**TRAINING UPDATE**

| | | | |
|---|---|---|---|
| **Lab Location:** | SGMC | **Date Distributed:** | 7/21/2016 |
| **Department:** | Core | **Due Date:** | 8/31/2016 |
| | | **Implementation:** | **9/1/2016** |

**DESCRIPTION OF PROCEDURE REVISION**

**Name of procedure:**

## Rotavirus Antigen Detection SGAH.M32 v4

## Rota Virus Quality Control Log AG.F34.4

**Description of change(s):**

| Section | Reason |
|---|---|
| 3.1 | Removed swab and Culturette |
| 6.3 | Changed ext. QC frequency to 31 days |
| 7.2 | Added vortex mixer |
| 12 | Added to Clinical Significance |
| 14.4 | Added sensitivity, specificity, predictive value |
| 17 | Update Package Insert revision date |

QC frequency on log changed to 31 days

This revised SOP & FORM will be implemented on September 1, 2016

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** |
| | | |
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## 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**<br>**-Other Acceptable** | Raw stool in a clean dry container | |
| **Collection Container** | Container or culturette | |
| **Volume - Optimum**<br>**- Minimum** | 1 mL<br>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 |

Form revised 10/31/02

| Criteria | |
|---|---|
| **Timing Considerations** | N/A |
| **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 |

## 4. REAGENTS

Refer to the Safety Data Sheet (SDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

### 4.1 Reagent Summary

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

### 4.2 Reagent Preparations and Storage

**NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation**.
**Refer to the Safety Data Sheet (SDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step**.

| 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.<br>Allow kit components to reach 21 - 25°C prior to use.<br>Gently mix liquid reagents prior to use.<br>Do not substitute reagents from other manufacturers or between different kit lot numbers |

## 5. CALIBRATORS/STANDARDS

### 5.1 Calibrators/Standards Used

N/A

### 5.2 Calibrator Preparations and Storage

N/A

### 5.3 Calibration Procedure

N/A

## 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

**NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation**.

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

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 Review Patient Data

N/A

### 6.6 Documentation

Document quality control data immediately on appropriate log sheet.

### 6.7 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 is required minimum personal protective equipment. Report all accidents to your supervisor**.

**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.**

| 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.<br>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** | |
| 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 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. |

## 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 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. |

### 10.6 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 Report 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 ImmunocardSTAT!, 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

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

## 16. RELATED DOCUMENTS

Current package insert for Immunocard STAT Rotavirus
Rota Virus Quality Control Log (AG.F34)

## 17. REFERENCES

Immunocard STAT Rotavirus package insert, Meridian Diagnostics, 02/15

## 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 |

## 19. ADDENDA

None

# ROTAVIRUS QUALITY CONTROL LOG

☐ Germantown Emergency Center
☐ Shady Grove Medical Center
☐ Washington Adventist Hospital

**Next external QC is due = *Month*** _____ *Circle day*

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31

1. **External Positive and Negative Controls** are tested and documented with each new kit lot number or shipment or every 31 days, whichever is more frequent.
2. **Internal Controls** must be documented each time the test is performed.
3. If QC results are not acceptable, document corrective action. Do not accept patient results before reviewing QC results for proper reactions.

| Temp. 21-25C | Date | Patient Name / MR# | Patient Result | Kit | Internal Negative Control | Internal Positive Control | External Positive Control | | External Negative Control | | Tech |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Lot # / Expire | | | Lot # / Expire | Result | Lot # / Expire | Result | |
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|---|---|---|
| Weekly review: | Weekly review: | Weekly review: |
| Weekly review: | Weekly review: | Monthly review: |