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Wellcolex* E. coli O157

REF R30959501.....50 tests **EN**

1. INTENDED USE

Wellcolex* E. coli O157 is a rapid latex agglutination test for the presumptive identification of *Escherichia coli* O157 isolates on laboratory media.

The Wellcolex* E. coli O157 Rapid Latex Test has been categorised as highly complex under the Clinical Laboratory Improvement Act (CLIA88 : Test System Code 40262; Analyte Code 1604).

2. SUMMARY AND EXPLANATION OF THE TEST

Verocytotoxin-producing *E. coli* O157 are an important cause of diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome[1,2]. The pathogenicity of this organism is due to the expression of verocytotoxin (VT1 and/or VT2)[3,4,5]. Enteritis due to other serotypes is not unusual but in contrast to the majority of isolates, most *E. coli* O157:H7 do not ferment sorbitol and this has been used as a differential feature for laboratory identification. In particular, MacConkey agar containing sorbitol instead of lactose (SMAC) is often used as a primary screening medium[6,7]. Other non-sorbitol fermenting enterobacteria can grow on SMAC. Testing suspect colonies with Wellcolex* E. coli O157 determines whether the isolate belongs to the O157 serogroup.

3. PRINCIPLE OF THE PROCEDURE

The Wellcolex* E. coli O157 Test Reagent consists of red latex particles coated with antibodies specific for *E. coli* O157. When a drop of the reagent is mixed on a card with a suspension of *E. coli* O157 organisms, rapid agglutination occurs through the interaction of specific IgG and O157 lipopolysaccharide antigen.

Some faecal coliforms can cause non-specific aggregation of latex particles particularly when grown on sugar containing media such as MacConkey. Therefore a Control Latex is provided to assist with the identification of non-specific reactions.

4. REAGENTS

KIT CONTENTS

1.	Test Latex	1 dropper bottle (Pink cap)
2.	Control Latex	1 dropper bottle (Grey cap)
3.	Positive Control	1 dropper bottle (Red cap)
4.	Negative Control	1 dropper bottle (Blue cap)
5.	Disposable Reaction Cards	1 pack
6.	Disposable Mixing Sticks	3 packs
7.	Instructions for Use	1

DESCRIPTION OF REAGENTS, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also **Warnings and Precautions**.

The latex suspensions and controls should be stored in an upright position at 2 to 8°C under which condition they will retain their activity until the expiry date of the kit. Do not freeze latex suspensions. Reaction Cards and Mixing Sticks may be stored at room temperature (18 to 30°C).

O157 TEST LATEX **Test Latex**

A buffered suspension of red polystyrene latex particles coated with rabbit IgG specific for *E. coli* O157. Contains 0.05% Bronidox® preservative.

O157 CONTROL LATEX **Control Latex**

A buffered suspension of red polystyrene latex particles coated with non-immune rabbit IgG. Contains 0.05% Bronidox® preservative.

The latex suspensions should be brought to room temperature (18 to 30°C) before use. After prolonged storage some aggregation or drying of the latex may have occurred around the top of the bottle. Under these circumstances the bottle of latex should be shaken vigorously for a few seconds until resuspension is complete.

CONTROL + **Positive Control**

A dilute suspension of heat killed *E. coli* O157 antigens. Contains 0.05% Bronidox® preservative.

CONTROL − **Negative Control**

A dilute suspension of heat killed *E. coli* O106 antigens. Contains 0.05% Bronidox® preservative.

5. WARNINGS AND PRECAUTIONS

IVD

For *in vitro* diagnostic use only.

For professional use only.

Caution: This product contains dry natural rubber.

Please refer to the manufacturer's safety data sheet and the product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

1. It is recommended that these reagents and test specimens be handled using established good laboratory working practices.
2. Non-disposable apparatus should be sterilised by any appropriate procedure after use, although the preferred method is to autoclave for at least 15 minutes at 121°C. Disposables should be autoclaved or incinerated. Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a standard bacterial disinfectant. Materials used to clean spills, including gloves, should be disposed of as biohazardous waste.
3. Wear a laboratory coat, disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
4. When used in accordance with the principles of Good Laboratory Practice, good standards of occupational hygiene and the instructions stated in this Instructions for Use, the reagents supplied are not considered to present a hazard to health.
5. The *E. coli* antigens used in the Positive and Negative Controls have been heat inactivated, however they should be handled as potentially infectious.

ANALYTICAL PRECAUTIONS

1. Do not use the reagents beyond the stated expiry date.
2. Latex reagents which show signs of aggregation or 'lumpiness' before use may have been frozen and should not be used.
3. It is important when using dropper bottles that they are held vertically and that the drop forms at the tip of the nozzle. If the nozzle becomes wet an incorrect volume will form around the end and not at the tip; if this occurs dry the nozzle before progressing.
4. Only use the Reaction Cards provided with this kit.
5. Do not touch the reaction areas on the cards.
6. Only test non-sorbitol fermenting cultures.
7. Do not interpret agglutination appearing after 30 seconds as a positive result.
8. A magnifying glass/lens should not be used to interpret the agglutination.

6. SPECIMEN COLLECTION AND STORAGE

For details of specimen collection and treatment a standard text book should be consulted.

Non-sorbitol fermenting cultures should be tested from sorbitol MacConkey agar.

THE USE OF FRESH CULTURES GROWN OVERNIGHT IS RECOMMENDED.

7. PROCEDURE

MATERIALS PROVIDED

Sufficient latex reagents are provided for 50 tests, see **Kit Contents**.

MATERIALS NOT PROVIDED

- A suitable pipette to measure 40 µl.
- Saline (0.85% NaCl w/v)

TEST PROCEDURE

Please read **Analytical Precautions** carefully before performing the test. Allow reagents to reach room temperature (18 to 30°C) before use.

Step 1	Shake the latex reagents	
Step 2	For each test sample place 40 µl of **saline** in two circles on a Reaction Card.	**40 µl Saline**
Step 3	Using a mixing stick, remove sufficient growth to just cover the blunt end of the stick. **NOTE: The latex is very sensitive, do not pick an excessive amount of culture.**	
Step 4	Emulsify the sample of culture in the **saline** by rubbing with the flat end of the stick. Mix thoroughly, but not too vigorously, or the surface of the card may be damaged. Some cultures may be difficult to emulsify and this should be noted. Lumps of a poorly emulsified culture can make the latex appear 'bitty' or 'stringy' on reading. Discard the mixing stick for safe disposal.	**Emulsify Sample**
Step 5	Using a separate stick, emulsify a similar amount of sample from the culture into the **saline** in the other circle. Discard the mixing stick for safe disposal.	**Emulsify Sample**
Step 6	For each test sample place one drop of **Test Latex** in one circle (with the emulsified culture) and one drop of **Control Latex** in the other circle with the emulsified culture. Ensure that the dropper bottles are held vertically to dispense an accurate drop.	**1 drop**
Step 7	Mix the contents of the circles, carefully spreading the latex over the entire area of the circle. Discard the mixing sticks for safe disposal.	
Step 8	Rock the card slowly for 30 seconds and observe for agglutination. The card should be held at normal reading distance (25 to 35 cm) from the eyes. Do not use a magnifying lens.	**30 seconds**
Step 9	Discard the used Reaction Card for safe disposal.	

8. RESULTS

READING OF RESULTS

A positive result is indicated by the development of an agglutinated pattern showing clearly visible clumping of the latex particles. The speed and appearance of the agglutination is dependent on the quality and quantity of the culture antigen.

In a negative result the latex does not agglutinate and the appearance of the suspension remains substantially unchanged throughout the period of rotation. Note: that faint traces of granularity may be detected in the negative results depending on the visual acuity of the operator.

In a non-specific result agglutination occurs in both the Test and Control Latexes.

QUALITY CONTROL

The following procedures should be carried out with each shipment of test kits and periodically throughout the life of the kit. Local regulations may require that quality control procedures are carried out with every run of testing. A run may be defined as a period of up to 24 hours.

Any departure from the expected results indicates that there may be a problem with the reagents, which must be resolved before further use with clinical samples.

Visual inspection
The latex suspensions should always be inspected for aggregation as they are dropped onto the Reaction Card. If there is evidence of clumping before addition of the test sample the suspension should not be used. After prolonged storage some aggregation or drying may have occurred around the top of the bottle. If this is observed the bottle should be shaken vigorously for a few seconds until resuspension is complete.

Control Procedure
When testing clinical specimens, the performance of the test kit reagents should be evaluated by testing both the Positive Control and a Negative Control included in each test kit. A Positive Control and a Negative Control should be included in each test run. Ensure each are mixed thoroughly before use.

Positive Control Procedure

Step 1	Place one drop of Test Latex in one circle on a Reaction Card.	**1 drop**
Step 2	Dispense one drop of Positive Control next to the Test Latex.	**1 drop**
Step 3	Mix using a Mixing Stick. Discard the Mixing Stick for safe disposal.	
Step 4	Rock the card for 30 seconds. After this time, definite agglutination should be visible in the Test Latex.	**30 seconds**

Negative Control Procedure

Step 1	Place one drop of Test Latex in one circle on a Reaction Card.	**1 drop**
Step 2	Dispense one drop of Negative Control next to the Test Latex.	**1 drop**
Step 3	Mix using a Mixing Stick. Discard the Mixing Stick for safe disposal.	
Step 4	Rock the card for 30 seconds. After this time, there should be no significant agglutination in the Test Latex.	**30 seconds**

The performance of the Test and Control Latex reagents can also be confirmed using fresh, overnight cultures of reference strains of bacteria, following the method described in Test Procedure. Suitable reference strains are shown below:

STRAIN	EXPECTED RESULT	
	Test Latex	Control Latex
Positive control *E. coli* O157 (ATTC 43895)	+	–
Negative control *E. coli* O14 (ATCC 19110)	–	–

INTERPRETATION OF RESULTS

Positive Results
Agglutination of the Test Latex accompanied by a lack of agglutination of the Control Latex indicates the presence of O157 antigen in the culture under test. Cultures that give a positive reaction should be identified biochemically as *E. coli*.

When *E. coli* O157 are identified, they should be further tested for H7 antigen and/or verocytotoxin production. *E. coli* O157:H7 or verocytotoxin producing isolates should be reported to local health departments for further evaluation and, if necessary, public health action to prevent further cases.

Negative Result
Lack of agglutination in both reagents means that the culture under test is unlikely to be *E. coli* O157. If minor 'aggregation' corresponding to unemulsified bacteria is seen in both latexes this can be considered as a negative reaction provided the majority of the latex is still present as a smooth, unagglutinated suspension.

A negative reaction does not eliminate the possibility of *E. coli* O157 being present in the specimen in low numbers.

Non-interpretable Result
Visible agglutination of the Control Latex indicates a non-specific reaction.

9. LIMITATIONS OF THE PROCEDURE

1. Only pure cultures should be tested. Positive cultures should be identified biochemically to confirm that they are *E. coli* and should be evaluated for verocytotoxin production.
2. This test only identifies *E. coli* with the O157 antigen. Neither Sorbitol MacConkey agar or this test confirms an isolate as being a verocytotoxin producing strain. Not all verocytotoxin-producing isolates belong to serotype O157 and not all O157 isolates produce verocytotoxin[4].
3. H7 antigen is not detected in this assay. Care should be taken when testing *E. coli* O157 for H7 antigen. *E. coli* vary in their expression of flagellar antigens and isolates that are initially negative in an H-serology test, should be retested after passage through appropriate motility media to enhance motility before it is concluded that they do not express the H7 antigen.

   If a strain is positive for the O157 antigen and is non-motile, it should be evaluated for verocytotoxin production to rule out haemorrhagic *E. coli*. Some strains of *E. coli* O157 do not produce verocytotoxins and have H-antigens other than H7.
4. Verocytotoxin-producing *E. coli* O157 which ferment sorbitol have been isolated. These may not be detected in protocols that rely on SMAC for the preliminary selection of isolates for screening with latex agglutination tests.

10. EXPECTED RESULTS

Strains expressing O157 antigen will give a strong, rapid agglutination with the Test Latex.

11. SPECIFIC PERFORMANCE CHARACTERISTICS

The performance of Wellcolex* E. coli O157 has been evaluated in one North American and two European clinical microbiology laboratories by testing colonies from 611 non-sorbitol fermenting cultures. The cultures were tested in parallel with at least two alternative commercial latex tests for the identification of *E. coli* O157. The results of the study are summarised in Table 1.

The study included 307 cultures which tested positive for *E. coli* O157 with Wellcolex* E. coli O157 and with the alternative tests. The sensitivity of Wellcolex* E. coli O157 on this group of cultures is therefore estimated to be 100% (307/307) with a lower 95% confidence limit of 98.8%.

A total of 299 other non-sorbitol fermenting Enterobacteria, including *E. hermannii*, were also tested with Wellcolex* E. coli O157 and the alternative tests. A false positive reaction was recorded with one *Providencia rettgeri* culture by Wellcolex* E. coli O157 and one of the alternative tests. The specificity of Wellcolex* E. coli O157 on this group of cultures is therefore estimated to be 99.7% (298/299) with lower and upper 95% confidence limits of 98.2% and 99.9% respectively.

The predictive values of a positive and negative result for Wellcolex* E. coli O157 for the population studied were 99.7% (307/308) and 100% (298/298) respectively.

Wellcolex* E. coli O157 gave a non-interpretable result with five non *E. coli* O157 cultures, which have been excluded from the summary above.

Table 1

	Wellcolex* E.coli O157 Result		
	Positive	Negative	Total
E. coli O157	307	0	307
Other non-sorbitol fermenting Enterobacteria	1	298	299
Total	308	298	606

12. BIBLIOGRAPHY

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13. PACKAGING

REF ZC60/R30959501.....50 tests

Symbol legend

REF	Catalogue Number
IVD	*In Vitro* Diagnostic Medical Device
	Consult Instructions for Use
	Temperature Limitation
LOT	Batch Code
	Use By
	Manufacturer

CE

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