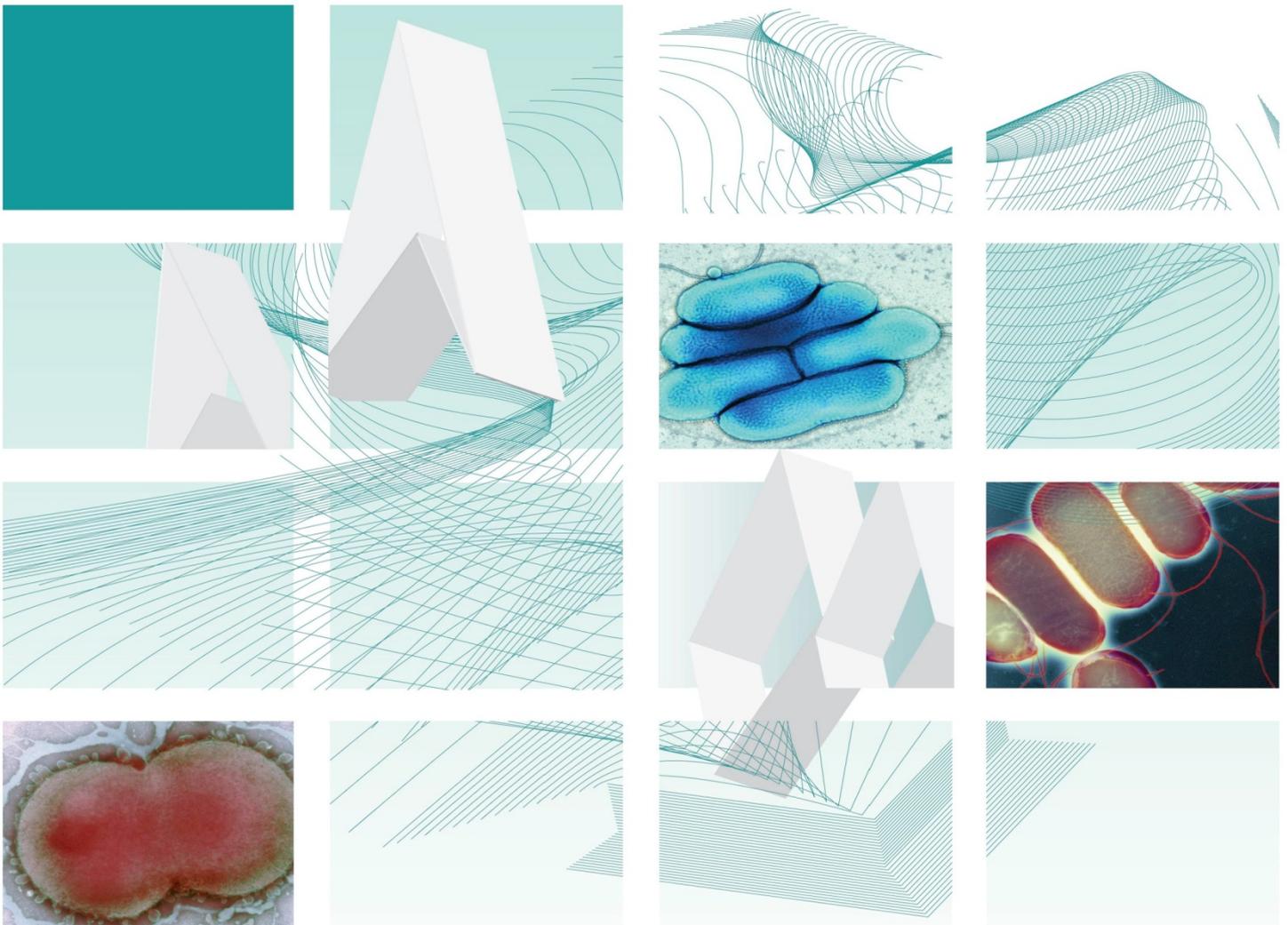




UK Standards for Microbiology Investigations

Changing the Phase of *Salmonella*



Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-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.

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UK Standards for Microbiology Investigations are produced in association with:



Logos correct at time of publishing.

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NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment Table

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

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	5/08.01.15
Issue no. discarded.	2.2
Insert Issue no.	3
Section(s) involved	Amendment
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Introduction.	This section has been updated and references added.
Technical information/Limitations.	Section updated.
Safety Considerations.	Information and references updated.
Reagents/Equipment.	This section has been updated and referenced.
Procedures and Results.	Information and references updated.
Flowchart.	This flowchart has been modified for easy guidance.
References.	Some references updated.

UK Standards for Microbiology Investigations[#]: Scope and Purpose

Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests.
- SMIs provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies.

The list of participating societies may be found at <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>.

Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

SMIs are developed, reviewed and updated through a wide consultation process.

[#]Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a 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 conditions.

The development of SMIs are subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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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.

This SMI should be used in conjunction with other SMIs.

Introduction

Most 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 plate¹⁻³). 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 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.

In some cases using a broth culture can expedite results.

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.

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

1 Safety Considerations⁴⁻²⁰

Most *Salmonella* 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 reported²¹. *S. Typhi* vaccination is available and guidance is given in the Department of Health immunisation policy²².

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

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 SMI.

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 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 method¹

Nutrient agar plate

Sterile filter paper strips

Sterile forceps

3 Quality Control Organisms

Positive Control

N/A

Negative Control

N/A

4 Procedure and Results

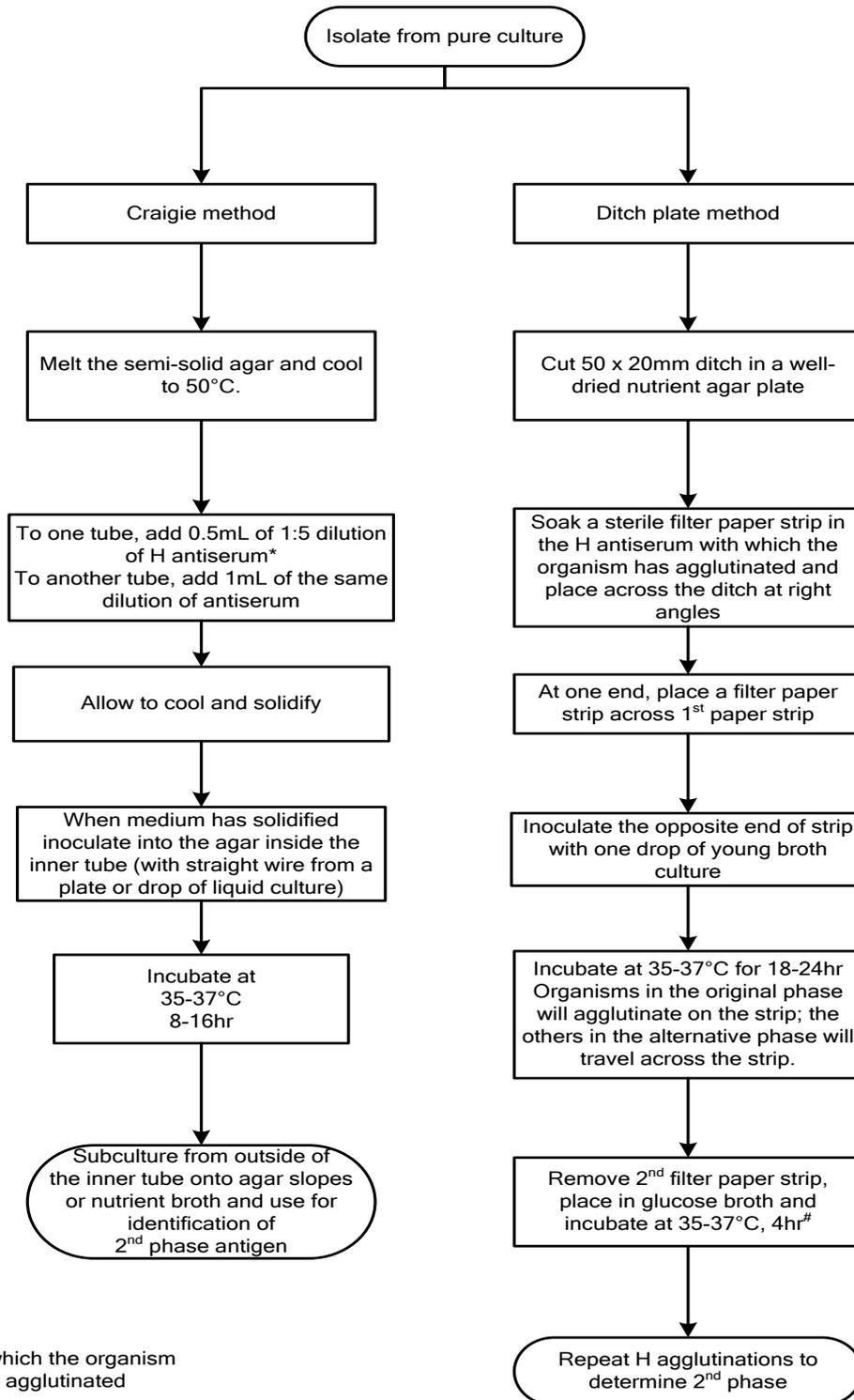
4.1 Craigie Method²

- Prepare tubes containing the semi-solid nutrient agar and a small 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 tube add 0.5mL of a 1:5 dilution of H antiserum (to which the organism has previously agglutinated) and to another 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 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 8-16hr
- 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 Method¹

- 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, place one drop of 0.5% thioglycollic acid to neutralize any preservative in the serum
- At the other end, place a second filter paper strip across the first paper strip
- Inoculate the opposite 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 second filter paper strip 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
- 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 uninoculated end of the strip can be investigated by agglutination with antisera

Appendix: Changing the phase of *Salmonella*



* H serum to 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 uninoculated end can be investigated with antisera

The flowchart is for guidance only.

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