ImmunoCard STAT! ROTAVIRUS

A RAPID IMMUNOASSAY FOR THE DETECTION OF ROTAVIRUS ANTIGEN IN HUMAN STOOL

REF **750030**

IVD

R_x Only

INTENDED USE
The Immuno*Card* STAT! Rotavirus Immunoassay is a rapid in vitro qualitative procedure for the detection of rotavirus antigen in human stool. The test can be used to aid in the diagnosis of rotavirus associated gastroenteritis.

SUMMARY AND EXPLANATION OF THE TEST
Rotavirus is a major cause of acute gastroenteritis, especially in children 6 to 24 months in age.[1-3] In addition, rotavirus infections can produce severe illness as well as asymptomatic infection in adults.[1, 2, 4, 5] The incubation period of rotavirus infection is usually one to three days, followed by gastroenteritis with an average duration of five to eight days. Virus titers in stool reach a maximum shortly after the onset of illness, then decline.[2, 6, 7] Due to inadequacies in existing culture methods, human rotavirus is not routinely isolated from rotavirus-containing specimens.[2, 8] For many years, electron microscopy has been the standard method for rotavirus detection. However, newly introduced enzyme immunoassays and latex agglutination assays with increased sensitivities and specificities are now the methods of choice.[9-15] The Immuno*Card* STAT! Rotavirus test offers a simple, rapid method for detecting rotavirus antigen in patient stool.

BIOLOGICAL PRINCIPLES
The Immuno*Card* STAT! Rotavirus assay detects the presence of rotavirus antigen in stool. Patient specimen is diluted 1:15 in Sample Diluent. The suspension is mixed and 150 µL is added to the sample port of the device. The sample mobilizes gold particles coated with monoclonal antibody to rotavirus and migrates along the membrane through the **Test** (polyclonal anti-rotavirus antibody) and **Control** zones. After ten minutes, the **Test** and **Control** zones are observed for the presence of red/purple lines across the membrane surface. If rotavirus is present in the sample, a complex is formed between the capture antibody and the monoclonal antibody-gold conjugate which can be seen visually as a red/purple line in the **Test** zone. No red/purple line in the **Test** zone indicates a negative result. The **Control** line serves as a procedural control to assure that the sample has migrated the appropriate distance along the membrane.

REAGENTS/MATERIALS PROVIDED
The maximum number of tests obtained from this kit is listed on the outer box.
1. **Immuno*Card* STAT! Rotavirus Devices** - Individually foil pouched devices containing immobilized rabbit anti-rotavirus antibody (**Test** zone), goat anti-mouse IgG antibody (**Control** zone) and embedded monoclonal antibody to rotavirus VP-6 conjugated to gold particles.
2. **Positive Control** -Inactivated rotavirus (SA-11) in a buffer containing 0.02% thimerosal as a preservative.
3. **Sample Diluent** - Buffer containing 0.1% sodium azide as a preservative.
4. **Transfer Pipettes**

MATERIALS NOT PROVIDED
1. 12 x75 mm test tubes
2. Applicator sticks (recommended for solid stools)
3. Timer

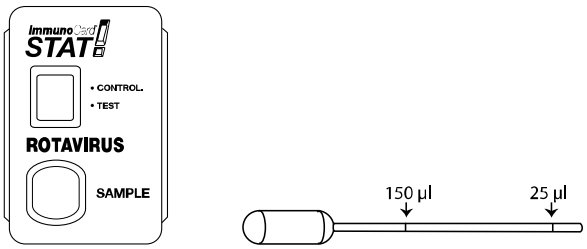
PRECAUTIONS
1. All reagents are for in vitro diagnostic use only.
2. Reagent concentration, incubation times and temperatures (21-25 C) have been optimized for sensitivity and specificity. Best results are obtained by adhering to these specifications. Once the assay has been started, complete all subsequent steps without interruption.
3. Patient specimens and used Immuno*Card* STAT! Rotavirus devices may contain infectious agents and should be handled at Biosafety Level 2 as recommended in the CDC/NIH manual "Biosafety in Microbiology and Biomedical Laboratories".
4. Positive Control reagent contains inactivated rotavirus. However, it should be handled as a potential biohazard.
5. All reagents should be gently mixed and at 21-25 C before use.
6. Do not interchange reagents from different kit lot numbers, or use expired reagents.
7. Hold Positive Control vial vertically to insure proper drop size and delivery. Do not allow the tips of the vial or pipette to touch the sample port.
8. Use only one transfer pipette per control or specimen. Discard after use. Do not attempt to reuse.
9. Stool must be mixed thoroughly (regardless of consistency) to insure a representative sample prior to pipetting. Do not use stools that have dried out.
10. Dilution of stool as described under **Sample Dilution** is important. Over-inoculation of stool into the Sample Diluent may restrict movement within the Immuno*Card* STAT! Rotavirus device so as to produce an invalid result.

WARNING: Some reagents in this kit contain sodium azide, which is a skin irritant. Avoid skin contact with reagents. Disposal of reagents containing sodium azide into lead or copper plumbing can result in the formation of explosive metal azides. This can be avoided by flushing with a large volume of water during such disposal.

HAZARD and PRECUATIONARY STATEMENTS
There are no known hazards associated with this product.

SHELF LIFE AND STORAGE
The expiration date is indicated on the kit label. Store the kit at 2-8 C.

PROCEDURAL NOTES
1. The Immuno*Card* STAT! Rotavirus format is diagrammed below.



2. Batch processing of samples or controls is possible provided correct incubation time is maintained for each device.
3. The **Control** zone of each device is a procedural control to assure that the sample has migrated sufficiently in the device to permit a valid test result to be read.

REAGENT PREPARATION
1. All reagents come ready to use (no further dilution is required).
2. Allow kit components to reach 21-25 C prior to use.
3. Gently mix liquid reagents prior to use.

SPECIMEN COLLECTION AND PREPARATION
For the best results, specimens should be collected after onset of symptoms. Several authors have reported declining numbers of rotavirus particles after day eight or nine, with peak counts occurring on days three through five. Samples collected after day eight or nine may be less reactive than those collected earlier in the course of the disease.[2, 6, 7] Specimens containing high levels of blood may fail to flow in the Immuno*Card* STAT! Rotavirus device, resulting in an invalid test result. Testing of an additional specimen is recommended under such circumstances.

Stool specimens may be collected by the method routinely utilized by the laboratory, providing no dilution of the specimen occurs. A sample may be collected into a clean, dry container free of detergent residue, or obtained from a diaper.

The specimen should be tested as soon as possible, but may be stored up to 72 hours at 2-8 C prior to testing. If testing cannot be performed within this time frame, specimen should be frozen in a non-defrosting freezer (-20 C or lower).

TEST PROCEDURE
Note: All components must be 21-25 C prior to use.
1. Sample Dilution
   a. Add 350 µL Sample Diluent to one 12 x 75 mm test tube for each specimen to be tested.
   b. Mix stool thoroughly, regardless of consistency.
   c. Liquid or semi-solid stool: Using a transfer pipette, draw stool to the 25 µL calibration point (first mark from tip of the pipette). Dispense the stool into the Sample Diluent in appropriate 12 x 75 mm tube. Using the same pipette, gently withdraw and expel the stool suspension several times, then vortex ten seconds. Leave transfer pipette in tube for further use. **Note:** Do not pipette more than 25 µL of stool. Over-inoculation with stool may produce invalid results. Proceed to Step 2 within 30 minutes.
   d. Solid stool (nonpipetable stool from a diaper): Using a wooden applicator stick, transfer a 2 mm diameter portion of stool into the Sample Diluent in the appropriate 12 x 75 mm tube. Emulsify the stool thoroughly using the applicator stick, then vortex ten seconds. Place transfer pipette in the tube. Proceed to Step 2 within 30 minutes.
2. Remove appropriate number of Immuno*Card* STAT! Rotavirus devices from their pouches. Label appropriately. Use one device per control or sample.
3. Vortex each diluted specimen for ten seconds. Using the original specimen transfer pipette, draw diluted sample to the 150 µL calibration point (second mark from tip of the pipette) and add to **Sample** port.
4. Incubate ten minutes at 21-25 C. **NOTE:** During the ten minute incubation, diluted specimen must move past the **Control** zone.
5. Visually read **Control** and **Test** zones for the presence or absence of a red/purple line at the end of the incubation period.

INTERPRETATION OF RESULTS
Positive Test Result: Visually detectable red/purple **Test** and **Control** lines. A positive result indicates the presence of rotavirus antigen.[1]
Negative Test Results: Visually detectable red/purple **Control** line. No red/purple **Test** line present. A negative result indicates that rotavirus antigen is absent or below the level of detection.
Invalid Test Result: No visually detectable red/purple **Control** line, with or without a visually detectable red/purple **Test** line.[2]

[1]Levels of the detection and capture antibodies were adjusted to achieve performance equivalence with the Premier Rotaclone EIA.

[2]Invalid test results may be due to a Reagent/Device problem, a procedural error, or over-inoculation of stool into Sample Diluent during specimen dilution. Re-dilute stool and repeat test. Stools containing high levels of blood may fail to flow properly, resulting in an invalid result. Testing with an additional specimen is recommended. On occasion, a stool may have very high levels of rotavirus antigen and will yield a visible **Test** line and no visible **Control** line. In such cases, the specimen may be diluted twofold or greater, beyond original 1:15 dilution (ex. 25 µL stool + 750 µL Sample Diluent) and retested.

QUALITY CONTROL
This test should be performed per applicable local, state or federal regulations or accrediting agencies.
The positive and negative controls should be assayed with each new kit lot or new shipment. Thereafter, Meridian recommends that the procedural control, performed with each test, is sufficient to verify reagent integrity and assay performance. The Negative and Positive Controls are intended to monitor for reagent failure, but will not ensure precision at the analytical assay cut-off. Additional controls may be tested in accordance with guidelines or requirements of local, state, and /or federal regulations and accrediting organizations.

Add three drops of Positive Control or, using a transfer pipette, add 150 µL Sample Diluent (Negative Control) directly to **Sample** port of appropriate device (do not dilute Positive Control).
1. The Positive Control should yield visually detectable red/purple **Test** and **Control** lines.
2. The Negative Control should yield a visually detectable red/purple **Control** line. No **Test** line should be present.

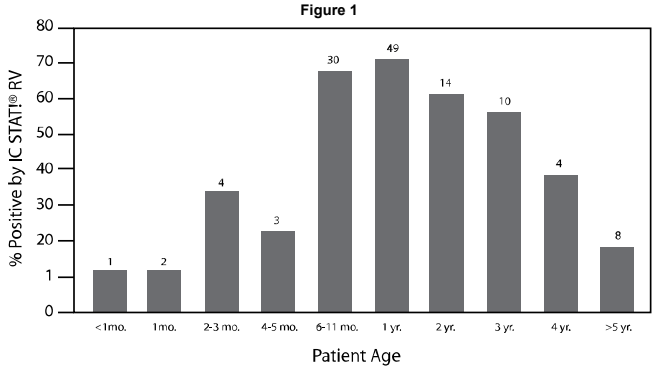
If the expected control reactions are not observed, repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor.

EXPECTED VALUES
The ImmunoCard STAT! Rotavirus test detects the presence of rotavirus antigen in stool. Expected values for a given population should be determined for each laboratory. The rate of positivity may vary depending on patient age, geographic location, season, method of specimen collection, handling and transportation, and a general health environment of the patient population under study.[2, 16]

It has been reported that in neonates, when rotavirus was present, the disease was mild or totally asymptomatic.[2, 6, 17] However, during cooler months, rotavirus may account for approximately 50% or more of the gastroenteritis found in hospitalized children.[3,16] In adults, the incidence of serious gastroenteritis caused by the virus is relatively low and when infected, adults tend to be asymptomatic.[2, 4] Studies from nursing homes and hospital geriatric wards show that this population is at an increased risk and susceptible to rotavirus associated disease.[2, 17]

Clinical studies with Immuno*Card* STAT! Rotavirus conducted at three different hospitals (two Children's Hospitals, Midwest and West Coast, and one General Hospital, East Coast) examined the incidence of rotavirus in different age groups (Figure 1). Values over the frequency bars indicate the number of Immuno*Card* STAT! Rotavirus positive specimens.

Figure 1



LIMITATIONS OF THE PROCEDURE
1. The Immuno*Card* STAT! Rotavirus test does not define the presence of rotavirus associated gastroenteritis, but only demonstrates the presence of the antigen in stool. As with all *in vitro* diagnostic procedures, test results should be interpreted by a physician in conjunction with other clinical information.
2. Limit of detection in stool specimens is 1.8-3.7x10^6 rotavirus particles (SA-11) per test volume.
3. The use of meconium stools in this assay is not recommended as their performance characteristics have not been evaluated.
4. A positive result does not preclude the presence of other infective organisms.

SPECIFIC PERFORMANCE CHARACTERISTICS
The Immuno*Card* STAT! Rotavirus test was evaluated on stools from pediatric patients submitted for rotavirus testing at three sites. All specimens were tested by Immuno*Card* STAT! Rotavirus, a competitor's flow-through membrane EIA, Premier Rotaclone® microwell EIA and electron microscopy (EM). In the table below, test results are compared to electron microscopy.[18]

Comparison of Immuno*Card* STAT! Rotavirus, Competitor's Membrane EIA and Premier Rotaclone® EIA to Electron Microscopy Results for Rotavirus (n=249)

Electron Microscopy Results	**Immuno*Card* STAT! Results**		**Competitor's Membrane EIA Results**		**Premier Rotaclone® Results**	
	Pos	Neg	Pos	Neg	Pos	Neg
Pos	121	9	122	8	122	8
Neg	5	115	11	109	7	113
Performance Statistic	Value	± 95% Conf.	Value	± 95% Conf.	Value	± 95% Conf.
Sensitivity	93.1%	4.4%	93.8%	4.1%	93.8%	4.1%
Specificity	95.8%	3.6%	90.8%	5.2%	94.2%	4.2%
Predictive Value Pos.	96.0%	3.4%	91.7%	4.7%	94.6%	3.9%
Predictive Value Neg.	92.7%	4.6%	93.2%	4.6%	93.4%	4.4%
Correlation	94.4%	2.9%	92.4%	3.3%	94.0%	2.9%

All five of the Immuno*Card* STAT! Rotavirus positive, electron microscopy negative specimens were positive by Premier Rotaclone® and the competitor's membrane EIA. Six of nine Immuno*Card* STAT! Rotavirus negative, electron microscopy positive specimens were negative by all three immunoassays. Furthermore, quantitative estimates by the electron microscopist indicated that eight of these specimens had few rotavirus particles.

Immuno*Card* STAT! Rotavirus Compared to Premier Rotaclone® Results (n=250)

Immuno*Card* STAT! Results	**Premier Rotaclone® Results**	
	Positive	Negative
Positive	126	0
Negative	3	122
Statistic	Value	± 95% Conf.
Relative Sensitivity	97.7%	2.6%
Relative Specificity	100.0%	0.0%
Relative Correlation	98.8%	1.3%

Two of three Immuno*Card* STAT! Rotavirus negative, Premier Rotaclone® positive specimens were negative by electron microscopy. One of the three was electron microscopy positive.

ASSAY SPECIFICITY
The ImmunoCard STAT! Rotavirus test was evaluated for specificity using the reference bacterial and viral strains listed below. A stool positive for rotavirus and a stool negative for rotavirus were spiked with bacteria (≥ 6 X 10^8 cfu/mL stool) or viruses (≥ 1 X $10^{2.7}$ $TCID_{50}$/mL).

Microorganism or Virus (# strains tested)

Aeromonas hydrophilia (1)
Bacillus cereus (1)
Bacillus subtilis (1)
Bacteroides fragilis (1)
Campylobacter coli (1)
Campylobacter fetus (1)
Campylobacter jejuni (1)
Citrobacter freundii (1)
Clostridium difficile (1)
Clostridium sordellii (1)
Enterobacter aerogenes (1)
Escherichia coli (1)
Escherichia coli (O157:H7) (1)
Escherichia coli (non-O157:H7, SLT+) (1)
Helicobacter pylori (1)
Klebsiella pneumoniae (1)
Proteus mirabilis (1)
Pseudomonas aeruginosa (1)
Salmonella (1)
Serratia liquefaciens (1)
Shigella dysenteriae (1)
Shigella flexneri (1)
Shigella sonnei (1)
Staphylococcus aureus (1)
Staphylococcus aureus (Cowan 1) (1)
Streptococcus faecalis (1)
Vibrio cholerae (1)
Vibrio parahaemolyticus (1)
Adenovirus, types 2, 40, 41
Coronavirus
Coxsackie types A9, B1, B6
Echovirus, types 22, 32
Enterovirus, type 69
Poliovirus, type 1

All organisms were found to be negative when spiked into the negative stool. In addition, they did not interfere with either the positive specimen or the procedural control.

Studies with different serotypes of group A rotavirus (the most common form) showed that the Immuno*Card* STAT! Rotavirus test detects serotypes 1, 2, 3, and 4. Reactivity with serotypes 8 and 9 has not been evaluated.

ASSAY PRECISION
Three physician office laboratories tested six specimens and the two controls, in triplicate, on each of three different days. The **Test** line of the Immuno*Card* STAT! Rotavirus device showed 100% reproducibility with negative and medium positive stools, and 96% reproducibility on low positive stools. **Control** line reproducibility was 100% with all specimens.