NATIONAL STANDARD METHOD

CHANGING THE PHASE OF SALMONELLA

BSOP TP 32

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections
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Email: standards@hpa.org.uk
CHANGING THE PHASE OF SALMONELLA SPECIES

SCOPE OF DOCUMENT

The majority of serotypes of Salmonella possess two phases of H (flagellar) antigens. If agglutination is obtained with one phase, the organism may be induced to change to the other phase.

INTRODUCTION

The phase can be changed using two methods: a Craigie’s tube or ditch plate (Jamieson’s plate). Both methods involve adding the test organism to the H anti-serum which it has already agglutinated with. Organisms in the original phase demonstrated, agglutinate with the H anti-serum, leaving the organisms in the alternative phase free to move in the culture.

TECHNICAL INFORMATION/LIMITATIONS

Some organisms eg Salmonella typhi and Salmonella Montevideo have only one phase.

Phase change is not always achieved at the first attempt. When necessary the procedure should be repeated before concluding that the organism has no alternative phase.

In some cases using a broth culture can expedite results.
1 SAFETY CONSIDERATIONS

Most *Salmonella* species are in hazard group 2 with important exceptions including *S. Typhi* and *S. Paratyphi A, B & C*. Work involving these organisms must be performed under containment level 3 conditions.

*S. Typhi*, *S. Paratyphi A, B & C* cause severe and sometimes fatal disease. Laboratory acquired infections have been reported. *S. Typhi* vaccination is available and guidance is given in the HPA immunisation policy.

Refer to the current guidance on the safe handling of all Hazard Group 2 organisms documented in this NSM.

All work likely to generate aerosols must be performed in a microbiological safety cabinet.

The above guidance should be supplemented with local COSHH and risk assessments.

Compliance with postal and transport regulations is essential.

2 REAGENTS AND EQUIPMENT

Discrete colonies growing on solid medium.

*Salmonella* H antisera.

Bacteriological straight wire/loop (preferably nichrome) or disposable alternative.

**Craigie’s tube method (semi-solid agar)**

Dispense the semi-solid agar in 12 mL amounts and add a piece of glass tubing (the tube must be longer than the depth of the medium)

**Ditch plate method**

Nutrient agar plate.

Sterile filter paper strips.

3 QUALITY CONTROL ORGANISMS

N/A
4 PROCEDURE AND RESULTS

4.2 CRAIGIE METHOD\textsuperscript{2}

- Melt two tubes of semi-solid agar and allow to cool to 50°C
- To one tube add 0.5 mL of a 1:5 dilution of H antiserum (to which the organism has previously agglutinated) and to the second tube add 1 mL of the same dilution of antiserum
- When the medium has solidified, inoculate the culture to the agar inside the inner tube (either with a straight wire from a plate, or add one drop of a liquid culture)
- Incubate at 35-37°C for the shortest period required for swarming eg 8-16 hours
- Subculture from the outside of the inner tube to agar slopes or nutrient broth and use this culture for identification of the second phase antigens

4.2 DITCH PLATE METHOD\textsuperscript{3}

- Cut a 50 x 20 mm ditch in a well-dried nutrient agar plate
- Soak a sterile filter paper strip in the H anti-serum with which the organism has agglutinated and place across the ditch at right angles
- At one end place a filter paper strip across the first paper strip
- Inoculate the other end of the strip with one drop of a young broth culture and incubate at 35-37°C for 18-24 hours. Organisms in the original phase will agglutinate on the strip, the others in the second phase will pass across it
- Remove the second filter paper strip and place it in glucose broth and incubate this at 35-37°C for 4 hours
- Repeat H agglutinations to determine the second phase
- The second strip is optional. If one end of the first strip is inoculated with a well-isolated colony and incubated, the resulting growth from the un-inoculated end of the strip can be investigated by agglutination with anti-sera
5 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology (http://www.hpa-standardmethods.org.uk/wg_bacteriology.asp). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

The National Standard Methods are issued by Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

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APPENDIX

Isolate from pure culture

Craige's method
Melt two tubes of semisolid agar and cool to 50°C
To one tube add 0.5 mL of 1:5 dilution of H antiserum
To other tube add 1 mL of the same dilution of the antiserum
When medium has solidified inoculate to the agar inside the inner tube (with straight wire from a plate, or a drop of liquid culture)
Incubate at 35-37°C 8-16 h
Subculture from outside of the inner tube to agar slopes or nutrient broth and use for identification of 2nd phase antigen

Ditch plate method
Cut 50 x 20 mm ditch in a well-dried nutrient agar plate
Soak a sterile filter paper strip in the H antiserum with which the organism has agglutinated, and place across the ditch at right angles
At one end place a filter paper strip across 1st filter paper strip
Inoculate other end of filter paper strip with one drop of young broth culture
Incubate at 35-37°C 18-24 hours
Organisms in the original phase will agglutinate on the filter paper strip, others in 2nd phase will pass across
Remove 2nd filter paper strip, place in glucose broth and incubate at 35-37°C for 4 hours
Repeat H agglutinations to determine 2nd phase

* the H serum in which the organism has previously agglutinated

# 2nd strip is optional – if one end of the 1st strip is inoculated, with isolated colony and incubated resulting growth from un-inoculated end can be investigated with antisera
REFERENCES


