UK Standards for Microbiology Investigations

Agglutination Test
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 http://www.hpa.org.uk/SMI/Partnerships. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see http://www.hpa.org.uk/SMI/WorkingGroups).

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:
### 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.

<table>
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<th>Amendment No/Date.</th>
<th>4/13.03.14</th>
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<td>Professional body logos have been reviewed and updated.</td>
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<td>Standard safety and notification references have been reviewed and updated.</td>
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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 http://www.hpa.org.uk/SMI/Partnerships. 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.

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1#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 http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313. 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.

SMIs are Crown copyright which should be acknowledged where appropriate.
Scope of Document

Agglutination tests are used to test an unknown organism against known antisera. They are used, for example, in the serotyping of *Salmonella* species and the grouping of streptococci\(^1,2\). This SMI should be used in conjunction with other SMIs.

Introduction

Bacteria, provided they form stable suspensions in saline, can be agglutinated directly by antibodies. Bacterial agglutination tests may be performed on a slide, in microtitre tray wells or in tubes. 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\(^3\). Microtitre trays may be used to reduce the volume of antisera used.

Standard bacterial suspensions and antisera may be obtained commercially. Latex agglutination preparations are available, and manufacturers' recommendations should be followed.

Technical Information/Limitations

For slide agglutinations the test cannot be performed if the bacterial suspension is granular, autoagglutinates or is sticky.

For slide agglutinations, growth on some solid media is not optimal for the formation of flagella. 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 using commercially manufactured antisera, check suitability of use for all methods.
1 Safety Considerations

Most Salmonella, Shigella and Escherichia species are Hazard Group 2, with important exceptions including: Salmonella enterica serovar Typhi, Salmonella enterica serovar Paratyphi A, B and C, E coli O157 and Shigella dysenteriae type 1. All work on S. Typhi, S. Paratyphi A, B and C, E. coli O157 and S. dysenteriae type 1 must be performed under Containment level 3 conditions.

S. Typhi, S. Paratyphi A, B and C, E. coli O157 and S. dysenteriae type 1 cause severe and sometimes fatal disease. Laboratory acquired infections have been reported. S. Typhi immunisation is available; guidance is given in the Department of Health immunisation policy.

Refer to current guidance on the safe handling of all organisms documented in the SMI.

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

2.1 Slide Agglutination

Known antisera.
Bacterial culture.
0.85% saline.
Glass slides.

Bacteriological straight wire loop (preferably nichrome) or disposable alternative.

2.2 Microtitre Agglutination

Somatic antigen suspension.
Flagellar antigen suspension.
1% formol saline.

U well microtitre plates.

2.3 Tube Agglutination

Somatic antigen suspension.
Flagellar antigen suspension.
Known antisera.
0.85% saline.
1% formol saline.

Glass tubes usually 75mm by 1cm.
Dreyer's tubes H antigen.

**Quality control organisms for tube and slide agglutinations**

**Positive Result**
Homologous organism to the antiserum.

**Negative Result**
Organism in saline only.

### 3 Quality Control Organisms

N/A

### 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 $10^9$ bacteria/ml (McFarland Standard)

#### 4.1.1 Preparation of O Suspensions
- Steam the O antigen broth culture for 30min
- Allow to cool and dilute with an equal volume of saline

#### 4.1.2 Preparation of H Suspension
- 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

#### 4.2 Microtitre Tray Tests
- Add 25µl of saline to all eight wells in a column in a microtitre tray
- Add 25µl of an antiserum to the top well and double dilute down to well seven. Discard the excess 25µl from well seven
- Well eight contains 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:

<table>
<thead>
<tr>
<th>Well:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>1/20</td>
<td>1/40</td>
<td>1/80</td>
<td>1/160</td>
<td>1/320</td>
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</table>

- 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.

### 4.3 Slide Agglutination Tests

Make two adjacent suspensions 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 and mix.

Examine for agglutination (clumping) of the suspension (with antiserum) and clearing of the saline.

**Positive Result**
Agglutination of the suspension.

**Negative Result**
Suspension remains turbid.

### 4.4 Tube Agglutination Test Procedure

- 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 two and seven

- Add 0.2ml of 1/5 antiserum to tubes one and two. Mix the contents of tube two and perform doubling dilutions to tube six and then discard 0.2ml instead of adding it to tube seven.

- Add 0.2ml of the respective bacterial O or H suspension to each tube

The final dilutions are:

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<thead>
<tr>
<th>Tube</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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- Incubate tests with O suspensions in a water-bath at 37°C for 4hr, then allow to stand overnight in a refrigerator

- Using a fine capillary pipette and starting from tube seven and working backwards to tube one, transfer the contents of each H tube to a Dreyer tube

- Incubate H tests for 2hr in a water-bath at 50-52°C

- 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
• The titre is the highest dilution with 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

Isolate discrete colony

Inoculate 2 tubes of brain heart infusion broth with either O or H antigen

Incubate at 37°C for 4-5 hr

Dilute each suspension to 10^9 cfu/ml

O Suspensions
H Suspensions

Steam the O antigen broth for 30min

Allow to cool and dilute with equal volume of saline

Add equal volume of 1% formol saline to broth

Leave to stand overnight

Tube agglutination

For each antigen set up row of 7 tubes and add 0.4ml saline to tubes 2 and 7

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 4hr and then leave in refrigerator overnight

Microtitre tray test

Add 25μl to column of 8 wells in a microtitre tray

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

Note: Well 8 = control

Add 25μl of O or H antigen to all wells and seal plate

O Antigen
H Antigen

Place in 50°C incubator over night

Place in 50°C waterbath for 2hr

Incubate H tests at 50-52°C in waterbath for 2hr

Examine each tube for agglutination

Positive Agglutination of suspension
Negative Suspension remains turbid

Incubate O suspension in a 37°C waterbath for 4hr and then leave in refrigerator overnight

Transfer tube contents using fine capillary pipette, to Dreyer tube starting with tube 7 and working backwards

Agglutination or suspension is rough

Smooth suspension

Discard slide

Positive Agglutination of suspension
Negative Suspension remains turbid

The flowchart is for guidance only.

Bacteriology – Test Procedures | TP 3 | Issue no: 2.2 | Issue date: 13.03.14 | Page: 13 of 15

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References


4. European Parliament. UK Standards for Microbiology Investigations (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".


