Global Salm-Surv

A global *Salmonella* surveillance and laboratory support project of the World Health Organization

Laboratory Protocols

Level 4 Training Course

Latex test for agglutination of *Escherichia coli* O157.

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Latex test for agglutination of *Escherichia coli* O157.

Introduction

The *Escherichia coli* O157 Latex Test is a highly sensitive and specific latex agglutination test for identification of *Escherichia coli* O157 (ref 2). This method is quick and simple to use, but is expensive compared to ordinary conventional agglutination tests.

Materials

Equipment

- Loop (1 µl)
- Bunsen burner
- Sterile Pasteur pipettes

Chemicals and reagents

- Sterile normal saline.
- The *Escherichia coli* O157 Latex Test consisting of disposable reaction cards, test latex, control latex, pos. Control suspension and neg. control suspension, (store at 2-8^{o}C upon arrival)

Bacterial strains

Strains for identification on non-selective agar e.g. Nutrient agar.

Safety

Several countries follow the CDC/NIH biosafety guidelines described in "Biosafety in Microbiological and Biomedical Laboratories", 4th Edition, 1999 (ref. 1) that recommend Biosafety Level 2 practices for all the *Escherichia coli* O157 work.

Carry out all procedures in accordance with local safety codes of practice.

1. CDC/NIH. Biosafety in Microbiological and Biomedical Laboratories (BMBL) - 4th edition, US Government Printing Office, Washington. http://www.cdc.gov/od/ohs/biosfty/biosfty.htm
2. Post, D.E. Food-borne pathogens monograph number 5 *Escherichia coli* and *Shigella species* and folio 415. Oxoid Limited, wade Road, Basingstoke, Hampshire RG24, UK.

Procedure

Day 1

1. Allow the latex reagent to gain room temperature before use and shake all of the reagents.

2. Dispense one drop of test latex onto one circle of the reaction card.

3. Place a drop of saline next to the drop of test latex. Make sure they do NOT mix.

4. Using a loop transfer some culture from the Nutrient agar plates to the drop of saline and mix it. Make sure all of the culture is completely emulsified and the suspension holds no lumps.

5. Mix the saline and the drop of test latex together and spread it around in the whole area of the circle.

6. Rock the reaction card up and down with your fingertips. Look for a positive agglutination within 1 minute.

7. If the culture agglutinates with the test latex then proceed testing the culture for auto-agglutination using the same procedure but instead of using test latex use control latex.
8. Remember to perform quality control on the test kit with the enclosed positive and negative control suspensions in order to verify the test kit is still working correctly. Appendix 1 illustrates the use of the different reagents.

9. Disinfect and discard the reaction card after use .

Theory / comments

The Latex test is best used in conjunction with a standard using SMAC-agar.
Presumption positive *Escherichia coli* O157 colonies should be inoculated from SMAC-agar to non-selective agar e.g. Nutrient agar. Test up to 10 colonies to ensure a high possibility of *Escherichia coli* O157 detection.

Some *Escherichia coli* O157 strains may be difficult to emulsify resulting in a reaction similar to the positive agglutination. This reaction should be ignored.

Do not rock the card for more than 1 minute and do not use a magnifying glass.
If the agglutination is absent after 1 minute the reaction should be interpreted as negative.
If a strain tests positive in the test without a proper identification this strain has to be identified by biochemical tests.

The result can NOT be interpreted if a reaction occurs in both control- and test-latex.

The test procedure should be performed every day before testing samples.

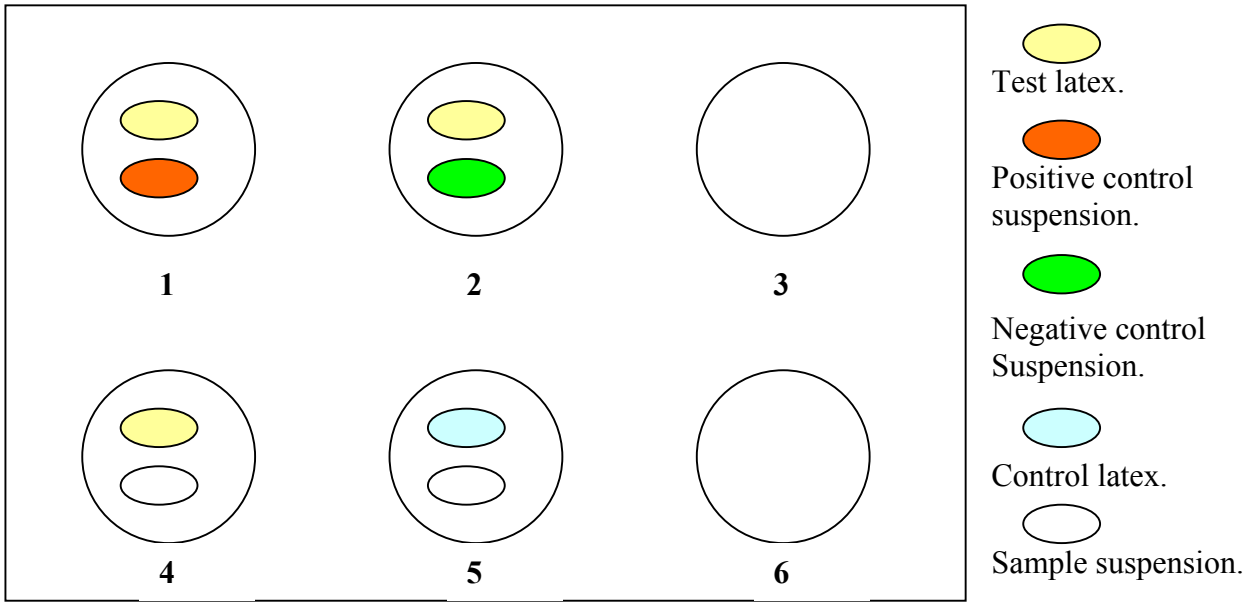
The latex test does NOT indicate if the strain is verotoxin producing.
Escherichia hermanii cross react with the *Escherichia coli* O157 sera and give a positive reaction. If this is indicated a further identification has to follow to differentiate the species.

References:

Post, D.E. Food-borne pathogens monograph number 5 *Escherichia coli* and *Shigella species* and Folio 415. Oxoid Limited, wade Road, Basingstoke, Hampshire RG24, UK.

APPENDIX 1

Illustration: use of the different reagents.



1: Test latex and positive control as Quality assurance.

2: Test latex and negative control as Quality assurance.

4: Test latex and sample suspension as sample testing.

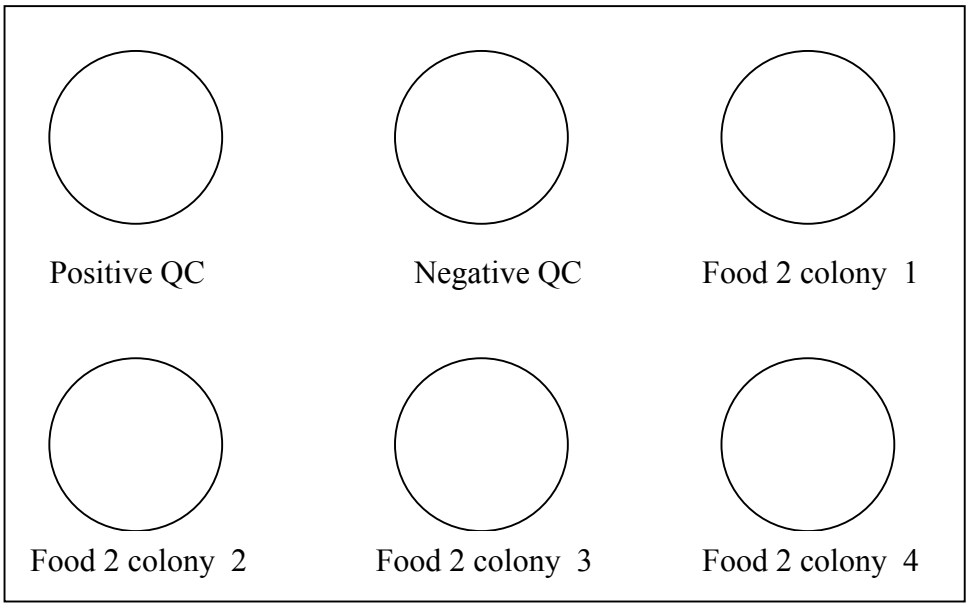
5: Control latex and sample suspension to check for autoagglutination.

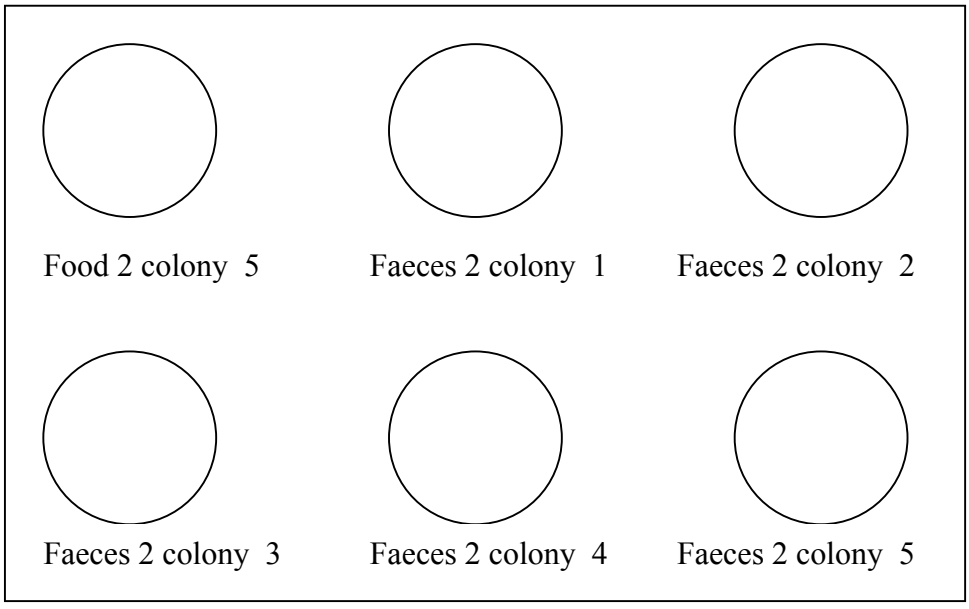
Date:_____

Initials:_____

Recordsheet:

Latex test for agglutination of *Escherichia coli* O157.





Overall result for food 2:

Overall result for faeces 2: