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.

For further information please contact us at:

Standards Unit
National Infection Service
Public Health England
61 Colindale Avenue
London NW9 5EQ
E-mail: standards@phe.gov.uk

Website: https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

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Logos correct at time of publishing.
Agglutination test for *Salmonella* species

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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.

<table>
<thead>
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<th>Amendment number/date</th>
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<tr>
<td></td>
<td>Commercial agglutination alternatives have been mentioned in the document and in the flowchart.</td>
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<tr>
<td></td>
<td>Technical limitations/information updated with subheadings.</td>
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<tr>
<td></td>
<td>Picture added to show positive and negative agglutination.</td>
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<tr>
<td></td>
<td>Flowchart updated.</td>
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<tr>
<td>References.</td>
<td>References updated and graded.</td>
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</table>

*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. 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 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](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.
Agglutination test for *Salmonella* species

**Suggested citation for this document**
Scope of document

The document covers the procedure for agglutination tests for *Salmonella* species. Agglutination tests are used to test an unknown organism against known antisera. They are used for example, in the serotyping of *Salmonella* species and serotyping of other organisms such as the Lancefield grouping of streptococci and in the differentiation of *Staphylococcus aureus* from other species of staphylococci\(^1-3\).

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

Introduction

Bacteria, provided they form stable suspensions in saline, can be agglutinated directly by antibody. Bacterial agglutination tests may be performed on a slide, in microtitre tray wells, in tubes or by using commercial alternatives. Tube agglutination tests are usually more sensitive than slide tests as they require a longer incubation period which allows more antigen and antibody to interact.

Slide agglutination tests are simple to use, require minimal equipment and are rapid.

Technical information/limitations

Interpretation of results

Slide agglutination tests cannot be performed if the bacterial suspension is granular, autoagglutinates or is sticky as the results will be uninterpretable.

Growth on solid media is not optimal for the formation of flagella and therefore not ideal for slide agglutinations of flagella antigen. False negative results may be obtained with H antisera. Inoculation of the pure culture to a wet nutrient agar slope will aid flagellum formation.

If a weak reaction is encountered in a slide agglutination assay, it is recommended that this should be confirmed with a tube agglutination assay\(^4\).

Isolates that show no agglutination must be identified by other methods.

Commercial agglutination preparations

Standard bacterial suspensions and antisera may be obtained commercially. Latex agglutination preparations are available and manufacturers’ recommendations should be followed. However, where there are any deviations from these recommendations, in-house validation must be performed and documented. If using commercially manufactured antisera, check suitability of use for all methods. The limitation of these commercially manufactured agglutination preparations is that they have limited shelf lives that place increased demands on procurement and distribution systems for laboratories.

Commercial agglutination alternatives

These commercial agglutination kits rapidly detect and presumptively identify *Salmonella* from culture by latex agglutination. They save testing time over traditional agglutination methods. Laboratories can use these to eliminate *Salmonella*-negative samples from further testing, reducing the number of samples requiring confirmatory testing.
Agglutination methods

The two agglutination methods (although being slowly phased out in many hospital laboratories) include tube and microtitre tray agglutination tests for serotyping; however, they do have their limitations. The tube agglutination tests are usually expensive due to the number of dilutions and large amounts of antigen required.

Agglutination with microtitre trays is easier to perform; saves time and space as well as reduces the volume of antisera used\textsuperscript{5,6}.
1 Safety considerations

Most *Salmonella* species are Hazard Group 2 with important exceptions including *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi A, B and C. All work on S. Typhi and S. Paratyphi A, B and C must be performed under Containment Level 3 conditions.

S. Typhi and S. Paratyphi A, B and C cause severe and sometimes fatal disease. Laboratory acquired infections have been reported\(^{25}\). S. Typhi vaccines are available; guidance is available from the Department of Health\(^{26}\).

Immunisation of laboratory workers may therefore:

- protect the individual and their family from an occupationally-acquired infection
- protect patients and service users, including vulnerable patients who may not respond well to their own immunisation
- protect other healthcare and laboratory staff
- allow for the efficient running of services without disruption

The most effective method for preventing laboratory-acquired infections is the adoption of safe working practices. Appropriate personal protective equipment (PPE) and techniques designed to minimise exposure of the laboratory workers should be worn and adhered to at all times.

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

For slide agglutination and microtitre tests, all slides/plates should be discarded appropriately after reading of results to avoid contaminating the fingers or workbench with live bacterial suspensions\(^{27}\).

Refer to current guidance on the safe handling of all 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

2.1 Slide agglutination

- Known antisera
- Bacterial culture
- 0.85% sterile saline
- Glass slides
- Bacteriological straight wire/loop (preferably nichrome) or disposable alternative

2.2 Microtitre agglutination

- Somatic (O) antigen suspension
Flagellar (H) antigen suspension
Known antisera
1% formol saline
U well microtitre plates

2.3 Tube agglutination
Somatic (O) antigen suspension
Flagellar (H) antigen suspension
Known antisera
0.85% saline
1% formol saline
Glass tubes usually 75mm by 1cm
Dreyer’s tubes H antigen

2.4 Commercial alternatives
Laboratories should adhere to manufacturers’ instructions when using commercial kits.

3 Quality control organisms

Quality control organisms for tube and slide agglutinations

Positive control
Homologous organism to the antiserum

Negative control
Organism in saline only

4 Procedure and results

4.1 Preparations of O and H suspensions
- for each organism inoculate two tubes of Brain Heart Infusion broth, one for O antigen and one for H antigen
- incubate at 37°C for 4-5hr
- dilute each suspension in formol saline so that there are approximately $10^8$ bacteria/mL (McFarland Standard)

4.1.1 Preparation of O Suspensions
- steam the O antigen broth culture at 100°C for 30 min
- allow to cool and dilute with an equal volume of saline
4.1.2 Preparation of H Suspensions

- add an equal volume of 1% formol saline to the H antigen broth culture
- allow to stand overnight or can use straight away if possible (necessary)

4.2 Slide agglutination test procedure

- place 2 drops of sterile saline on a divided slide and emulsify a colony in each to make a milky suspension

  OR

  alternatively, place 2 drops of previously prepared milky suspension of the test organism in drops of saline on a slide

- if auto-agglutination occurs or the suspension is rough in saline then discard the slide. The test can only be performed with smooth suspensions
- add a drop of antiserum to one suspension only, the other acts as the control, and mix by tilting the slide to and fro for 30-60 sec
- examine for agglutination (clumping) of the suspension (with antiserum) and clearing of the saline under a good light against a black background with the naked eye

Positive result
Agglutination of the suspension (clumping)

Negative result
Suspension remains turbid

4.3 Microtitre tray test procedure

- add 25μL of saline to all 8 wells in a column in a microtitre tray
- add 25μL of 1/10 prediluted antiserum to the top well and double dilute down to well 7. Discard the excess 25μL from well 7 instead of adding it to well 8
- well 8 contain saline only as an antigen control
- add 25μL of respective O or H diluted antigen to all wells. Seal the microtitre plate

The final dilutions are:

A. Positive agglutination reaction
B. Negative agglutination reaction

(Adapted from Smith, SK et al28.)
• incubate the O antigens in an incubator at 50°C overnight before examining for agglutination

• incubate the H antigens in a water bath at 50°C for 2hr before examining for agglutination

Positive result
Agglutination of the suspension.

Negative result
Suspension remains turbid.

Antigen control well
Suspension remains turbid.

Note:
1. care must be taken to avoid shaking of the microtitre plates during and after incubation to allow settling of the antigen
2. it should be noted that the dilution and time of incubation will vary depending on the antiserum that is used

4.4 Tube agglutination test procedure
Note: The O and H antigen tests are carried out in parallel

• for each O and H antigen tested against each antiserum set up a row of seven tubes and add 0.4mL of saline to tubes 2 and 7

• add 0.2mL of 1/5 antiserum to tubes 1 and 2. Mix the contents of tube 2 and perform doubling dilutions to tube 6 and then discard 0.2mL instead of adding it to tube 7

• add 0.2mL of the respective bacterial O or H suspension to each tube

The final dilutions are:

<table>
<thead>
<tr>
<th>Tube</th>
<th>1</th>
<th>2</th>
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<th>6</th>
<th>7</th>
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<td>1/10</td>
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<td>1/80</td>
<td>1/160</td>
<td>1/320</td>
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</table>

• incubate tests with O suspensions in a water-bath at 37°C for 4-6hr, then allow to stand overnight in a refrigerator

• using a fine capillary pipette and starting from tube 7 and working backwards to tube 1, transfer the contents of each H tube to a Dreyer tube

• incubate H tests for 2 - 4hr in a water-bath at (37°C) and read after standing on the bench for half an hour. For some bacteria, incubation at 50°C is preferable

• examine each tube for agglutination of the bacterial suspension. If necessary, rotate the tube to swirl-up the granules from the deposit, but do not shake the tube
Agglutination test for *Salmonella* species

- examine the control tube 7 without the serum to confirm that autoagglutination has not taken place. And if it has, disregard positive results in the other tubes
- the titre taken is the highest dilution with clearly visible agglutination

For practical purposes, it is usual to set up a range of different O antisera at 1/20 and then titrate the positives.

**Positive result**
Agglutination of the suspension

**Negative result**
Suspension remains turbid

**Antigen control tube**
Suspension remains turbid
Appendix: Agglutination test for *Salmonella* species

1. **Isolate discrete colony**
2. **Inoculate 2 tubes of brain heart infusion broth for O or H antigen respectively and incubate at 37°C for 4-5hr**
3. **Dilute each suspension to 10⁶cfu/mL**
4. **Steam the O antigen broth suspension at 100°C for 30 min**
5. **Allow to cool and dilute with an equal volume of saline**
6. **Add equal volume of 1% formol saline to the H suspension broth**
7. **Leave to stand overnight or use straight away if necessary**

**Tube agglutination**

- **For each antigen, set up row of 7 tubes and add 0.4mL saline to tubes 2 and 7**
- **Double dilute down to well 7 discarding excess 25µL from well 7 NB. Well 8 = control**
- **Add 25µL of O or H diluted antigen to all wells and seal plate**

**Microtitre tray test**

- **Add 25µL saline to column of 8 wells in a microtitre tray**
- **Add 25µL antiserum to well 1**
- **Double dilute down to well 7 discarding excess 25µL from well 7**
- **Add 25µL of O or H diluted antigen to all wells and seal plate**
- **Carry out doubling dilutions from tubes 2-6 discarding remaining 0.2mL**
- **Add 0.2mL of either O or H suspension to each tube**
- **Incubate O suspension in a 37°C waterbath for 4-6hr and then leave in refrigerator overnight**
- **Transfer tube contents of H suspension into Dreyer tube, and incubate at 37°C in waterbath for 2-4hr**

**Examining for agglutination**

- **Examine for agglutination**
- **Positive Agglutination of suspension**
- **Negative Suspension remains turbid**

**Commercial alternatives**

- **Follow manufacturers' instructions.**
  - **Note:** Users should use the instructions given by manufacturer. Do not use instructions from different commercial kits to perform and interpret test results.

The flowchart is for guidance only.

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UK Standards for Microbiology Investigations | Issued by the Standards Unit, Public Health England
References

Modified GRADE table used by UK SMIs 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 SMIs 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.

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


10. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive 2013. 1-35. A, VI


16. European Parliament. UK Standards for Microbiology Investigations (UK SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes". 1998. A, VI


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