.933
Standard deviation	0.008	0.023	0.100
C.V. (%)	12.3	11.4	10.7

CROSSREACTIVITY
Common intestinal pathogens and other organisms occasionally present in feces were tested by PREMIER Rotaclone. Suspensions containing 10^7 to 10^9 organisms were prepared in normal stool extracts with and without rotavirus. Results are shown in Table 3. A similar experiment was performed with a variety of viruses. These results are shown in Table 4.

Table 3. Crossreactivity Study

	A_{450}*	A_{450}**
Microorganisms Tested	**Organism Alone**	**Organism & Virus**
Pseudomonas aeruginosa	0.08	0.81
Staphylococcus aureus	0.06	0.66
Staph. aureus (Cowan's)	0.05	0.71
Candida albicans	0.06	0.71
Enterobacter aerogenes	0.06	0.69
Enterobacter cloacae	0.06	0.72
Klebsiella pneumoniae	0.05	0.74
Proteus mirabilis	0.06	0.77
Serratia marcescens	0.07	0.72
Escherichia coli	0.07	0.81
Rotavirus neg stool	0.08	–
Rotavirus pos stool	–	0.78

Table 4.

	A_{450}*	A_{450}**
Virus Tested	**Organism Alone**	**Organism & Virus**
Echo 22	0.02	1.06
Echo 32	0.02	1.01
Coxsackie A-9	0.02	1.11
Coxsackie B-1	0.02	1.01
Coxsackie B-6	0.02	1.00
Adeno 2	0.02	1.05
Adeno 40	0.02	1.05
Adeno 41	0.02	1.06
Rotavirus neg stool	0.02	–
Rotavirus pos stool	–	1.07

* Rotavirus negative 10% stool suspension used as diluent.
** Rotavirus positive 10% stool suspension used as diluent.