**DrySpot E. coli O157**

**Latex Test**

**1. INTENDED USE**

The Oxoid DrySpot E. coli O157 test is a latex agglutination test for the identification of E. coli serogroup O157.

**2. PRINCIPLE OF THE TEST**

Enterohaemorrhagic E. coli belong to a number of O serogroups with O157 being most significant in human disease. The potentially verocytotoxin (VT)-producing strains are associated with a range of symptoms from non-bloody diarrhoea, fever and vomiting to cases of haemorrhagic colitis (HC) and associated with a range of symptoms from non-bloody diarrhoea, to cases of haemorrhagic colitis (HC) and associated with a range of symptoms from non-bloody diarrhoea, fever and vomiting to cases of haemorrhagic colitis (HC) and with O157 being most significant in human disease.

**3. COMPONENTS OF THE KIT (DR0120M)**

**DR0121M**
DrySpot E. coli O157 Test Reagent Cards

A suspension of synthetic blue latex particles coated with antibody specifically reactive with the E. coli O157 serogroup antigen. Four pouches each containing 10 cards and a moisture absorbent sachet. There are 3 test and 3 control reaction areas on each card – 120 tests in total.

**DR0122M**
Positive control strips (10 sticks – pink spots)

Pink-dyed inactivated antigenic extract of E. coli O157.

**DR0123M**
Negative control strips (10 sticks – green spots)

Green-dyed inactivated antigenic extract of E. coli O116.

Mixing paddles

Instructions for Use

**Materials required but not provided**

Saline (0.9% NaCl prepared using distilled or deionised water)

Microbiological loop and bunsen burner

A suitable laboratory disinfectant

A timer.

**4. PRECAUTIONS**

**IVD**

This product is for in vitro diagnostic use only. Specimen materials may contain pathogenic organisms. Handle with the appropriate precautions.

**5. STORAGE AND OPENING**

This kit must be stored between 2°C and 25°C. If stored in a cold environment, allow pouches to reach room temperature before opening to prevent condensation of moisture on the cards. The dry reagents will deteriorate and give false results if they are allowed to absorb moisture. Open the pouches by cutting with scissors just below the seal. Once opened, remove the number of cards required for immediate testing (testing within the next 10 minutes) and re-seal the pouch immediately by clamping the open end of the bag between the two halves of the plastic clip.

If fewer tests are required cut the reaction cards along the indicated lines and return the unused portions to the pouch. Do not return used portions because they will cause contamination of remaining cards in the pouch.

The control sticks are also provided in a moisture impermeable pouch. Ensure that the same techniques are used to avoid moisture damage. Under these conditions the reagents will retain their activity until the expiry date shown on the kit box.

**6. CONTROL PROCEDURES**

The dried control sticks provided should be used in the following way to check the correct working of the latex reagent each day before routine tests are performed. Add a 50 μl drop of saline to the small circle at the base of the test oval reaction area. Remove a positive control card stick from the pouch by tearing one off from the others on the strip, take care to avoid touching the flexible end where the dry spots are located. Re-seal the inner bag and pouch. Turn the stick over so that the colour spots are at the bottom and place the stick on the card with the spots touching the liquid. Push down so that the end bends at the hinge and mix in a circular manner for 10 seconds to rehydrate the dried reagent. Continue to use the stick to mix the liquid into the DrySpot O157 test reagent until all the reagents are fully rehydrated and homogeneous. Rock the card and look for agglutination. This procedure should be repeated using a negative control stick.

The positive control DR0122M must show agglutination with the dried reagent within 1 minute. The negative control DR0123M must show no agglutination within 1 minute.

Do not use the test if reactions with the control reagents are incorrect.

**7. IMPORTANT PROCEDURE NOTES**

Do not touch the circles on the reaction cards as this may cause agglutination and thereby affect the reaction. In a high humidity environment do not leave pouches open for more than 2 minutes. If there is evidence of moisture in the spots do not use.

Do not place the drop of saline directly onto the dry latex spots. Pouch clips should be retained for future use to allow multiple packs to be opened. Although suitable for room temperature storage the kit or pouches must not be stored near heat sources. They should not be stored where exposure to sunlight may cause increased temperatures.

The controls provided should be used to check the correct working of the latex reagents each day before routine tests are performed. The positive control must cause visible agglutination with the latex reagent immediately. The negative control must not cause agglutination within 1 minute. Do not use the test if reactions with the control suspensions are incorrect.

**8. CULTURE MATERIAL**

Non-sorbitol fermenting (NSF) colonies may be taken from Sorbitol MacConkey Agar or Sorbitol MacConkey Agar with Cefixime Tellurite Supplement.

Alternatively NSF isolates may be inoculated onto non-selective media such as Nutrient Agar for testing. It is necessary to test up to 10 separate NSF colonies to ensure a high probability of detecting any O157 strains which may be in mixed culture with NSF E. coli of other serotypes. The use of the control latex will ensure that the isolate is not an auto-agglutinating strain.

**9. TEST METHOD**

1. Add a drop (50 μl) of saline (0.9%) to the small ring (at the bottom of each oval) in both the test and control reaction areas ensuring that the liquid does not mix with the dried latex reagents.

2. Use a sterile loop (or one of the paddles provided) to pick up a portion of the suspect colony from a culture media plate and carefully emulsify in the saline drop. Ensure that the resulting suspension is smooth.

3. Using the provided paddle mix the suspension into the dry latex spots until completely suspended and spread to cover the reaction area. Discard the loop-paddle appropriately.

4. Using a separate loop or paddle, proceed in the same way with the Control Latex.

5. Pick up and rock the card for up to 60 seconds and look for agglutination under normal lighting conditions. Do not use a magnifying glass.

6. When the test is completed dispose of reaction cards safely into disinfectant.

**10. READING AND INTERPRETATION OF RESULTS**

**Positive Result**

A result is positive if agglutination of the latex particles occurs within 1 minute. This indicates the presence of E. coli serogroup O157.

**Negative Result**

A negative result is obtained if no agglutination occurs and a smooth blue suspension remains after 60 seconds in the test area.

Reactions occurring after 60 seconds should be ignored.

**Uninterpretable Result**

The test is uninterpretable if the control reagent shows agglutination. This indicates that the culture causes auto-agglutination.

**Granular or Stringy Reactions**

Occasionally granular or stringy reactions may be seen due to the particulate nature of the test material. When such reactions are seen to occur they should be interpreted using the following criteria:

The result is positive when, using the test reagent, greater clearing of the blue background occurs compared with the reaction of the control reagent. The result is negative, when there are no significant changes in the clearing of the blue background using the test and control reagents.

If stringiness is found to be too severe for a correct judgement to be made then another colony should be suspended in 0.3 ml of saline. Allow the lumps to settle and re-test this smooth supernatant.

**11. LIMITATIONS OF THE TEST**

Mixing paddles provided are not sterile. These may be sterilised locally if required.

If a positive result is obtained on a colony of unknown species, then biochemical tests should be performed to confirm that the organism is an E. coli strain.

Neither the Sorbitol MacConkey Agar nor the E. coli O157 latex test can reliably confirm the isolate as a toxin-producing strain.

Other sorotypes have been found which produce the vero-cytotoxin.

Strains of *Escherichia hermanii* cross react with *E. coli* O157 sera and the latex test due to a shared antigen.

In addition other bacterial strains have been identified with epitopes that mimic those of *E. coli* O157 lipopolysaccharide.

*E. hermanii* may be differentiated from *E. coli* by the former’s fermentation of cellobiose growth in the presence of KCN, negative reaction in the 4-Methylumbelliferone glucuronide (MUG) assay and yellow pigmentation, which may be delayed.

**12. PERFORMANCE CHARACTERISTICS**

The performance of Oxoid DrySpot E. coli O157 kit was evaluated at one clinical microbiology laboratory in Great Britain. Previously characterised strains of *E. coli* O157 and other non-sorbitol fermenting enterobacteriaceae were tested with this kit and with two other commercially available latex tests.

The sensitivity of Oxoid DrySpot E. coli O157 kit was calculated to be 98.3%.

The specificity of Oxoid DrySpot E. coli O157 kit was calculated to be 100%.

The results of the evaluation are summarised below:

<table>
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<th>Organism</th>
<th>Total tested</th>
<th>Oxoid DrySpot E. coli O157 test result</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
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<tr>
<td><em>E. coli O157</em></td>
<td>60</td>
<td>59</td>
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* A false negative reaction was recorded for an *E. coli* O157:H9 (VT) which gave a negative result with all latex kits tested.
REFERENCES


SYMBOL LEGEND

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