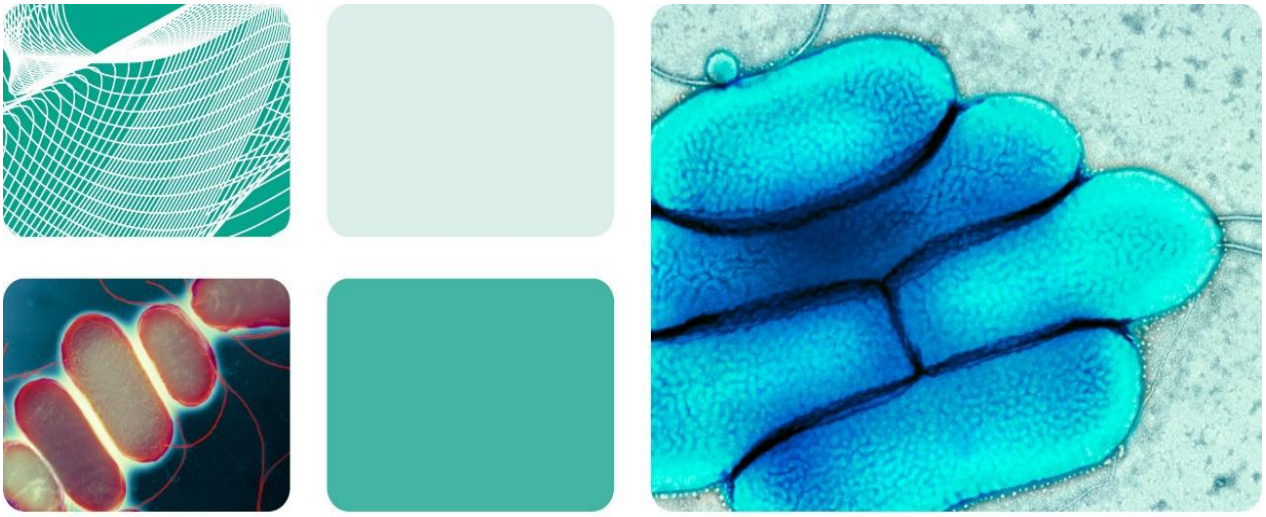
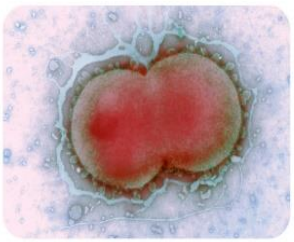
UK Health Security Agency

UK Standards for Microbiology Investigations

Changing the phase of *Salmonella*





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Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee.

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

UK SMIs are produced in association with:

Displayed logos correct as of December 2024

Contents

Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	7/12.03.25
Issue number discarded	4
Insert issue number	4.1
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 15/05/2019.** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.

Amendment number/date	6/15.05.19
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	15.05.22
Section(s) involved	**Amendment**
Whole Document	Document updated and references graded. Technical limitations updated with subheadings.

	Ditch plate method was written for clarification.

*Reviews can be extended up to 5 years where appropriate

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

The majority of serotypes of *Salmonella* possess two phases of H (flagellar) antigens. This test is used to induce phase change in *Salmonella* serotypes, if agglutination has been obtained with one phase.

This UK SMI should be used in conjunction with other UK SMIs.

4 Introduction

Most *Salmonella* isolates exist in two phases: phase 1, phase 2 or may have both phases simultaneously. Cultures that are not expressed in one phase upon primary culture may be switched to the other phase using 2 methods: a Craigie's tube or ditch plate (Jamieson's plate[1-3]). Both methods involve adding the test organism to the H anti-serum to which it has already agglutinated. 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.

5 Technical information/limitations

5.1 Phase change

Some serotypes eg *Salmonella* Typhi and *Salmonella* Montevideo have only one phase and so these should be sent to the Reference Laboratory.

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[2].

5.2 Broth culture

In some cases, using a broth culture can expedite results.

5.3 Culture media

Bacterial culture should be performed using non-selective media eg nutrient agar. If selective media are used, antigen production may be insufficient or autoagglutination may occur.

5.4 Quality control

It is essential that the formulation of the antisera used are checked and validated prior to use.

6 Safety considerations[4-21]

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

S. Typhi, *S.* Paratyphi A, B and C cause severe and sometimes fatal disease. Laboratory acquired infections have been reported[22]. *S.* Typhi vaccination is available and guidance is given in the Department of Health immunisation policy[23].

Appropriate personal protective equipment (PPE) and techniques designed to minimise exposure of the laboratory workers should be worn and adhered to at all times.

The most effective method for preventing laboratory-acquired infections is the adoption of safe working practices.

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

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

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

Compliance with postal and transport regulations is essential.

7 Reagents and equipment

Discrete colonies growing on solid medium

Salmonella H antisera

Bacteriological straight wire/loop or disposable alternative

Craigie's tube method (0.2 – 0.3% semi-solid agar)[2]

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

Ditch plate method[1]

Nutrient agar plate

Sterile filter paper strips

Sterile forceps

8 Quality control organisms

Positive control:

N/A

Negative control:

N/A

9 Procedure and results

9.1 Craigie method[2]

- prepare bottles or test tubes containing the semi-solid nutrient agar and a small hollow inner tube open at both ends with the upper end projecting well above the agar
- melt the semi-solid agar and allow to cool to 50°C
- to one test tube, add 0.5mL of a 1:5 dilution of H antiserum (to which the organism has previously agglutinated) and to another test tube, add 1mL of the same dilution of antiserum
- allow to cool and solidify
- when the medium has solidified, inoculate the culture to the agar inside both of the inner tubes (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 8-16hr
- subculture from the outside of the inner tubes to agar slopes or nutrient broth and use this culture for identification of the second phase antigens

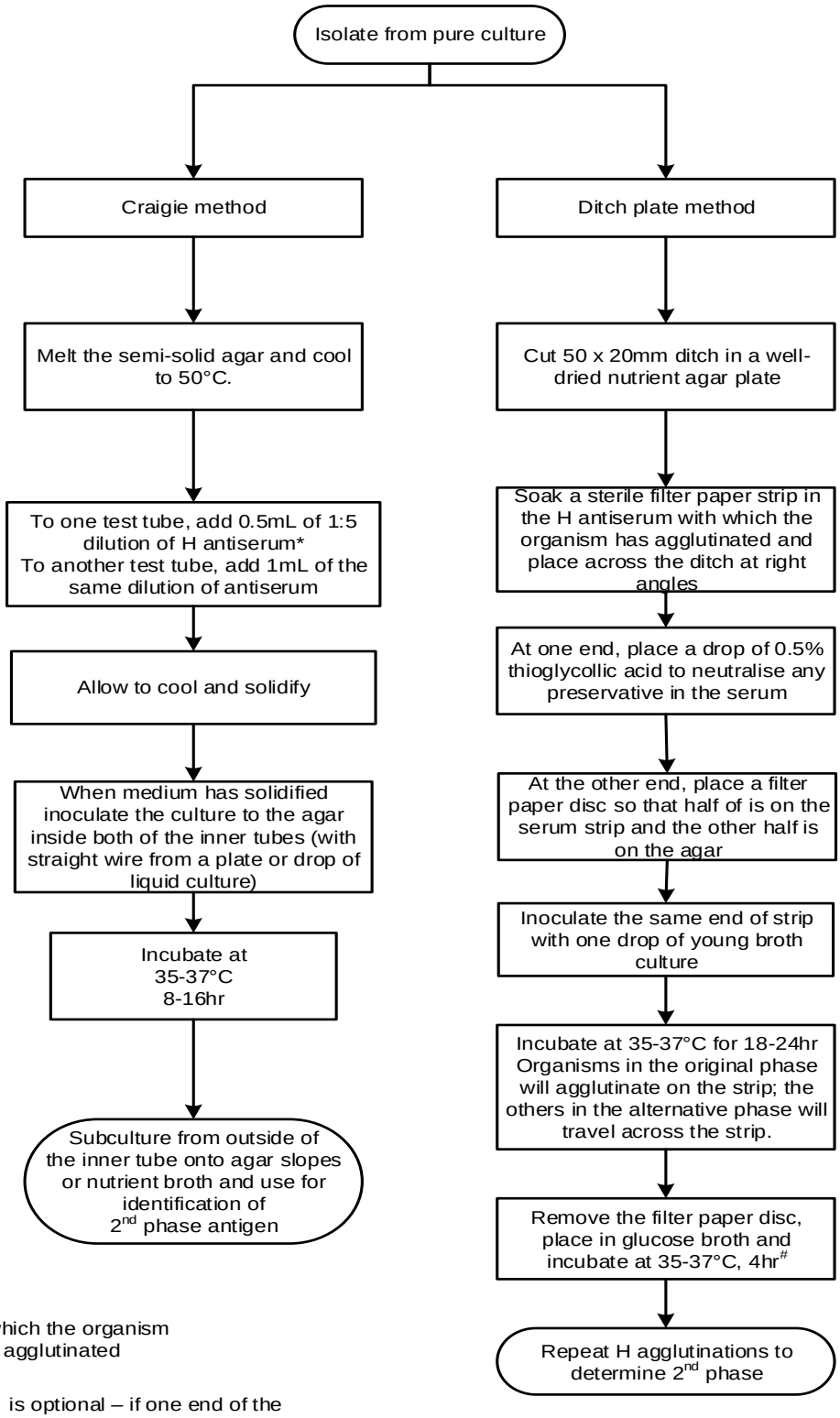
9.2 Ditch plate method[1]

- cut a 50 x 20mm 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 of the filter strip, place one drop of 0.5% thioglycollic acid to neutralise any preservative in the serum
- at the other end of the filter strip, place a filter paper disc, about 7mm diameter, so that half of it is on the serum strip and the other half on the agar. Inoculate the same end of the strip with one drop of a young broth culture and incubate at 35-37°C for 18-24hr. Organisms in the original phase will agglutinate on the strip; the others in the alternative phase will travel across the strip
- remove the filter paper disc using sterile forceps and place it in glucose broth and incubate this at 35-37°C for 4hr
- repeat H agglutinations to determine the second phase

  Note: It should be noted that the use of the second strip/disc is optional. If one end of the first strip is inoculated with a well-isolated colony and incubated, the

resulting growth from the uninoculated end of the strip can be investigated by agglutination with antisera

Algorithm: Changing the phase of *Salmonella*



* H serum to which the organism has previously agglutinated

2nd strip/disc is optional – if one end of the 1st strip is inoculated with isolated colony and incubated resulting growth from uninoculated end can be investigated with antisera

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An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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