TRAINING UPDATE

Lab Location:	SGMC	**Date Distributed:**	8/3/2018
Department:	Core Lab	**Due Date:**	8/31/2018
		Implementation:	**8/20/2018**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Rotavirus Antigen Detection SGAH.M32 v5

Description of change(s):

Section	Reason
4,6	Removed individual section labeling instructions and add general one
8.2.3	Clarified dilution (added example)
10.5	Review data moved from section 6
15	Updated to new standard wording
17	Updated PI

This revised SOP will be implemented on August 20, 2018

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

Title	**Rotavirus Antigen Detection**
Prepared by	Ron Master Date: 12/14/2009
Owner	Ron Master Date: 12/14/2009

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name	**Signature**	**Date**

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Rotavirus antigen	Immunoassay	ROTA

Synonyms/Abbreviations
None

Department
Microbiology

Form revised 10/31/02

2. ANALYTICAL PRINCIPLE

Rotavirus is a major cause of acute gastroenteritis, especially in children 6 to 24 months in age. In addition, Rotavirus infections can produce severe illness as well as asymptomatic infection in adults. 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 onset of illness then decline.

The ImmunoCard 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 ul 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.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Raw stool in a clean dry container
Special Collection Procedures	Specimens should be collected after onset of symptoms.
Other	N/A

3.2 Specimen Type & Handling

Criteria		
Type -Preferred **-Other Acceptable**	Raw stool in a clean dry container	
Collection Container	Container or culturette	
Volume - Optimum **- Minimum**	1 mL 50 uL	
Transport Container and Temperature	Container or culturette at room temperature	
Stability & Storage Requirements	Room Temperature:	Test as soon as possible
	Refrigerated:	2 – 8 °C up to72 hours
	Frozen:	≤ -20° C up to 30 days
Timing Considerations	N/A	

Form revised 10/31/02

Criteria	
Unacceptable Specimens & Actions to Take	The testing of meconium stools in this assay is not recommended, as their performance characteristics have not been evaluated. Reject sample and request recollection
Compromising Physical Characteristics	Specimens containing high levels of blood may fail to flow in the ImmunoCard STAT Rotavirus device, resulting in an invalid test result. Testing of an additional specimen is recommended under such circumstances.
Other Considerations	N/A

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

4. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
ImmunoCard STAT	Meridian Diagnostics 750030

4.2 Reagent Preparations and Storage

Assay Kit	
Reagent a	ImmunoCard STAT test devices in individual foil pouches
Reagent b	Positive control (1.8ml)
Reagent c	Sample diluent (10.5ml)
Storage	2 – 8°C
Stability	Expiration date printed on kit label. Do not use reagents beyond expiration dates.
Preparation	All reagents come ready to use. Allow kit components to reach 21 - 25°C prior to use. Gently mix liquid reagents prior to use. Do not substitute reagents from other manufacturers or between different kit lot numbers

5. CALIBRATORS/STANDARDS

Not applicable

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
External Positive Control	included in kit
External Negative Control	sample diluent

The ImmunoCard STAT Rotavirus test contains a built-in procedural control. The Control line serves as an internal positive control. A visually detectable red/purple Control line must be present. The presence of this Control line verifies reagent integrity and assay performance. The presence of a clear background in the Test and Control zone serves as an internal negative control.

6.2 Control Preparations and Storage

See section 4.2.

6.3 Frequency

External positive and negative controls are tested with each new kit lot number or shipment or every 31 days, whichever is more frequent.

The internal procedural controls are recorded for each test.

Add three drops of positive control or, using a transfer pipette, add 150 ul sample diluent directly to sample port of appropriate device (do not dilute positive control).

6.4 Tolerance Limits and Criteria for Acceptable QC

The external positive control should yield detectable red/purple test and control lines.

The external negative control should yield a visually detectable red/purple control line. No test line should be present.
Patient results are not to be reported if the controls do not perform as expected.

- Re-analyze in accordance with Laboratory Quality Control Program.
- Corrective action must follow the Laboratory Quality Control Program.

6.5 Documentation

Document quality control data immediately on appropriate log sheet.

6.6 Quality Assurance Program

The laboratory participates in CAP proficiency testing.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

N/A

7.2 Equipment

Vortex mixer

7.3 Supplies

ImmunoCard STAT device
Diluent
Transfer pipettes
12 X 75 test tubes
Applicator sticks
Timer

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1	Specimen / Reagent Preparation
1.	Add 350 µl of sample diluent to one 12 X 75 mm test tube for each specimen to be tested.
2.	Mix stool thoroughly, regardless of consistency.

8.2	Test Run
8.2.1	**Liquid or Semi-solid Stool**
1.	Using a transfer pipette, draw stool to the 25 µl calibration point.
2.	Dispense the stool into the sample diluent in appropriate 12 X 75 mm tube.
3.	Using the same pipette, gently withdraw and expel the stool suspension several times.
4.	Vortex ten seconds.
5.	Leave transfer pipette in tube for further use. Proceed to the next step within 30 minutes.
6.	Do not pipette more than 25 µl of stool. Over-inoculation with stool may produce invalid results.
8.2.2	**Solid Stool**
1.	For solid stool: 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. For swabs: mix the swab into the diluent, ring out diluent from the swab.

2.	Emulsify the stool thoroughly using the applicator stick.
3.	Vortex ten seconds.
4.	Place transfer pipette in the tube. Proceed to the next step within 30 minutes.
8.2.3	**Testing Process**
1.	Remove appropriate number of ImmunoCard STAT Rotavirus devices from their pouches.
2.	Label appropriately. Use one device per control or sample.
3.	Vortex each diluted specimen for ten seconds.
4.	Using the original specimen transfer pipette, draw diluted sample to the 150 µl calibration point and add to Sample port.
5.	Incubate ten (10) minutes at 21 - 25°C. During the ten-minute incubation, diluted specimen must move past the Control zone.
6.	In a well-lit area, visually read Control and Test zones for the presence or absence of a red/purple line at the end of the incubation period.
7.	On occasion, a stool may have 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 and re-tested (ex., 25 µl stool + 750 µl Sample Diluent).

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen

9. CALCULATIONS

N/A

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

10.1.1 Positive test result: visually detectable red/purple Test and Control lines. A positive result indicates the presence of rotavirus antigen.

10.1.2 Negative test result: 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.

10.1.3 Invalid test result: no visually detectable red/purple Control line, with or without a visually detectable red/purple Test line.

10.2 Rounding

N/A

10.3 Units of Measure

N/A

10.4 Clinically Reportable Range (CRR)

N/A

10.5 Review Patient Data

N/A

10.6 Repeat Criteria and Resulting

IF the result is …	THEN…
Red/purple test and control lines	Positive
Red/purple control line. No red/purple test line present.	Negative
Red/purple test line. No red/purple control line present.	Invalid test. Perform an additional twofold dilution (1:30 final dilution) and repeat test.
No red/purple test or control lines	Invalid test. Run external controls and if acceptable, repeat test.

10.7 Reporting

Use LIS function **MEM** to enter results.

Enter Shift: (1, 2, or 3)
Worksheet: Use SIM2
Test: <Enter>
Enter "A" (Accept)
Enter Accession number
Press <Enter> until Result screen is displayed

Enter Results as listed below:

IF the result is …	THEN report with LIS code
Positive	POS
Negative	NEG

11. EXPECTED VALUES

11.1 Reference Ranges

Negative

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

The ImmunoCard 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. Rotavirus is a major cause of acute gastroenteritis, especially in children 6 to 24 months in age. In addition, Rotavirus infections can produce severe illness as well as asymptomatic infection in adults. 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 onset of illness then decline.

13. PROCEDURE NOTES

- **FDA Status**: Approved
- **Validated Test Modifications:** None

The ImmunoCard STAT Rotavirus test does not define the presence of rotavirus-associated gastroenteritis, but only demonstrates the presence of the antigen in stools. As with all *in vitro* diagnostic test procedures, test results should be interpreted by a physician in conjunction with other clinical information.

A positive result does not preclude the presence of other infective organisms.

A negative test result does not exclude the possibility of Rotavirus infection as too small quantities of virus, obtaining sample too late in infection, or inadequate and improper sampling techniques may cause a false negative result.

Intestinal infection with bacterial pathogens may be present simultaneously with Rotavirus infection. Therefore, perform bacterial testing in parallel with the Rotavirus assay.

Results of this test should be interpreted in conjunction with information available from the clinical evaluation of the patient.

The ImmunoCard STAT Rotavirus test does not define the presence of rotavirus-associated gastroenteritis, but only demonstrates the presence of the antigen in stool.

The rate of positivity may vary depending on patient age, geographic location, season, method of specimen collection, handling, transport and general health environment of the patient population under study.

It has been reported that in neonates, when rotavirus was present, the disease was mild or totally asymptomatic. However, during cooler months, rotavirus may account for approximately 50% or more of the gastroenteritis found in hospitalized children.

In adults, the incidence of serious gastroenteritis caused by the virus is relatively low and when infected, adults tend to be asymptomatic. Studies from nursing homes and hospital geriatric wards show that this population is at an increased risk and susceptible to rotavirus associated disease.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

N/A

14.2 Precision

N/A

14.3 Interfering Substances

The use of meconium stools in this assay is not recommended as their performance characteristics have not been evaluated.

14.4 Clinical Sensitivity/Specificity/Predictive Values

The ImmunoCardSTAT! Rotavirus test was evaluated on stools from pediatric patients submitted for rotavirus testing at three sites. All specimens were tested by The ImmunoCcardSTAT!, Competitior's Membrance EIA, and Premier Rotaclone EIA.

Sensitivity – 93.1%

Specificity – 95.8%

Predictive Value of Positive – 96.0%

Predictive Value of Negative – 92.7%

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

Current package insert for ImmunoCard STAT Rotavirus
Rotavirus Quality Control Log (AG.F34)

17. REFERENCES

ImmunoCard STAT Rotavirus package insert, Meridian Bioscience, Inc., 12/16

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SGAH.M008.003		
000	12/14/2010	6.3	Corrected QC frequency	R. Master	R. Master
000	12/14/2010	11.2	Title change to local terminology	L. Barrett	R. Master
000	12/14/2010	16	Moved Current PI to related docs	L. Barrett	R. Master
001	11/9/2012	6.1	Added description of internal control	R. Master	R. Master
002	4/22/2014	6.3	Changed external QC frequency	R. Master	R. Master
002	4/22/2014	10.6	Added detail for LIS reporting	L. Barrett	R. Master
002	4/22/2014	16	Log moved from section 19	L. Barrett	R. Master
002	4/22/2014	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	L. Barrett	R. Master
3	6/28/16	3.1	Removed swab and Culturette	R. Master	R. Master
3	6/28/16	6.3	Changed ext. QC frequency to 31 days	L. Barrett	R. Master
3	6/28/16	7.2	Added vortex mixer	R. Master	R. Master
3	6/28/16	12	Added to Clinical Significance	R. Master	R. Master
3	6/28/16	14.4	Added sensitivity, specificity, predictive value	R. Master	R. Master
3	6/28/16	17	Updated PI date	R. Master	R. Master
4	7/17/18	4,6	Removed individual section labeling instructions and add general one	L. Barrett	R. Master
4	7/17/18	8.2.3	Clarified dilution (added example)	R. Master	R. Master
4	7/17/18	10.5	Review data moved from section 6	L. Barrett	R. Master
4	7/17/18	15	Updated to new standard wording	L. Barrett	R. Master
4	7/17/18	17	Updated PI	R. Master	R. Master

19. ADDENDA

None