Global Salm-Surv

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

Laboratory Protocols

Level 4 Training Course

Conventional agglutination of enterohaemorrhagic *Escherichia coli* O157.

1st Ed. February 2003

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1. Conventional agglutination of enterohaemorrhagic *Escherichia coli* O157.

Introduction

Conventional agglutination with antiserum O157 is a cheap and easy agglutination test for identification of *Escherichia coli* O157. The method is quick and simple to use, but producing the antiserum is laborious and could cause problems.

Materials

Equipment

- Inoculation loops (1 µl)
- Bunsen burner
- Sterile Pasteur pipettes
- Slide

Media

- Sterile normal saline.
- Nutrient agar plates.
- Antiserum O157

Bacterial strains

- *E.coli* CCUG 29889

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

Procedure

O-typing

Day 1

Place a drop of saline on a slide.

From the inoculated nutrient agar plate transfer a loop full of growth and mix it into the drop of saline on the slide.

Ensuring a smooth, opaque suspension in the drop.

Check the appearance of the drop. If it has lumps in it, the culture is autoagglutinating and no further agglutination is possible. (Negative control test)

Place a drop of O157 antiserum next to the first drop.

Mix antiserum and culture (antigen) with a loop or stick and rock the slide with the sample gently for a maximum of 1 minutes.

A homogenous suspension is a negative reaction. Lumping is a positive reaction.

O157 detected are noted. Both positive and negative reactions are noted.

Theory / comments

Agglutination will be seen as particulate matter or “lumps” forming within the drop Autoagglutinating cultures may be referred to as “rough” strains of *Escherichia coli*. If a strain autoagglutinates, subculture on blood agar or Mueller-Hinton agar in order to recover the smooth state of the strain, and repeat the agglutination test.

Detection of the O- antigen is performed by slide agglutination. Antibodies in the specific sera agglutinate with the bacteria when the corresponding antigens are present.

2. Composition and preparation of culture media and reagents

If no reference is given, it is the procedure used at DVL.
The media and reagents are available from several companies including Oxoid, Merck and Difco. The composition of the dehydrated media given below is an example and may vary a little among the different manufacturers. Also, the media should be prepared according to the manufacturers description if it differs from the description given here.

Nutrient agar (ref. 1)

Meat extract	3.0 g
Peptone	5.0 g
Agar	12 g to 18 g[1)]
Water	1000 ml

[1)] Depending on the gel strength of the agar.

Preparation:
Dissolve the dehydrated medium in the water by heating if necessary. Adjust pH to ~7.0 after sterilisation, transfer into bottles and autoclave at 121°C for 20 min. Pour 15 ml of melted medium into each plate.

References

1. Post D. E. (1997) Food-borne pathogens monograph number 5 *Salmonella*. Oxoid limited, Hampshire, England.

Date:_____

Initials:_____

Recordsheet:
Conventional agglutination of *Escherichia coli* O157.

Sample:	**Antigenic reaction:**
Food 2 – colony 1	
Food 2 – colony 2	
Food 2 – colony 3	
Food 2 – colony 4	
Food 2 – colony 5	
Faeces 2 – colony 1	
Faeces 2 – colony 2	
Faeces 2 – colony 3	
Faeces 2 – colony 4	
Faeces 2 – colony 5	

Overall result for sample food 2:

Overall result for sample faeces 2: