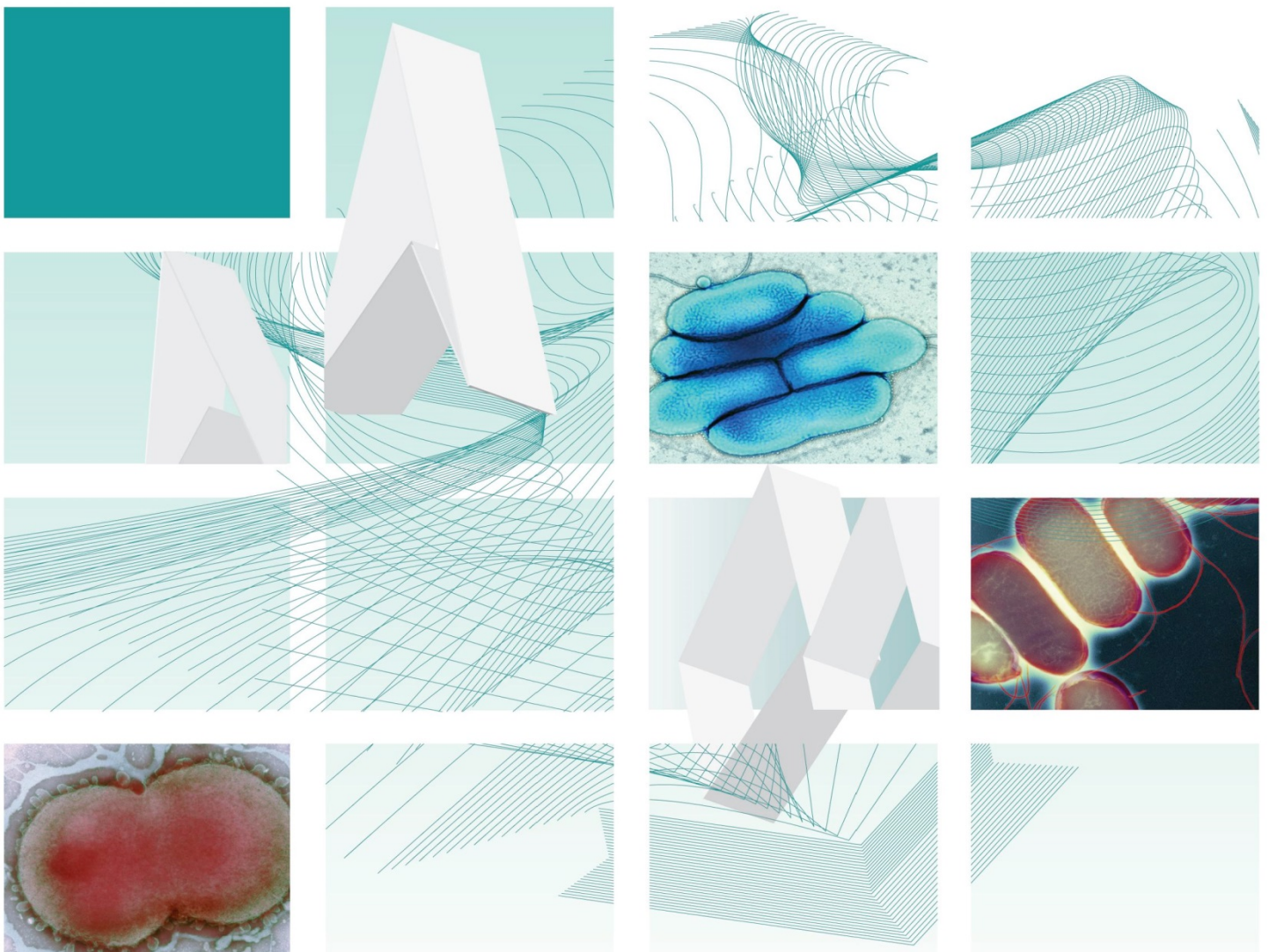




Protecting and improving the nation's health

# UK Standards for Microbiology Investigations

## Identification of *Salmonella* species



## 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 <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. 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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PHE Publications gateway number: 2015013

UK Standards for Microbiology Investigations are produced in association with:



Logos correct at time of publishing.

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NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

For full details on our accreditation visit: [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

## 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](mailto:standards@phe.gov.uk).

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	6/25.06.15
Issue no. discarded.	2.3
Insert Issue no.	3
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	Hyperlinks to gov.uk added.
Page 2.	Updated logos added.
Introduction.	The taxonomy of <i>Salmonella</i> species has been updated. More information has been added to the Characteristics section. The medically important species of <i>Salmonella</i> are mentioned. Section on Principles of Identification has been updated to include the MALDI-TOF.
Technical information/limitations.	Addition of information regarding quality control, commercial identification systems and MALDI-TOF MS.
Safety considerations.	This section has been updated on the handling of <i>Salmonella</i> species as well as laboratory acquired infections.
Target organisms.	The section on the Target organisms has been updated and presented clearly.
Identification.	Updates have been done on 3.2, 3.3 and 3.4 to reflect standards in practice. Section 3.4.2, 3.4.3 and 3.4.4 has been updated to include Commercial Identification Systems, MALDI-TOF MS and NAATs with references. Subsection 3.5 has been updated to include the Rapid Molecular Methods.
Identification flowchart.	Modification of flowchart for identification of <i>Salmonella</i> species has been done for easy guidance.

Reporting.	Subsection 5.3 has been updated to reflect the information required on reporting practice.
Referral.	The addresses of the reference laboratories have been updated.
References.	Some references updated.

## UK Standards for Microbiology Investigations<sup>#</sup>: scope and purpose

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

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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<sup>#</sup>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 <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>. 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

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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.

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**Suggested citation for this document**

Public Health England. (2015). Identification of *Salmonella* species. UK Standards for Microbiology Investigations. ID 24 Issue 3. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>



## Scope of document

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This SMI describes the identification of *Salmonella* species. The majority of Salmonellae are isolated from faeces but the organism may be isolated from other specimens such as blood, bone marrow and urine.

This SMI should be used in conjunction with other SMIs.

## Introduction

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### Taxonomy

There are currently 2 validly published species and 6 subspecies. They are *Salmonella bongori*, *Salmonella enterica* and its subspecies - *Salmonella enterica* subspecies *arizonae*, *Salmonella enterica* subspecies *diarizonae*, *Salmonella enterica* subspecies *enterica*, *Salmonella enterica* subspecies *houtenae*, *Salmonella enterica* subspecies *indica* and *Salmonella enterica* subspecies *salamae*<sup>1</sup>.

All validly published names, even if they are not cited in the above list can still be used by bacteriologists. These include *Salmonella* Enteritidis, *Salmonella* Paratyphi, *Salmonella* Typhi and *Salmonella* Typhimurium (even though they have been classed as serotypes). *Salmonella subterranean* was recently removed from the genus because it is closely related to *Escherichia hermannii* and does not belong to the genus *Salmonella*<sup>1</sup>.

The nomenclature adopted in this SMI follows the advice from the Judicial Commission of the International Committee on Systematics of Prokaryotes<sup>2</sup>. It is likely however, that laboratories will continue to report serotypes as species for some time to come<sup>3</sup>.

### Characteristics

Cells are rod-shaped, non-spore-forming, and predominantly motile by means of peritrichous flagella with diameters of around 0.7-1.5µm and lengths of 2-5µm with a few exceptions. On blood agar, colonies are 2-3mm in diameter. Colonies are generally lactose non-fermenters. They obtain their energy from oxidation and reduction reactions using organic sources, and are facultative anaerobes. They produce acid from glucose usually with the production of gas, and are oxidase negative<sup>3</sup>. Most produce hydrogen sulphide except *Salmonella* Paratyphi A and *Salmonella* Typhi, which is a weak producer. They are identified with a combination of serological and biochemical tests.

*Salmonella* species are classified and identified into serotypes according to the White-Kauffmann-Le Minor scheme; there are more than 2,500 *Salmonella* serotypes that have been described and reported<sup>4,5</sup>. Presently, new serotypes are being discovered each year, adding to the complexity of this large bacterial population. Primary subdivision is into "O" serogroups (those which share a common somatic antigen), and these are then subdivided on the basis of "H" (flagella) antigens<sup>4</sup>. Strains of *Salmonella* Typhi may produce Vi antigen, which is an acidic polysaccharide layer outside the cell wall. When fully developed, it renders the bacteria agglutinable with Vi antiserum and inagglutinable with "O" antiserum. Antigens similar to Vi may also be found in some strains of *Salmonella* Paratyphi C and *Salmonella* Dublin.

All *Salmonella* serotypes are considered potentially pathogenic. Some serotypes are host-specific, but the majority can affect different hosts. Most *Salmonella* serotypes possess two phases of H (flagellar) antigens: 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 a Craigie's tube or another medium to enhance motility before the H antigens can be detected<sup>6,7</sup>.

*Salmonella* Enteritidis and *Salmonella* Typhimurium are the two dominant serotypes of salmonellosis transmitted from animals to humans in most parts of the world. *Salmonella* Typhi and *Salmonella* Paratyphi A, B and C are the most common causes of enteric fever in humans.

*Salmonella* species are found in faeces, blood, bile, urine, food and feed and environmental materials.

The type species is *Salmonella enterica*.

## Principles of identification

Isolates are identified by a combination of colonial appearance, serology (agglutination with specific antisera) and biochemical testing. If confirmation of identification is required, isolates should be sent to the Reference Laboratory.

## Technical information/limitations

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### Quality control

If using commercially manufactured antisera, check suitability of use for all methods. Each new lot or shipment of antisera/commercial identification systems should be tested and validated for positive and negative reactivity using known control strains, ensuring it is fit for purpose. Laboratories must follow manufacturer's instructions when using these products.

### Agglutination test

*Salmonella* species should agglutinate with Polyvalent O antiserum. Some serotypes eg *Salmonella* Typhi may produce a Vi antigen, which can prevent agglutination with Polyvalent O antiserum. Not all O serotypes are included in Polyvalent O antisera. H antigens may not be well developed on some solid agars and so sub-culturing onto a semi-solid agar may be necessary. Where results are inconclusive, it may be necessary to perform additional biochemical tests<sup>6,7</sup>.

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.

### MALDI-TOF MS

As a tool for subspecies and serovar typing of *Salmonella*, MALDI-TOF MS shows significant promise but will require additional studies and modifications to existing protocols before the method can be used as a stand-alone mechanism<sup>8,9</sup>.

# 1 Safety considerations<sup>10-26</sup>

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

*S. Typhi*, and *S. Paratyphi* A, B & C cause severe and sometimes fatal disease. The infectious dose varies with the serotype. Laboratory acquired infections have been reported- until 1974, 258 cases and 20 deaths due to laboratory-acquired typhoid fever were reported. Forty eight cases of salmonellosis were reported until 1976. Sixty four cases and 2 deaths due to *Salmonella* species infections were reported between 1979 and 2004, most of them associated with *S. Typhi*<sup>27-29</sup>. *S. Typhi* vaccines are available; guidance is available from the Department of Health<sup>30</sup>.

Refer to current guidance on the safe handling of all Hazard Group 2 organisms documented in this SMI.

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.

Laboratory procedures that give rise to infectious aerosols must be conducted 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 and should be verified.

## 2 Target organisms

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### Most common serotypes of *Salmonella* isolated and known to cause infections in humans

*Salmonella* Enteritidis (1,9,12:g, m:-), *Salmonella* Typhimurium (1,4,5, 12:i: 1,2), *Salmonella* Virchow (6,7:r: 1,2), *Salmonella* Hadar (6,8:z10: e, n, x), *Salmonella* Heidelberg (1,4,5,12:r: 1,2), *Salmonella* Newport (6,8:e, h: 1,2), *Salmonella* Infantis (6,7:r: 1,5), *Salmonella* Agona (4,12:f, g, s:-), *Salmonella* Paratyphi A (1,2,12:a: 1,2), *Salmonella* Paratyphi B (1,4,5,12:b: 1,2), *Salmonella* Paratyphi C (6,7,Vi: c: 1,5), *Salmonella* Typhi (9,12,Vi: d:-)

## 3 Identification

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### 3.1 Microscopic appearance

Gram stain [TP 39 - Staining procedures](#)

Gram negative rods

### 3.2 Primary isolation media

Blood agar incubated in 5-10% CO<sub>2</sub> at 35–37°C for 18-24hr.

Cystine-lactose-electrolyte deficient (CLED) agar incubated in air at 35–37°C for 18-24hr.

Xylose-lysine-desoxycholate agar (XLD) agar incubated in air at 35–37°C for 18-24hr.

Desoxycholate citrate (DCA) agar incubated in air at 35–37°C for 18-24hr.

Brilliant Green agar (BGA) incubated in air at 35–37°C for 18-24hr.

Other commercial validated media may be used.

### 3.3 Colonial appearance

Blood agar - Colonies are moist and 2-3mm in diameter.

CLED agar - *Salmonella* species are non-lactose fermenters (some serotypes eg *Salmonella* Arizonae and *Salmonella* Indiana may ferment lactose).

XLD agar – Colonies are red, and usually with a black centre (some serotypes eg *Salmonella* Paratyphi A and *Salmonella* Typhi may not produce a black centre).

DCA agar - Colonies are colourless, and usually with a black centre (some serotypes eg *Salmonella* Paratyphi A and *Salmonella* Typhi may not produce a black centre).

BGA agar - Colonies appear as red-pink, 1-3mm in diameter, surrounded by brilliant red zones in the agar.

### 3.4 Test procedures

#### 3.4.1 Agglutination

**Agglutination Test for *Salmonella* species** ([TP 3 - Agglutination test for \*Salmonella\* species](#))

*Salmonella* species should agglutinate with Polyvalent O antiserum. Some serotypes eg *Salmonella* Typhi may produce a Vi antigen, which can prevent agglutination with Polyvalent O antiserum. Not all O serotypes are included in Polyvalent O antisera. H antigens may not be well developed on some solid agar and so sub-culturing onto a semi-solid agar may be necessary. Where results are inconclusive, it may be necessary to perform additional biochemical tests<sup>6,7</sup>.

The following limited ranges of antisera are adequate for routine use:

Polyvalent O

Single factor O (2, 4, 6:7, 8, 9, 3:10)

Polyvalent H

Rapid H sera (RSD 1, 2, 3)

Polyvalent H phase 2 (1-7)

Single factor H (a, b, c, d, E, G, i, r)

**Changing the Phase of *Salmonella*** ([TP 32 - Changing the phase of \*Salmonella\*](#)) - optional

The majority of serotypes of *Salmonella* possess two phases of H (flagellar) antigens - phase 1, phase 2 or may have both phases simultaneously. If agglutination is obtained with only one phase, the organism may be induced to change to the other

phase. Some serotypes eg *Salmonella* Typhi and *Salmonella* Montevideo have only one phase and so these should be sent to the Reference Laboratory<sup>6,7</sup>.

### 3.4.2 Biochemical tests

#### Urease [TP 36 - Urease test](#)

*Salmonella* species do not produce urease

#### Oxidase [TP 26 - Oxidase test](#)

*Salmonella* species are oxidase negative

#### Indole Test [TP19 – Indole test](#)

*Salmonella* species are indole negative

### Commercial identification systems

Many rapid confirmation and identification methods have been developed for *Salmonella* and a large number have been developed into commercial products.

Biochemical confirmation can be accomplished using commercial identification systems.

Rapid immunological identification and confirmation tests based on latex agglutination, enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) have been developed for *Salmonella*, and simple-to-use lateral flow test strips using immunochromatographic technology have also been developed into commercial products by a number of manufacturers.

Laboratories should follow manufacturer's instructions and rapid tests and kits should be validated and be shown to be fit for purpose prior to use.

### 3.4.3 Matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF MS)

Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS), which can be used to analyse the protein composition of a bacterial cell, has emerged as a new technology for species identification. This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis. The advantage of MALDI-TOF as compared with other identification methods is that the results of the analysis are available within a few hours rather than several days. The speed and the simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high-throughput use<sup>31</sup>.

This has been utilised to aid in both the detection and species-level identification of *Salmonella*. It has also been used in discriminating *S. enterica* serovar Typhi from other *Salmonella* serovars. As a tool for subspecies and serovar typing, MALDI-TOF MS shows significant promise but will require additional studies and modifications to existing protocols before the method can be used as a stand-alone mechanism<sup>8,9</sup>.

### 3.4.4 Nucleic acid amplification tests (NAATs)

PCR is usually considered to be a good method for bacterial detection as it is simple, rapid, sensitive and specific. The basis for PCR diagnostic applications in microbiology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes. However, it does have limitations.

Although the 16S rRNA gene is generally targeted for the design of species-specific PCR primers for identification, designing primers is difficult when the sequences of the homologous genes have high similarity.

This has been used successfully in the identification of *Salmonella* subspecies I, *Salmonella enterica* serovars Typhimurium, Typhi and Enteritidis as well as *Salmonella enterica* subspecies *arizonae* and *diarizonae* (rapidly and accurately without the need for serological testing)<sup>5,32-34</sup>.

Multiplex PCR may sometimes be complex and lack reproducibility between laboratories because of the specific conditions needed for simultaneous amplification of several regions. However, more evaluation needs to be done with varieties of *Salmonella* serovars in conjunction with other laboratories in order to demonstrate accuracy of *Salmonella* identification in epidemiological and taxonomical studies<sup>5,35</sup>.

### 3.5 Further identification

#### Rapid molecular methods

Molecular methods have had an enormous impact on the taxonomy of *Salmonella*. Analysis of gene sequences has increased understanding of the phylogenetic relationships of *Salmonella* and related organisms; and has resulted in the recognition of numerous new species. Molecular techniques have made identification of many species more rapid and precise than is possible with phenotypic techniques.

A variety of subtyping methods have been developed for isolates from clinical samples; these include molecular techniques such as Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Multiple-Locus Variable Number Tandem Repeat Analysis (MVLA), SNP assays and Whole Genome Sequencing (WGS). All of these approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility.

However, some of these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

#### Pulsed field gel electrophoresis (PFGE)

PFGE detects genetic variation between strains using rare-cutting restriction endonucleases, followed by separation of the resulting large genomic fragments on an agarose gel. PFGE is known to be highly discriminatory and a frequently used technique for outbreak investigations and has gained broad application in characterizing epidemiologically related isolates. However, the stability of PFGE may be insufficient for reliable application in long-term epidemiological studies. However, due to its time-consuming nature (30hr or longer to perform) and its requirement for special equipment, PFGE is not used widely outside the reference laboratories<sup>36,37</sup>.

This has been used successfully to identify and discriminate between species of the family *Enterobacteriaceae* – for example, it has been used in tracking the source of *Salmonella* infections for different serotypes and is considered the gold standard for *Salmonella* molecular typing<sup>4,38</sup>.

#### Multilocus sequence typing (MLST)

MLST measures the DNA sequence variations in a set of housekeeping genes directly and characterizes strains by their unique allelic profiles. The principle of MLST is simple: the technique involves PCR amplification followed by DNA sequencing.

Nucleotide differences between strains can be checked at a variable number of genes

depending on the degree of discrimination desired. The technique is highly discriminatory, as it detects all the nucleotide polymorphisms within a gene rather than just those non-synonymous changes that alter the electrophoretic mobility of the protein product. One of the advantages of MLST over other molecular typing methods is that sequence data are portable between laboratories and have led to the creation of global databases that allow for exchange of molecular typing data via the Internet<sup>39</sup>.

MLST has been extensively used as the one of the main typing methods for analysing the genetic relationships within the *Enterobacteriaceae* population especially the genus *Salmonella*<sup>4,38</sup>. MLST was found to provide better discrimination of *Salmonella* serotype Enteritidis strains than PFGE and to differentiate accurately outbreak strains and clones of the *Salmonella* serovars most commonly associated with human disease. It has also been useful for typing non-typhoidal *Salmonella* strains<sup>40</sup>.

The drawbacks of MLST are the substantial cost and laboratory work required to amplify, determine, and proofread the nucleotide sequence of the target DNA fragments, making the method hardly suitable for routine laboratory testing.

### **Multiple-locus variable number tandem repeat analysis (MVLA) also known as “VNTR”**

Multiple-Locus Variable number tandem repeat Analysis (MLVA) is a method used to perform molecular typing of particular microorganisms. It utilizes the naturally occurring variation in the number of tandem repeated DNA sequences found in many different loci in the genome of a variety of organisms. The molecular typing profiles are used to study transmission routes, to assess sources of infection and also to assess the impact of human intervention such as vaccination and use of antibiotics on the composition of bacterial populations.

This has been used successfully in the subtyping of *Salmonella enterica* subsp *enterica* serovar Typhimurium, Enteritidis, Typhi, Infantis, Newport, Paratyphi A, Saintpaul, and Gallinarum isolates<sup>4,40-42</sup>. The method has proven very useful for detecting and investigating outbreaks, since it has the capacity to differentiate closely related strains. It is technically simple and inexpensive to perform. However, it has no usefulness for serovar assignment or for global phylogenetic studies because the scope of each MLVA is commonly restricted to a unique serovar.

### **Whole genome sequencing (WGS)**

This is also known as “full genome sequencing, complete genome sequencing, or entire genome sequencing”. It is a laboratory process that determines the complete DNA sequence of an organism's genome at a single time. There are several high-throughput techniques that are available and used to sequence an entire genome such as pyrosequencing, nanopore technology, Illumina sequencing, Ion Torrent sequencing, etc. This sequencing method holds great promise for rapid, accurate, and comprehensive identification of bacterial transmission pathways in hospital and community settings, with concomitant reductions in infections, morbidity, and costs.

This technique has equally been used to characterise *Salmonella enterica* serovar Typhi and to discover its recently acquired genes, such as those encoding the Vi antigen, by horizontal transfer events and it has provided new insights into how this pathogen has evolved to cause invasive disease in humans<sup>38</sup>.

***rpoB* Single nucleotide polymorphism (*rpoB* SNP) assay**

*rpoB* gene is a single-copy chromosomal gene encoding the RNA polymerase  $\beta$ -subunit. This gene has been previously used in phylogenetic analysis for bacterial species and genus delineation, since it is highly conserved across organisms<sup>43</sup>. However, the 16SrRNA gene has been used widely and its usefulness has been greatly enhanced through the establishment of public domain databases but its sensitivity has been questioned particularly among *Enterobacteriaceae* and so when the *rpoB* gene was used as an alternative for detection based on a Single Nucleotide Polymorphism, it was found to be more compatible with the currently accepted classification of *Enterobacteriaceae* and a powerful identification tool which may be useful for universal bacterial identification.

This has been used to detect *Salmonella enterica* serovar Typhimurium<sup>35</sup>.

The distinct advantage of SNP and other nucleotide sequence-based methods over profile-generating methods is that genetic relationships can be established on the basis of discrete data that are directly suitable for biocomputing and statistical analysis<sup>4</sup>.

**Other specialized tests****Phage typing**

Phage typing has proven to be an important tool for strain characterisation and the results obtained have been used in surveillance, source attribution and outbreak investigations. Phage typing is, however, also a phenotypical method that depends very much on the experience of the individual laboratory and on support from the reference centre that coordinates the maintenance of phages and the updating of the system. It is only when the phage typing method is harmonised and the performance in different laboratories is controlled, can the results be regarded as definitive and comparable between laboratories<sup>44</sup>. However, it will remain for some time as a useful tool to strengthen global *Salmonella* surveillance.

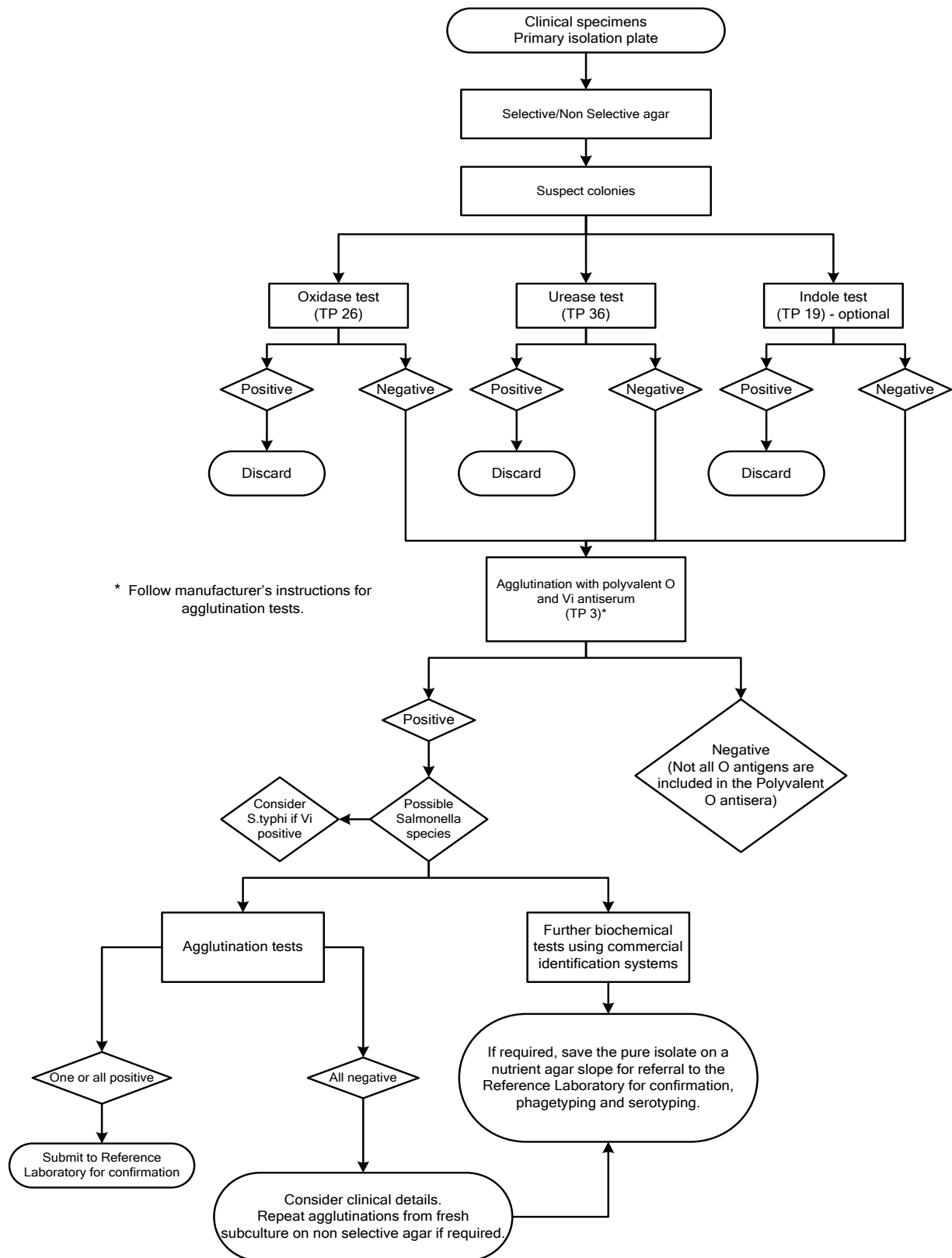
This technique has also been used successfully in the characterisation of several clusters of *Salmonella* Typhimurium<sup>44</sup>.

**3.6 Storage and referral**

If required, save the pure isolate on nutrient agar slopes for referral to the Reference Laboratory.



## 4 Identification of *Salmonella* species



The flowchart is for guidance only.

**Note:** Short biochemical screen panels could be used.

## 5 Reporting

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### 5.1 Presumptive identification

If appropriate growth characteristics, colonial appearance, urease and serology results are demonstrated.

### 5.2 Confirmation of identification

Further biochemical tests and/or molecular methods and/or reference laboratory report.

### 5.3 Medical microbiologist

According to local protocols inform the medical microbiologist at least of all positive cultures from sites normally sterile and of all presumptive or confirmed *Salmonella* Typhi and *Salmonella* Paratyphi isolates.

The medical microbiologist should be informed of a presumptive or confirmed *Salmonella* species when the request bears relevant information for example,

- pyrexia/fever of unknown origin (PUO, FUO)
- septicaemia
- enterocolitis, especially with ulceration and possible perforation of the bowel
- rose spots
- history of substance abuse, alcoholism, immunodeficiency or other serious underlying disorder, such as cancer or persons receiving treatment for cancer, inducing neutropenia and/or mucositis
- laboratory work
- food handler
- investigation of outbreaks or carrier state

Follow local protocols for reporting to clinician.

### 5.4 CCDC

Refer to local Memorandum of Understanding.

### 5.5 Public Health England<sup>45</sup>

Refer to current guidelines on CIDSC and COSURV reporting.

### 5.6 Infection prevention and control team

Inform the infection prevention and control team of presumptive and confirmed isolates of *Salmonella* species.

## 6 Referrals

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### 6.1 Reference laboratory

Contact appropriate devolved national reference laboratory for information on the tests available, turn around times, transport procedure and any other requirements for sample submission:

Gastrointestinal Bacteria Reference Unit  
Bacteriology Reference Department  
Public Health England  
61 Colindale Avenue  
London  
NW9 5EQ

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

England and Wales

<https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services>

Scotland

<http://www.hps.scot.nhs.uk/reflab/index.aspx>

Northern Ireland

<http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm>

## 7 Notification to PHE<sup>45,46</sup> or equivalent in the devolved administrations<sup>47-50</sup>

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The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

**Note:** The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAs) and Creutzfeldt–Jakob disease (CJD) under

'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

<https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010>

Other arrangements exist in [Scotland](#)<sup>47,48</sup>, [Wales](#)<sup>49</sup> and [Northern Ireland](#)<sup>50</sup>.

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