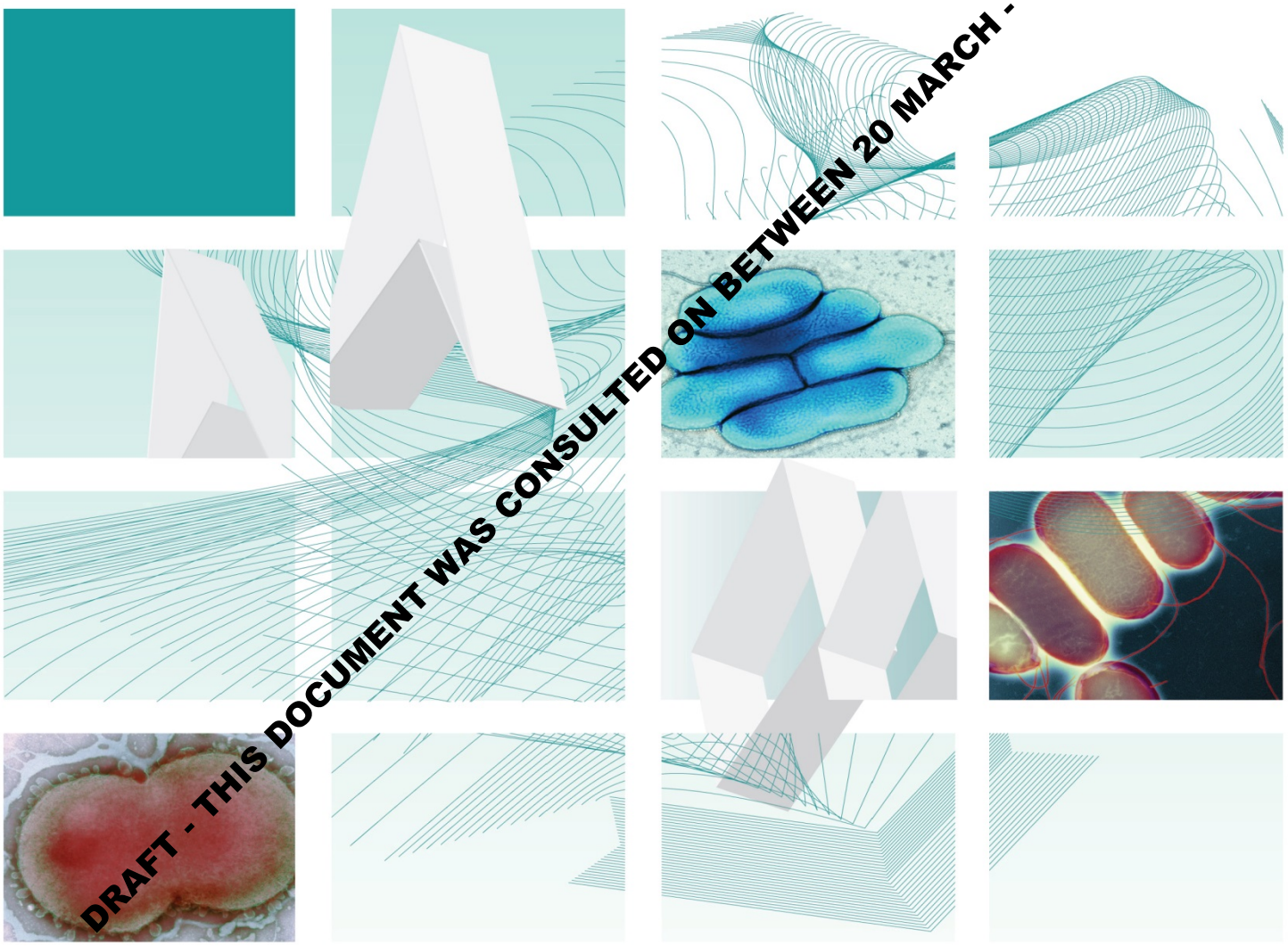
Public Health England

NHS

UK Standards for Microbiology Investigations

Agglutination test for *Salmonella* species



NICE accredited
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"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.**"

Issued by the Standards Unit, National Infection Service, PHE

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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UK Standards for Microbiology Investigations are produced in association with:

Logos correct at time of publishing.

Contents

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	
Issue number discarded	
Insert issue number	
Anticipated next review date*	
Section(s) involved	**Amendment**
Whole document	Document updated. Technical limitations/information updated with subheadings. Picture added to show positive and negative agglutination.
References	References updated and graded.

*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, 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

[#] 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.

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

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 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. They are equally expensive due to the number of dilutions and large amounts of antigen required.

Slide agglutination tests are simple to use, requires no equipment and is rapid.

Microtitre trays may be used as they are easier to perform, saves time and space as well as reduces the volume of antisera used[4,5].

Technical information/limitations

Interpretation of results

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

For slide agglutinations, growth on 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 a weak reaction is encountered in a slide agglutination assay, it is recommended that this should be confirmed with a tube agglutination assay[6].

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. If using commercially manufactured antisera, check suitability of use for all methods. The limitation to these commercially manufactured agglutination preparations is that they have limited shelf lives that place increased demands on procurement and distribution systems.

1 Safety considerations[7-24]

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 test, 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

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[27]

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 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 Microtitre tray test procedure

- Add 25µL of saline to all 8 wells in a column in a microtitre tray
- Add 25µL of an 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:

Well:	1	2	3	4	5	6	7	8
Dilution:	1/10	1/20	1/40	1/80	1/160	1/320	1/640	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 2hr before examining for agglutination

Positive Result

Agglutination of the suspension.

Negative Result

Suspension remains turbid.

Antigen control well

Suspension remains turbid.

Note: Care must be taken to avoid shaking of the microtitre plates during and after incubation to allow settling of the antigen.

4.3 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

4.4 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:

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 4-6hr, 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
- 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

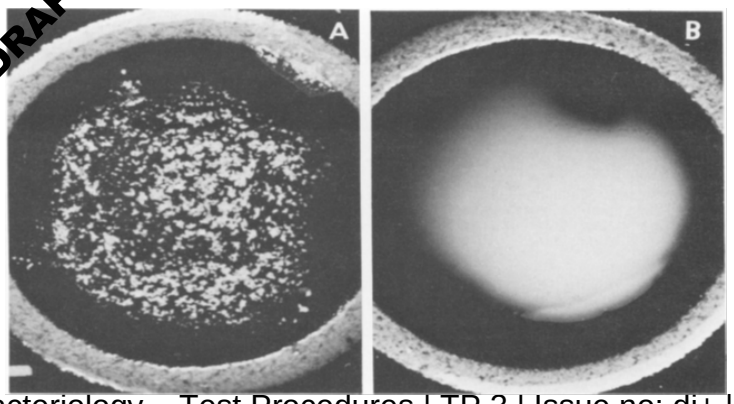
Agglutination of the suspension

Negative Result

Suspension remains turbid

Antigen control tube

Suspension remains turbid

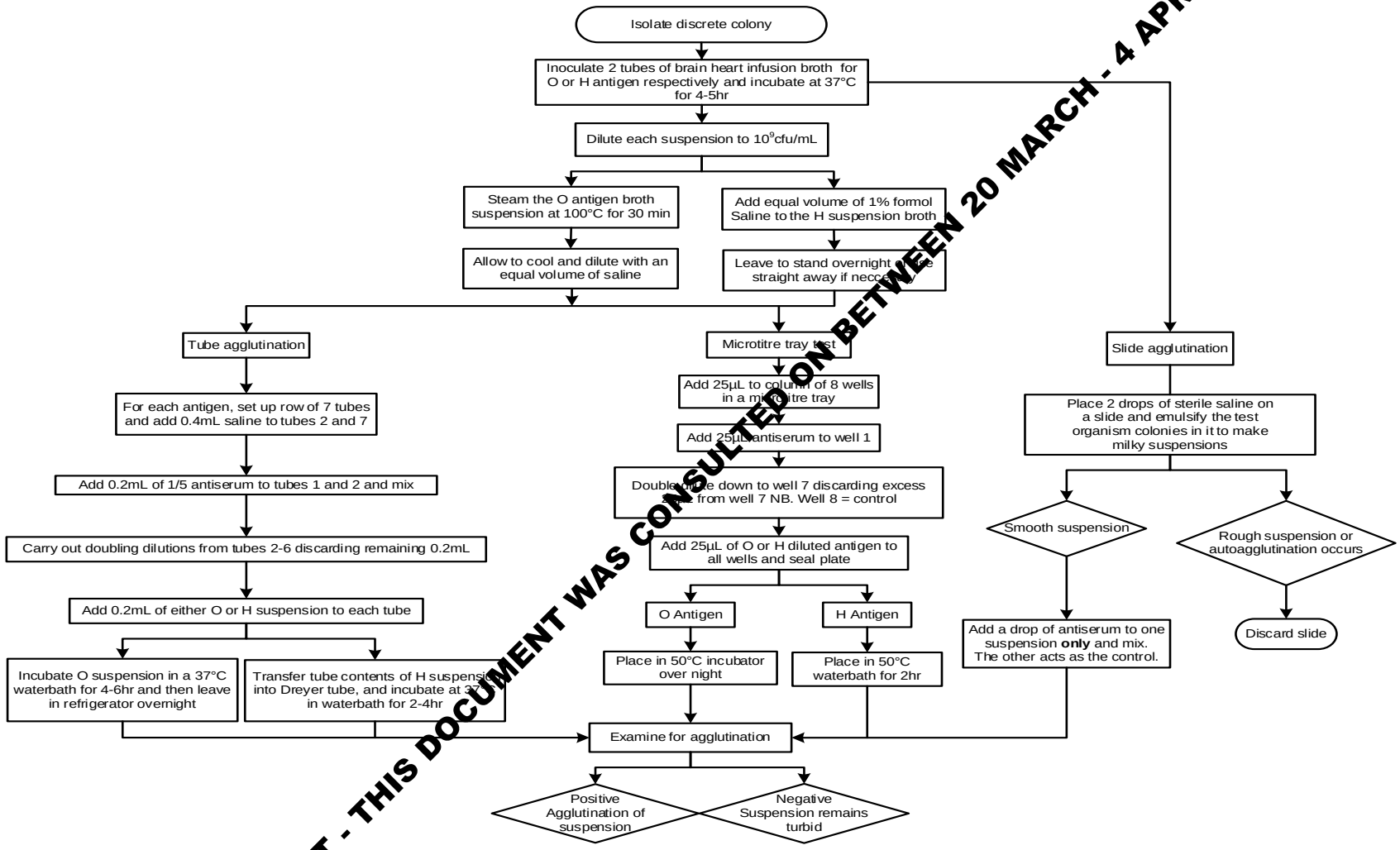


A. Positive agglutination reaction

B. Negative agglutination reaction

(Adapted from Smith, SK et al[28].)

Appendix: Agglutination test for *Salmonella* species



The flowchart is for guidance only.

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-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation	Quality 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
C Weakly recommended: seek alternatives	III Non-analytical studies, for example, case reports, reviews, case series
D Never recommended	IV Expert opinion and wide acceptance as good practice but with no study evidence
	V Required by legislation, code of practice or national standard
	VI Letter or other

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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, V**

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