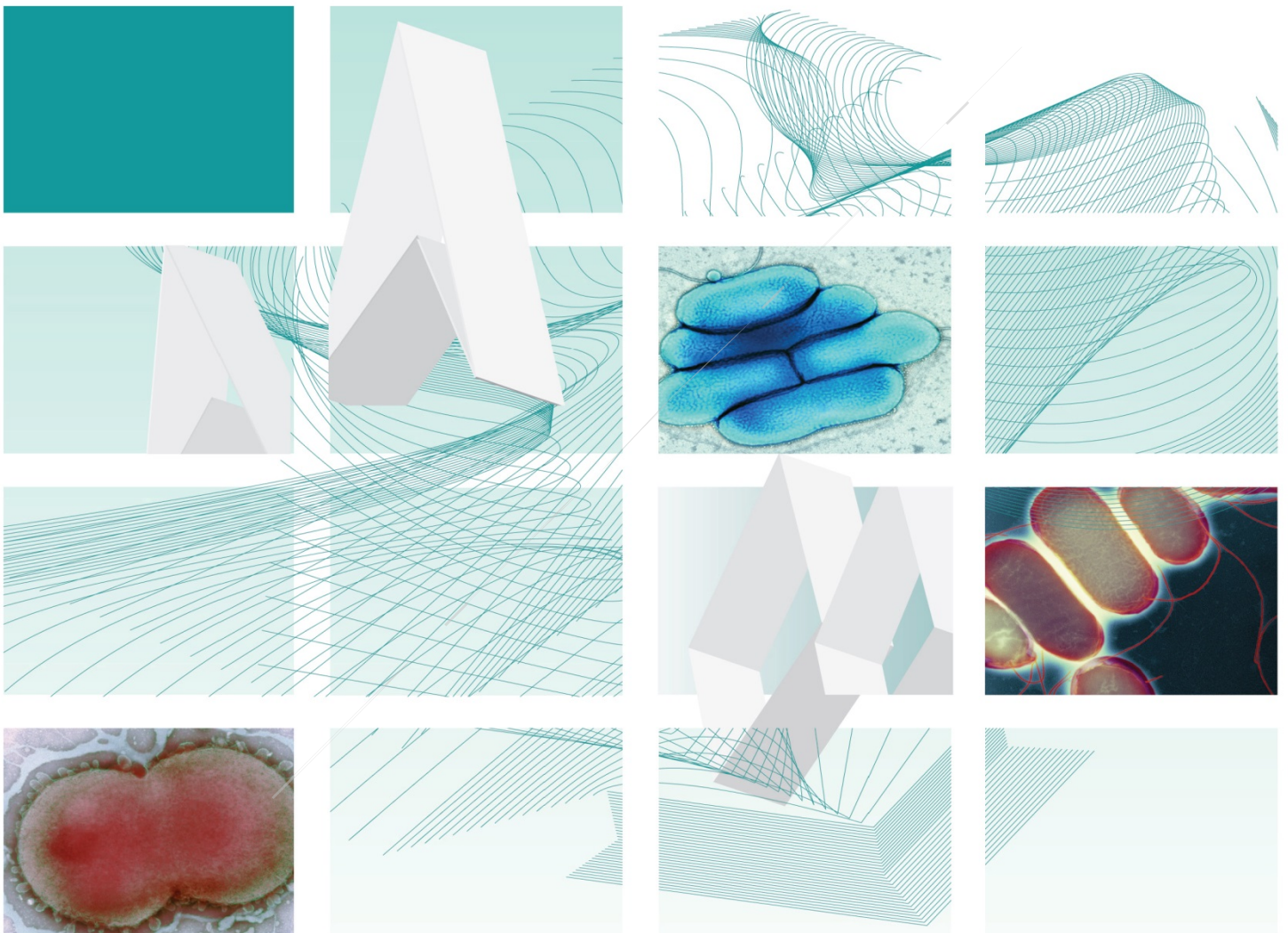




UK Standards for Microbiology Investigations

Changing the phase of *Salmonella*



"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

Acknowledgments

UK Standards for Microbiology Investigations (UK 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>. UK 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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Logos correct at time of publishing.

Contents

Acknowledgments	2
Contents	3
Amendment table	4
UK SMI: scope and purpose.....	5
Scope of document.....	8
Introduction.....	8
Technical information/limitations	8
1 Safety considerations	9
2 Reagents and equipment	9
3 Quality control organisms	9
4 Procedure and results.....	10
Appendix: Changing the phase of <i>Salmonella</i>	11
References	12



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Amendment table

Each UK 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 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 five years subject to resources available.

UK SMI[#]: scope and purpose

Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also 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. The documents also 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 UK SMIs

UK 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. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK 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

UK 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><http://www.hpa-standardmethods.org.uk/>.

Inclusion of a logo in an UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK 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. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE

[#] 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.

accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK 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 UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK 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 UK SMIs is subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>.

The UK 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

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, 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 UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date 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.

UK SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested citation for this document

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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.

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 plate¹⁻³). 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.

Technical information/limitations

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 phase².

Broth culture

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

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.

Quality control

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

1 Safety considerations⁴⁻²¹

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 reported²². *S. Typhi* vaccination is available and guidance is given in the Department of Health immunisation policy²³.

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 UK 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

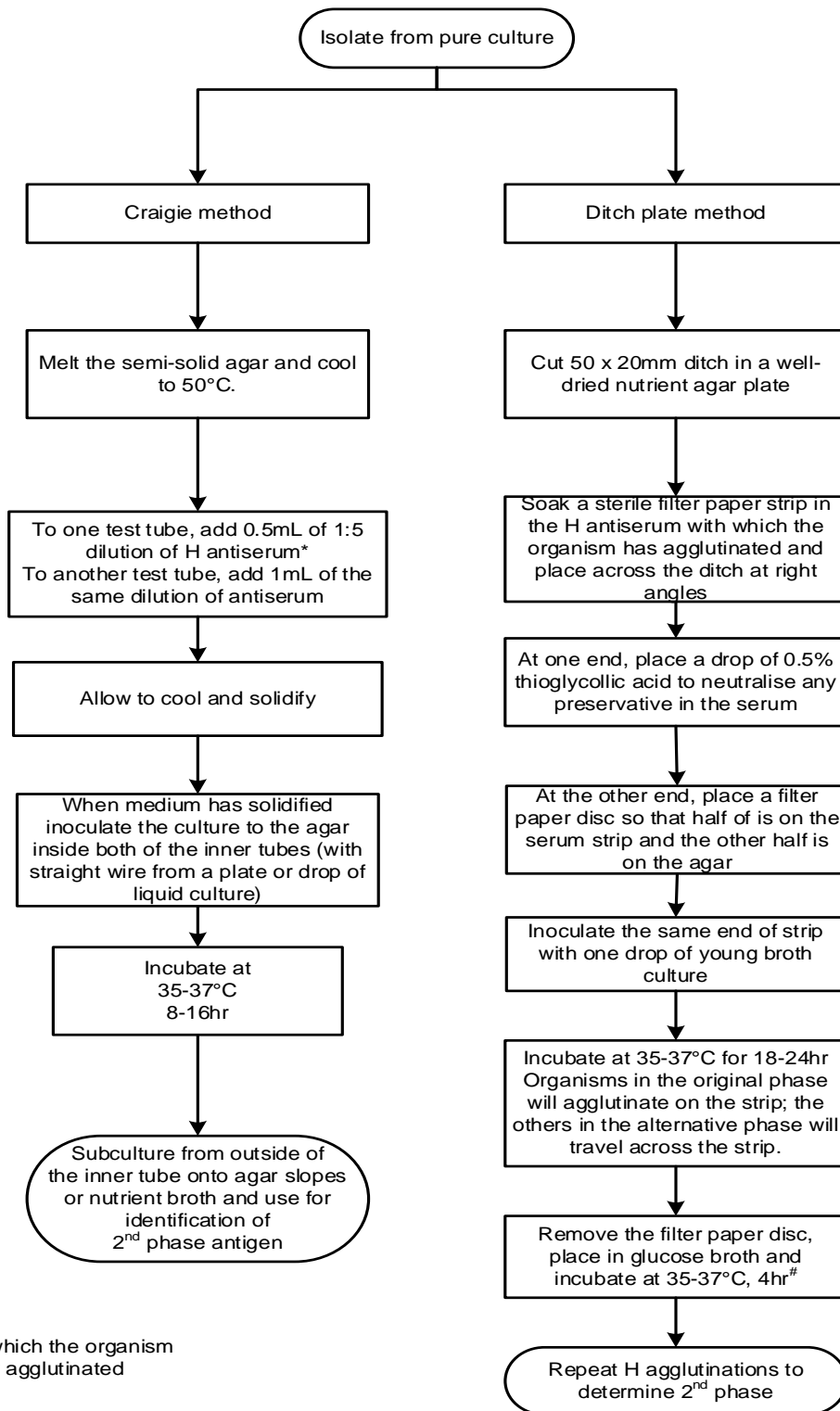
- prepare bottles or test tubes containing the semi-solid nutrient agar and a small hollow inner tube open at both ends with the upper end projecting well above the agar
- melt the semi-solid agar and allow to cool to 50°C
- to one test tube, add 0.5mL of a 1:5 dilution of H antiserum (to which the organism 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

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 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

Appendix: 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

The flowchart is for guidance only.

References

Modified GRADE table used by UK SMI's when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMI's for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VIII). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Quality/certainty of evidence	Types of evidence
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B* Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
	III Evidence from documents describing techniques, methods or protocols
C* Weakly recommended: seek alternatives	IV Non-analytical studies, eg case reports, reviews, case series
D Never recommended	V Expert opinion and wide acceptance as good practice but with no study evidence
	VI Required by legislation, code of practice or national standard/ guideline
	VII Letter /short communication /editorials /conference communication
	VIII Electronic citation

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