

NATIONAL STANDARD METHOD

CHANGING THE PHASE OF *SALMONELLA*

BSOP TP 32

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections







CHANGING THE PHASE OF *SALMONELLA*

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STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing standards@hpa.org.uk.

The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

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The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at www.hpa.org.uk.

The HPA aims to be a fully Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions¹.

More details can be found on the website at www.evaluations-standards.org.uk. Contributions to the development of the documents can be made by contacting standards@hpa.org.uk.

The reader is informed that all taxonomy in this document was correct at time of issue.

Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

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AMENDMENT PROCEDURE

Controlled document reference	BSOP TP 32
Controlled document title	Changing the phase of <i>Salmonella</i>

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
2/ 07.05.10	1.1	2	All	Whole document	Document reviewed, no updates required

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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)^{2,3}. 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.

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1 SAFETY CONSIDERATIONS⁴⁻¹⁰

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

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4 PROCEDURE AND RESULTS

4.2 CRAIGIE METHOD²

- 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 METHOD³

- 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

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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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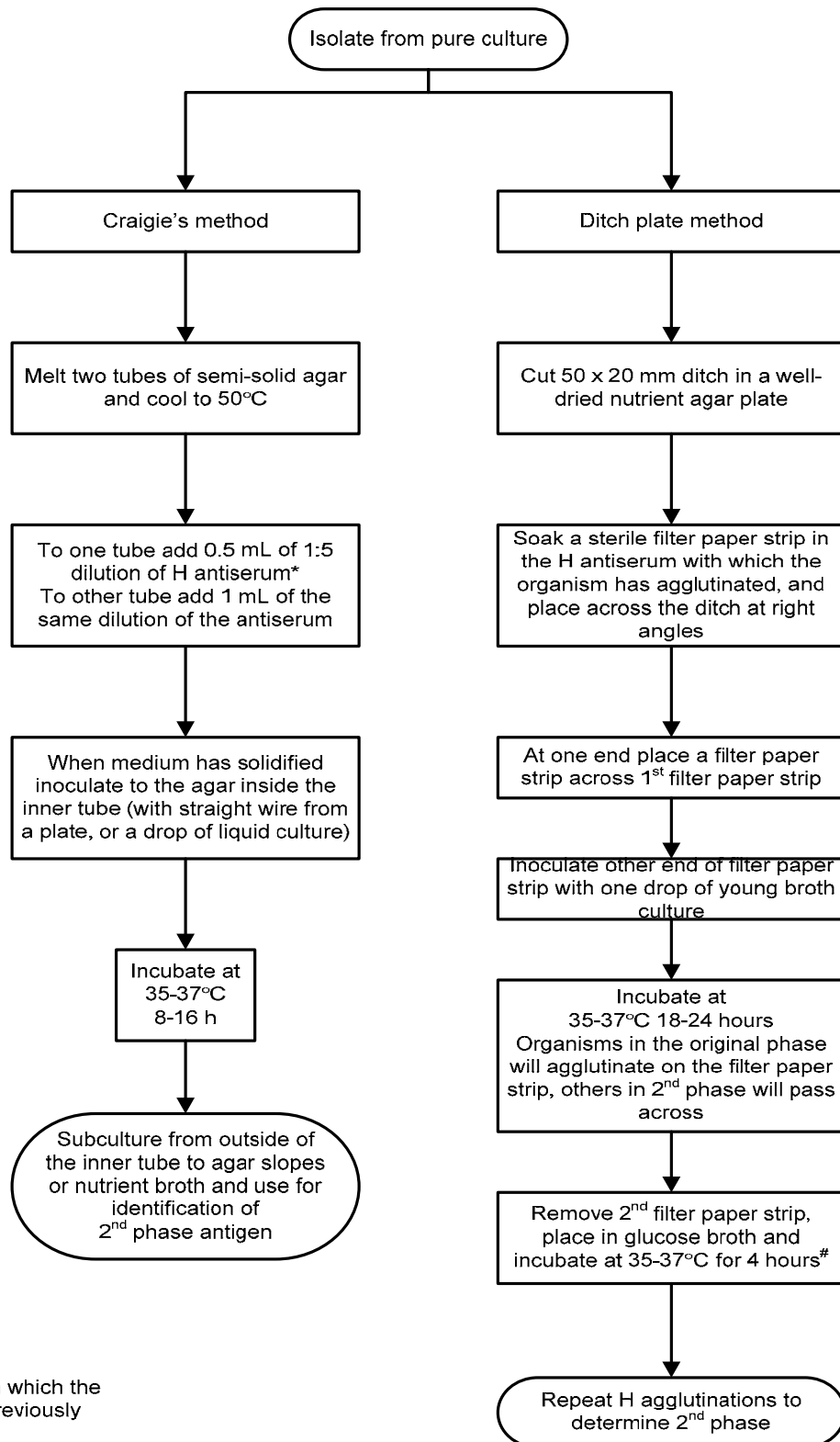
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APPENDIX



* 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

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