**SUMMARY AND EXPLANATION**

*Escherichia coli* O157:H7 was first recognized as a cause of hemorrhagic colitis in 1982. Since then, the verocytotoxin produced by *E. coli* O157:H7 has also been identified as an agent of hemolytic-uremic syndrome (HUS). E. coli serotypes other than O157:H7 also produce verocytotoxins, but are usually not associated with bloody diarrhea.

*E. coli* O157:H7 is difficult to detect with methods ordinarily used in clinical laboratories. Most strains of *E. coli* O157:H7 ferment lactose rapidly and are indistinguishable from non-O157:H7 *E. coli* on conventional enteric media. *E. coli* O157:H7 does not ferment sorbitol, unlike most strains of non-O157:H7 *E. coli*, *Salmonella*, and *Citrobacter*. The use of a selective/differential medium such as Sorbitol MacConkey Agar (SMAC) has been found to facilitate detection of *E. coli* O157:H7. Sorbitol-fermenting colonies form pink to red colonies on SMAC; nonsorbitol-fermenters are colorless.

**PRINCIPLE**

*RIM™* E. coli O157:H7 Latex Test includes 3 latex reagents. The particles in each reagent are coated with a different antibody: one against *E. coli* serotype O157, another against *E. coli* serotype H7, and the third with normal rabbit globulin, to serve as the control latex. When Test Latex particles are mixed with fresh colonies of O157 and/or H7 strains of *E. coli*, an immunochemical reaction occurs, resulting in agglutination. No agglutination indicates the test isolate is not *E. coli* O157:H7. The Control Latex reagent identifies non-specific agglutination.

**PRECAUTIONS**

This product is for In Vitro diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

Control Latex contains sodium azide (0.1%) which is toxic if swallowed and harmful by inhalation or skin contact, may be toxic to the aquatic environment, and cause long-term effects. Refer to Material Safety Data Sheet for additional information on reagent chemicals.

**STORAGE**

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Do not freeze. Do not incubate prior to use.

**PRODUCT DETERIORATION**

This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

**SPECIMEN COLLECTION, STORAGE, AND TRANSPORT**

Specimens should be collected and handled following recommended guidelines.7,8

**MATERIALS SUPPLIED**

- *E. coli* O157 Test Latex (green cap) 4.0 ml - one dropper vial containing latex particles coated with *E. coli* O157 pooled rabbit antibody, suspended in buffered solution with preservative
- *E. coli* H7 Test Latex (blue cap) 4.0 ml - one dropper vial containing latex particles coated with *E. coli* H7 pooled rabbit antibody, suspended in buffered solution with preservative
- *E. coli* Control Latex (neutral cap) 4.0 ml - one dropper vial containing latex particles coated with pooled normal rabbit globulin, suspended in buffered solution with 0.1% sodium azide
- *E. coli* O157:H7 Positive Control (red cap) 3.0 ml - one dropper vial containing formalin-killed cell suspension of *E. coli* O157:H7 in buffered saline
- *E. coli* (not O157:H7) Negative Control (yellow cap) 3.0 ml - one dropper vial containing a formalin-killed cell suspension of non-toxigenic *E. coli* in buffered saline
- Plastic Stirring Sticks (2 vials)
- Disposable Slides (35)
- Instructions for Use (IFU)

**MATERIALS REQUIRED BUT NOT SUPPLIED**

- Loop sterilization device
- Inoculating loop, needle, collection containers
- Incubators, alternative environmental systems
- Supplemental media
- Quality control organisms (optional)
- Boiling water bath
- McFarland standard #1 (REF R20411) or equivalent
- Sterile saline (0.85%)
- Glass test tubes

**PROCEDURE**

**Note:** Allow reagents to equilibrate to room temperature before use. Mix latex reagents thoroughly by gentle agitation. Hold the vials vertically and dispense only free-falling drops. Do not interchange components among different lot numbers of test kits.

1. Acceptable isolates and media include only:
   - Non-sorbitol fermenting colonies (NSFC) isolated on Sorbitol MacConkey Agar (SMAC) to test for *E. coli* O157
   - A subculture of NSFC growing on blood agar to test for *E. coli* H7

2. For each isolate to be tested dispense one drop of Test Latex (green cap) into a well of the test slide.

3. In like manner, dispense one drop of *E. coli* Control Latex (neutral cap) into a separate well of the test slide.

4. Using a plastic stick (provided), remove a portion of the NSFC from the SMAC plate and emulsify in *E. coli* O157 Test Latex on the slide. Spread over two-thirds of the reaction area. Discard the plastic stick. Using a fresh plastic stick, repeat the process with remaining NSFC and emulsify in *E. coli* Control Latex on the slide.

5. Rotate slide using circular motions through 3 planes for up to 1 minute or until agglutination appears.

6. If agglutination occurs with the *E. coli* O157 Test Latex and the Control Latex is negative, streak the isolate to a blood agar plate, incubate overnight, and proceed to step 7. If agglutination occurs with both the Test Latex and the Control Latex, proceed to step 8.

7. After overnight incubation (18-24 hours) repeat the test. Emulsify a sweep of growth from the blood agar plate in a drop of *E. coli* H7 Test Latex (blue cap). The Control Latex is omitted in this step. Do not use boiled cells with the H7 Test Latex.

   a. Agglutination indicates the presence of *E. coli* O157:H7.
   b. If no agglutination occurs with the H7 Latex, retest the isolate after passage through appropriate motility media (followed by subculture to a blood agar plate) before concluding that the H7 antigen is absent.

8. Any test isolate that agglutinates with both *E. coli* O157 Test Latex and *E. coli* Control Latex should be treated as follows:
   a. Suspend the test isolate in 0.5 ml of sterile saline in a glass tube. The suspension turbidity should be ≥ a #1 McFarland Standard or equivalent.
   b. Place tube in a boiling water bath for 10 minutes. Allow tube to cool to room temperature. Retest the isolate, using 30-50 µl of the suspension, with O157 Test Latex and the Control Latex.
      i) If agglutination occurs with *E. coli* O157 Test Latex and not with Control Latex, subculture the isolate to a blood agar plate. After overnight incubation, test with the H7 Test Latex as in step 7.
      ii) If agglutination occurs with both *E. coli* O157 Test Latex and Control Latex after boiling, the test is inconclusive. Send isolates biochemically confirmed as *E. coli* to a reference laboratory for further testing.

**INTERPRETATION OF THE TEST**

Positive Result - Agglutination* of Test Latex (O157 or H7) accompanied by no agglutination of Control Latex within 1 minute

Negative Result - No agglutination of the Test Latex and the Control Latex within 1 minute

Inconclusive - Agglutination of the O157 Test Latex and the Control Latex within 1 minute

*Large black clumps visible to the unaided eye, with clearing of the background.

<table>
<thead>
<tr>
<th>O157 Test Latex</th>
<th>H7 Test Latex</th>
<th>Control Latex</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td><em>E. coli</em> O157 not H7 present</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td><em>E. coli</em> O157:H7 present</td>
</tr>
<tr>
<td>-</td>
<td>Not tested</td>
<td>+</td>
<td>Non-specific agglutination (boil and retest O157)</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td><em>E. coli</em> O157:H7 not present</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Inconclusive results</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td><em>E. coli</em> O157:H7 not present</td>
</tr>
</tbody>
</table>
Isolates that are positive with RIM™ E. coli O157:H7 should always be confirmed biochemically as E. coli.6 Because bacteria vary in their expression of flagellar antigens, E. coli O157 positive isolates that are initially negative in E. coli H7 Test Latex should be retested after passage through appropriate motility media to enhance motility before concluding that the H7 antigen is not present. Strain variations and the concentration of the cell suspension will affect the degree of agglutination. This test is considered negative when there is no agglutination in the E. coli Test Latex and the E. coli Control Latex.

QUALITY CONTROL
A Positive and Negative Control is included with each kit. Test Controls with each new lot number and shipment or following applicable regulatory guidelines. Test the Positive Control (red cap) with O157 Test Latex, H7 Test Latex, and Control Latex. In like manner, test the Negative Control (yellow cap) with all 3 latex reagents. The controls should perform as indicated in the table below. If aberrant quality control results are noted, patient results should not be reported.

<table>
<thead>
<tr>
<th>INTERPRETATION OF QUALITY CONTROL</th>
<th>O157 Latex</th>
<th>H7 Latex</th>
<th>Control Latex</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control (+)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Satisfactory performance</td>
</tr>
<tr>
<td>Positive Control (-)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Potency dropped, do not report test results</td>
</tr>
<tr>
<td>Negative Control (+)</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>Satisfactory performance</td>
</tr>
<tr>
<td>Negative Control (-)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Loss of specificity, do not report test results</td>
</tr>
</tbody>
</table>

Agglutination of Positive Control with both Test Latex reagents indicates reagent sensitivity has been retained. No agglutination of Negative Control with both Test Latex reagents indicates reagent specificity has been retained.

PERFORMANCE CHARACTERISTICS
RIM™ E. coli O157:H7 Latex Test was compared to other reagents made and used at a large U.S. government reference laboratory and to commercial reagents used at a state health laboratory (Study #1). The strains were from stock culture collections and the test was performed following the procedure in this IFU. The results are outlined below.

Study #1 (n=162):

<table>
<thead>
<tr>
<th>REFERENCE REAGENTS</th>
<th>(+) O157:H7</th>
<th>(-) NSFC not E. coli O157:H7</th>
<th>Inconclusive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remel (+)</td>
<td>64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIM™ E.coli Test (-)</td>
<td>5**</td>
<td>89</td>
<td>4***</td>
</tr>
</tbody>
</table>

Relative sensitivity >99% Relative specificity >99%
**As compared to combined biochemical and reference serological identification.
**Five isolates were positive for agglutination with E. coli H7 Test Latex after passage through motility medium.
***Four strains of E. coli agglutinated with the Control Latex and were scored as inconclusive. None of these isolates were confirmed as E. coli O157.

Study #2: (n=127):
A second study was performed in three clinical laboratories to evaluate the relative specificity of RIM™ E. coli O157:H7 Latex Test compared to biochemical identification and another commercially available E. coli O157 Latex Test, using fresh isolates. A total of 127 NSFC clinical isolates from fresh human stool specimens were tested. These isolates were identified using both biochemical and serological methods. Of the isolates tested, 126 were negative by both methods (specificity >99%). One isolate was identified as E. coli O157 by both latex reagents and as E. coli O157:H7 by the RIM™ method.

Both studies included 22 different sorbitol-nonfermenting genera and species.

LIMITATIONS
1. Test only pure cultures initially grown on SMAC agar for O157.
2. Some brands of wooden applicators have been reported to affect results and should not be used in this test.
3. Nonmotile isolates positive for the O157 antigen should be evaluated for Shiga toxin production to rule out enterohemorrhagic E. coli.
4. The use of SMAC agar and/or RIM™ E. coli O157:H7 Latex Test will not determine nor confirm that an E. coli isolate is a verocytotoxin-producing strain. Other serotypes of E. coli have been identified which produce verocytotoxin.
5. Young, fast-growing cultures more readily express antigens identified by this test. Isolates removed from the agar surface of an overnight culture will yield the best results.
6. Blood agar may enhance the production of flagella and affords better detection of flagellar antigens.
7. When infections of E. coli O157:H7 are identified, they should be reported to local health departments for further evaluation and, if necessary, public action to prevent the spread of disease.7
8. Citrobacter freundii and Salmoneillla Group N (O30) are antigenically related to E. coli O157 and have been reported to yield positive results with RIM™ E. coli O157:H7 Latex Test. These strains readily ferment sorbitol and are biochemically distinguishable from E. coli. Isolates that are positive with RIM™ E. coli O157:H7 should always be confirmed biochemically as E. coli.6
9. When making a diagnosis, test results must be interpreted in the context of patient illness, history, and physical examination. Laboratory testing should not be used as the sole basis for the institution of antibiotic therapy.
10. Strains of E. coli O157:H7 that do not produce Shiga toxin have been reported.10

BIBLIOGRAPHY

PACKAGING
REF R24250, RIM™ E. coli O157:H7 Latex Test ............... 50 Tests/Kit

Symbol Legend
RIM™ is a trademark of Remel Inc.
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