

NATIONAL STANDARD METHOD

AGGLUTINATION TEST

BSOP TP 3

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections







AGGLUTINATION TEST

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National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

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The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

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The HPA aims to be a fully 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 conditions¹.

More details can be found on the website at www.evaluations-standards.org.uk. Contributions to the development of the documents can be made by contacting standards@hpa.org.uk.

The reader is informed that all taxonomy in this document was correct at time of issue.

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AMENDMENT PROCEDURE

| | |
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| Controlled document reference | BSOP TP 3 |
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Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

| Amendment Number/ Date | Issue no. Discarded | Insert Issue no. | Page | Section(s) involved | Amendment |
|------------------------|---------------------|------------------|------|-----------------------|--|
| 2/ 11.03.2010 | 1 | 2 | | Whole document | Document reviewed, no updates required |

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AGGLUTINATION TEST

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^{2,3}.

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 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 interact⁴. 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.

AGGLUTINATION TEST

1 SAFETY CONSIDERATIONS⁵⁻¹⁰

Most *Salmonella*, *Shigella* and *Escherichia* species are Hazard Group 2 with important exceptions including *Salmonella* Typhi, *Salmonella* 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 HPA immunisation policy.

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

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 75 mm by 1 cm

Dreyer's tubes H antigen

AGGLUTINATION TEST

Quality control organisms for tube and slide agglutinations

Positive control: homologous organism to the antiserum

Negative control: organism in saline only

3 QUALITY CONTROL ORGANISMS

N/A

4 PROCEDURE AND RESULTS¹¹

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 4-5 hours
- Dilute each suspension in formol saline so that there are approximately 10⁹ bacteria/mL (McFarland Standard)

4.1.1. PREPARATION OF O SUSPENSIONS

- Steam the O antigen broth culture for 30 minutes
- 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

4.2 MICROTITRE TRAY TESTS

- Add 25 µL of saline to all 8 wells in a column in a microtitre tray
- Add 25 µL of an anti serum to the top well and double dilute down to well 7. Discard the excess 25 µL from well 7
- Well 8 contains saline only as an antigen control.
- Add 25 µL of respective O or H diluted antigen to all wells. Seal the microtitre plate.

The final dilutions are:

| | | | | | | | |
|------------------|------|------|------|------|-------|-------|---|
| Well: | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Dilution: | 1/10 | 1/20 | 1/40 | 1/80 | 1/160 | 1/320 | 0 |

- 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 2 hours before examining for agglutination.

Positive result: agglutination of the suspension

Negative result: suspension remains turbid

Antigen control well: suspension remains turbid

AGGLUTINATION TEST

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 0.4 mL of saline to tubes 2 and 7
- Add 0.2 mL of 1/5 anti-serum to tubes 1 and 2. Mix the contents of tube 2 and perform doubling dilutions to tube 6 and then discard 0.2 mL 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:

| | | | | | | | |
|-----------------|------|------|------|------|-------|-------|---|
| Tube | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Dilution | 1/10 | 1/20 | 1/40 | 1/80 | 1/160 | 1/320 | 0 |

- Incubate tests with O suspensions in a water-bath at 37°C for 4 h, 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 h 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

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5 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology (http://www.hpa-standardmethods.org.uk/wg_bacteriology.asp). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

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For further information please contact us at:

Standards Unit
Department for Evaluations, Standards and Training
Centre for Infections
Health Protection Agency
Colindale
London
NW9 5EQ

E-mail: standards@hpa.org.uk

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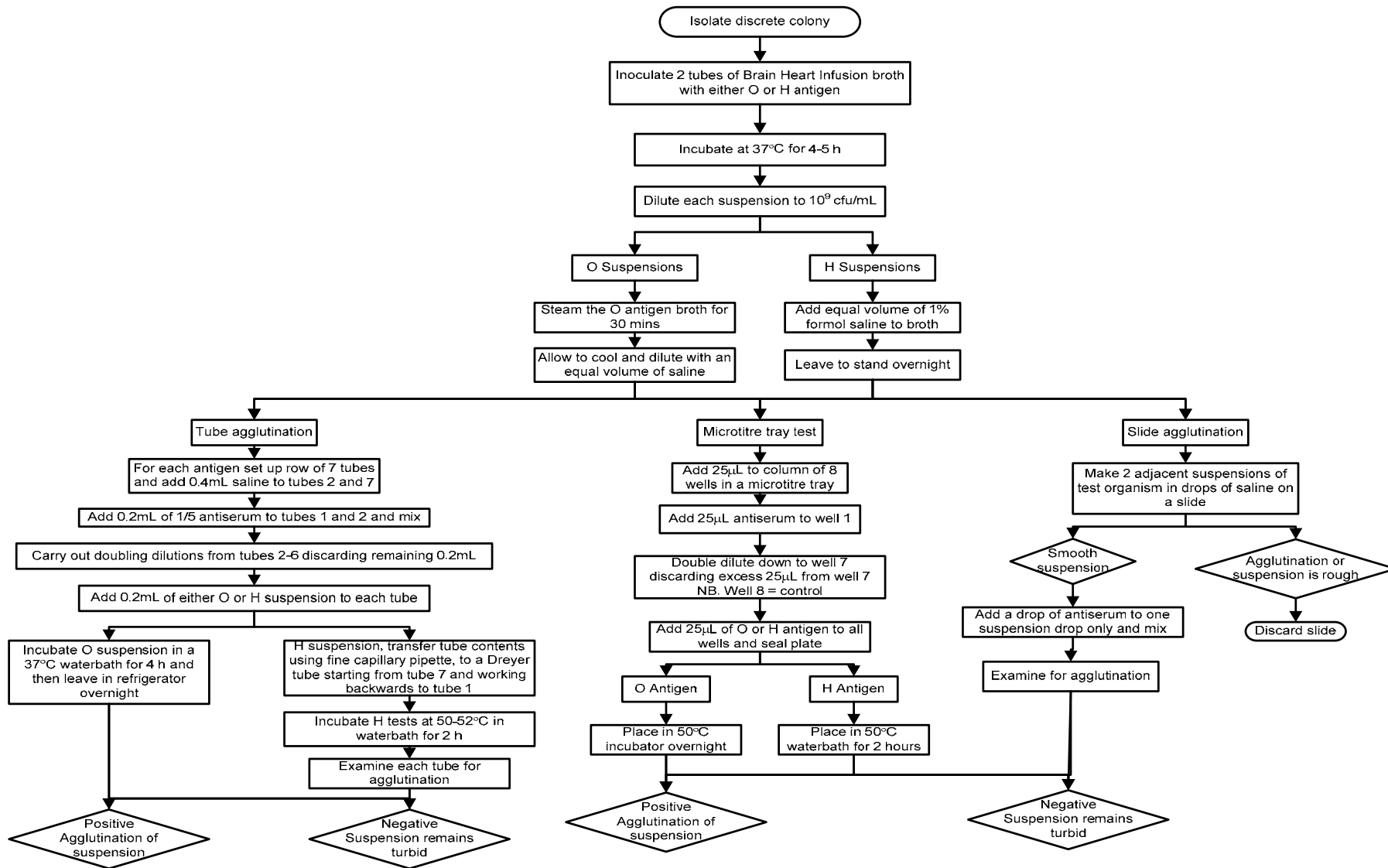
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APPENDIX: AGGLUTINATION TEST FLOWCHART



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