NATIONAL STANDARD METHOD

IDENTIFICATION OF SALMONELLA SPECIES

BSOP ID 24

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

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IDENTIFICATION OF SALMONELLA SPECIES

SCOPE OF DOCUMENT
This National Standard Method (NSM) 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.

INTRODUCTION

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 (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.

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

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. 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 Kauffmann-White scheme, 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. 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. Antigens similar to Vi may also be found in some strains of Salmonella Paratyphi C and Salmonella Dublin.

Laboratory acquired infections of salmonella including Salmonella Typhi have been reported.

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
N/A
1 SAFETY CONSIDERATIONS

Most *Salmonella* species are in Hazard Group 2 with important exceptions including *Salmonella Typhi* and *Salmonella Paratyphi A, B & C*.

All work on *Salmonella Typhi* and *Salmonella Paratyphi A, B & C* must be performed under Containment level 3 conditions.

*Salmonella Typhi*, and *Salmonella Paratyphi A, B & C* cause severe and sometimes fatal disease. Laboratory acquired infections have been reported and a *Salmonella Typhi* vaccination is available.

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

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

Commonest serotypes of *Salmonella* isolated in the UK

- *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:-)

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 IDENTIFICATION

3.1 MICROSCOPIC APPEARANCE

Gram's stain (see BSOPTP 39 - Staining Procedures)

Gram-negative rods

3.2 PRIMARY ISOLATION MEDIA

Blood agar incubated in air at 35 – 37 ºC for 18 - 24 hours
CLED agar incubated in air at 35 – 37 ºC for 18 - 24 hours
XLD agar incubated in air at 35 – 37 ºC for 18 - 24 hours
DCA incubated in air at 35 – 37 ºC for 18 - 24 hours
3.3 **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 *Salmonella Arizonae* and *Salmonella Indiana* may ferment lactose).

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

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

3.4 **TEST PROCEDURES**

3.4.1 **AGGLUTINATION**

Agglutination with polyvalent O and H antiserum (see BSOP 3 - 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 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 (see BSOP 36 - Urease Test) *Salmonella* species do not produce urease

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

Commercial identification kit
In-house identification kit

3.5 **FURTHER IDENTIFICATION**

N/A

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* – FLOW CHART

Clinical specimens
Primary isolation plate

- Blood agar
- CLED agar
- XLD agar
- DCA

- Moist colonies
  2-3 mm in diameter
- Non-lactose fermenter
- Red colonies
  usually with a black centre
- Colourless colonies
  usually with a black centre

Oxidase test

- Positive
  - Discard
- Negative
  - Agglutination with polyvalent O and Vi antiserum
    - Positive
      - Urease
        - (37°C for up to 4 h in air)
        - CLED purity plate
          - (37°C for 18 h in air)
      - Urease negative
        - Biochemical tests
          - CLED purity plate
            - (37°C for 18 h in air)
          - Check pure culture
          - Interpret biochemical tests
            - Possible
              - *Salmonella* species
            - Not
              - *Salmonella* species

- Agglutination tests
  - from CLED plate *
  - One or all positive
    - Single factor O
      - (2, 4, 6, 7, 8, 9, 3, 10)
    - Rapid H sera (RSD 1, 2, 3)
    - Polyvalent phase 2 (1-7)
  - All negative
    - Consider clinical details.
      - Repeat agglutinations from fresh subculture on non-selective agar if required.
      - If required, save the pure isolate on a nutrient agar slope for referral to the Reference Laboratory for confirmation, phagetypeing and serotyping

* Follow manufacturer’s instructions for agglutination tests. Not all O antigens are included in the Polyvalent O antiserum.
5 REPORTING

5.1 PRESumptive IDENTIFICATION

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

5.2 CONFIRMATION OF IDENTIFICATION

Following use of commercial or in-house identification kit results and/or the 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.

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 outbreaks or carrier state

Follow local protocols for reporting to clinician

5.4 CCDC

Refer to local Memorandum of Understanding.

5.5 CENTRE FOR INFECTIONS

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 REFERRALS

6.1 REFERENCE LABORATORY

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory refer to: http://www.hpa.org.uk/cfi/lep/default.htm

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7 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.

The National Standard Methods are issued by Standards Unit, Evaluations and Standards Laboratory, Centre for Infections, Health Protection Agency, London.

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REFERENCES


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