STATUS OF NATIONAL STANDARD METHODS

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 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 conditions².

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Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

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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.

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency.

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INTRODUCTION

Scope of document

This SOP 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.

Taxonomy

Serotypes of Salmonella and Arizona belong to the family Enterobacteriaceae and are now considered to belong to two species Salmonella bongori (formerly subspecies V) and Salmonella enterica\(^1,2,3\) (comprising six subspecies: I = enterica, II = salamae, IIIa = arizonae, IIIb = diarizonae, IV = houtenae, and VI = indica). Most (>99.5%) salmonella isolates from humans are serotypes of Salmonella enterica.

It is likely that laboratories will continue to report serotypes as species for some time to come\(^4\) and this is the approach adopted in this SOP.

Characteristics

Salmonella species are Gram-negative rods. On blood agar, colonies are 2-3 mm in diameter. Colonies are generally lactose non-fermenters. Salmonella species are motile (with a few exceptions), facultatively anaerobic, produce acid from glucose usually with the production of gas, and are oxidase-negative\(^5\). Most produce hydrogen sulphide except Salmonella typhi and Salmonella paratyphi A, 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 Kauffmann-White scheme\(^6\), which currently contains in excess of 2000 serotypes. 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\(^6\). 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 by “O” antiserum\(^7\). Antigens similar to Vi may also be found in some strains of Salmonella paratyphi C and Salmonella dublin.

Laboratory acquired infections of salmonella including S. typhi have been reported\(^8\).

Principles of identification

Isolates from culture are identified using 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.
1.0 SAFETY CONSIDERATIONS

1.1 Most Salmonella species are in Hazard Group 2 with important exceptions including S. typhi and S. paratyphi A, B & C. All work on 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. Laboratory acquired infections have been reported. S. typhi vaccination is available; guidance is given in the PHLS immunisation policy.

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

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.

2.0 TARGET ORGANISMS

2.1 Commonest serotypes of Salmonella isolated in the UK (1998)

Salmonella enteritidis (1,9,12:g, m)
Salmonella typhimurium (1,4,5: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:-)

2.2 Serotypes of Salmonella which cause enteric fever

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:-)
3.0 IDENTIFICATION – TESTS AND TEST RESULTS

3.1 Primary isolation media

Blood agar incubated in air at 35-37°C for 18-24 hours
CLED agar incubated in air at 35-37°C for 18-24 hours
XLD agar incubated in air at 35-37°C for 18-24 hours
DCA incubated in air at 35-37°C for 18-24 hours

3.2 Colonial appearances

Colonies on blood agar are moist and 2-3 mm in diameter

*Salmonella* species on CLED agar are lactose non-fermenters (some serotypes eg *S. arizonae* and *S. indiana* may ferment lactose)

*Salmonella* species on XLD agar produce red colonies usually with a black centre (some serotypes eg *S. paratyphi* A and *S. typhi* may not produce a black centre)

Colonies on DCA are colourless usually with a black centre (some serotypes eg *S. paratyphi* A and *S. typhi* may not produce a black centre)

3.3 Microscopic appearance

**Gram stain** (BSOP SP 8)
Gram-negative rods

3.4 Test procedures

3.4.1 Agglutination

Agglutination with polyvalent O and H antiserum (BSOP TP 3)

*Salmonella* species should agglutinate with Polyvalent O antiserum. Some serotypes eg *S. 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. Subculture to semi-solid agar if necessary.

The following limited range 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)

3.4.2 Biochemical tests

**Urease** (BSOP TP 36)
*Salmonella* species do not produce urease

**Oxidase** (BSOP TP 26)
*Salmonella* species are oxidase-negative

Commercial identification kit
In-house identification kit
3.5 Storage and referral

If required, save the pure isolate on nutrient agar slopes for referral to the Reference Laboratory.
4.0 IDENTIFICATION OF SALMONELLA - GUIDANCE

Clinical specimen
Primary isolation plate (blood agar, CLED agar, XLD agar or DCA)
Blood agar - moist colonies 2-3 mm in diameter
CLED agar - non-lactose fermenter
XLD agar – red colonies usually with a black centre
DCA - colourless colonies usually with a black centre

Oxidase (optional)

Oxidase positive
Discard

Oxidase negative

Agglutination with polyvalent O and Vi antiserum (optional)*
Positive agglutination

Urease
(37°C for up to 4h in air)

CLED purity plate
(37°C for 18h in air)

Urease negative
Biochemical tests
CLED purity plate
(37°C for 18h in air)
Check pure culture
Interpret biochemical tests
Possible Salmonella spp
Not Salmonella spp
Discard

From CLED plate
Polyvalent O agglutination*
Polyvalent H agglutination*
Vi agglutination*

One or all +ve
All –ve†

Single factor O
(2,4,6,7,8,9,3,10)
Rapid H sera (RSD 1,2,3)
Polyvalent phase 2 (1-7)
Single factor H
(a, b, c, d, E, G, i, r)

* Follow manufacturer’s instructions for agglutination tests. Not all O antigens are included in the Polyvalent O antiserum
† Consider clinical details. Repeat agglutinations from fresh subculture on non-selective agar if required
If required, save pure isolate on a nutrient agar slope for referral to the Reference Laboratory for confirmation phagetyping and serotyping

The flow chart is for guidance only
5.0 INTERPRETATION AND REPORTING OF RESULTS

5.1 Presumptive identification may be made

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

5.2 Confirmation of identification may be made

Following use of commercial or in-house identification kit results and/or the Reference Laboratory report

5.3 To 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 S. typhi and S. paratyphi isolates

According to local protocols, the medical microbiologist should be informed of a presumptive or confirmed Salmonella species when the request card bears relevant information eg

- pyrexia/fever of unknown origin (PUO, FUO)
- septicaemia
- enterocolitis, especially with ulceration and possible perforation of the bowel
- features of the above, plus subacute neurological dysfunction / toxic confusional states or rash (“rose spots”)
- foreign travel
- urinary infection secondary to schistosomiasis
- 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 poisoning, especially involving unusual or imported foods
- food handler
- investigation of outbreak situations or carrier state

Follow local protocols for reporting to clinician

5.4 CCDC

Refer to local Memorandum of Understanding

5.5 CDSC²¹

Refer to current guidelines on CDSC and COSURV reporting

5.6 Infection control staff

Inform the infection control team of presumptive and confirmed isolates of Salmonella species
6.0 REFERRALS

6.1 Examination required

Confirmation of identity serotyping and phage typing

6.2 Transport procedure

Cultures should be sent on nutrient agar slopes

Compliance with postal and transport regulations is essential

6.3 Other requirements

Always notify the Reference Laboratory when sending urgent cultures

6.4 Reference Laboratory

Laboratory of Enteric Pathogens
Specialist and Reference Microbiology Division
Health Protection Agency
61 Colindale Avenue
London
NW9 5HT

Contact SRMD main switchboard: Tel. +44 (0) 20 8200 4400
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