1. Introduction

There are two distinct syndromes caused by *Salmonella*:

A. Typhoid and paratyphoid fevers (enteric fevers)

B. Gastroenteritis/salmonellosis

These will be discussed as separate entities in this document since there are differences in both laboratory diagnosis and clinical manifestations of the infections.

- **Typhoid fever**: Causative organism *Salmonella* Typhi

- **Paratyphoid Fevers**: Causative organisms *Salmonella* Paratyphi A and *Salmonella* Paratyphi B

The term 'enteric fever', coined to embrace both typhoid and paratyphoid, has been defined (Levine et al. 1983) as 'a generalized infection of the reticuloendothelial system and intestinal tissue accompanied by sustained fever and bacteraemia'. The definition encompasses a range of severity, with typhoid tending to the severe, and paratyphoid to the milder, parts of the spectrum. However, each may range between an asymptomatic infection and a fatal illness. In 1998 there were 7, 32 and 3 notifications of S.Typhi, S.Paratyphi A and S.Paratyphi B respectively (Anonymous, 1999). The majority of infections were acquired by Australians travelling outside Australia.

**Gastroenteritis/salmonellosis**

Gastroenteritis is caused by salmonellae (over 2300 serovars/types), other than S.Typhi, and the Paratyphii. Extraintestinal infections, particularly blood stream infections, may occur. In 1998, of the 5982 cases of Australian acquired salmonellosis notified to the National Enteric Pathogens Surveillance Scheme, 5719, 131, 100 and 32 were from faeces, blood, urine and unusual sites respectively (Anonymous, 1999).
2. Laboratory diagnosis /tests

2.1 Culture for *Salmonella* Species

While salmonellae are classed as non fastidious organisms, because of the nature of the specimens for culture, Salmonella detection is still highly dependent on employing appropriate culture media. Variation between the serovars of *Salmonella enterica* requires the use of more than a single medium.

2.1.1 Media

There are many different plating media available to isolate salmonellae from faeces/rectal swabs. These media are differential and may vary from slightly selective to highly selective eg.

- Low selectivity - MacConkey (MAC), eosin methylene blue (EMB)
- Intermediate selectivity - xylose-lysine-desoxycholate (XLD), desoxycholate citrate agar (DCA), Salmonella Shigella agar (SS) and hektoen enteric agar (HE)
- Highly selective - bismuth sulphite agar (BS) and brilliant green agar (BG)
- BS, XLD and HE all have H$_2$S indicator systems, which are necessary for the detection of lactose-positive Salmonella.

Most laboratories use either HE or XLD because both of these media may also be used for the isolation of Shigella, together with a slightly selective medium such as MAC ( Murray et al., 1999)

- For the investigation of a typhoid outbreak or follow up of cases or screening of carriers BS would be the most suitable medium to use.
- Maximal recovery of Salmonella from faeces/rectal swab is obtained by using an enrichment broth also. Of the three most common selective broths (tetrahionate, brilliant green and selenite), selenite (SEL) is the most widely used as it has the advantage in that it also may be used for the recovery of *Salmonella Typhi* and *Shigella*, although its value as an enrichment for the latter has not clearly been established.

2.1.2 Suitable Specimens

Blood, urine, faeces /rectal swab, sterile sites (food and water may also be sent for culture in the event of a suspected outbreak, however this is beyond the scope of this document)

Faeces / rectal swab, sterile sites (food samples relevant to cases of gastroenteritis may also be sent, however this is beyond the scope of this document)

Blood, faeces, urine collected in an appropriate sterile container, transported to laboratory
ASAP (if longer than 2h keep at 4°C), store at 4°C for 24 hours (Murray et.al., 1999). Rectal swabs put into a tube transport medium containing modified Stuart's medium, transport to laboratory ASAP, store at RT for 24 hours. (Murray et. al., 1999).

2.1.3 Test sensitivity

Mathematical data not available. Most studies only deal with a known number of specimens, particular media used and then only comment on how each medium fared with regard to isolation of Salmonella species. The number of false negatives is not known.

Depends on the quality of the specimen, the type of media chosen and the number of organisms present, the time between the onset of diarrhoea and collection of the sample, and the time between collection and culture of the specimen. Antibiotic treatment may affect the recovery of salmonellae.

Examples:

- DCA - able to detect 50 S.Typhimurium/ ml of M/100 phosphate buffer (Anon. 1966). Only 5% of stool samples artificially inoculated with $10^3$ salmonella per gram of faeces grew salmonella when 0.4g of sample was cultured onto Liefson desoxycholate citrate agar (Banffer et al., 1993).

- Selenite - An inoculum of 3-7 S.Paratyphi B in 9ml of selenite broth could be detected after 24 hr incubation. In the presence of $10^7$ E.coli an inoculum of 30 SPB could be detected. In presence of Pseudomonas aeruginosa even an inoculum of $10^4$ SPB could not be detected (In this instance tetrathionate and Rappaport –10 broths more appropriate for isolation of SPB) (Patil and Parhad, 1986).

- HE and XLD - good differentiation of typical Salmonella strains including Salmonella Typhi. Salmonella Paratyphi A may not be recognised on XLD

2.1.4 Test specificity

The isolation of a Salmonella - like organism must be confirmed by both specific biochemical and screening serological techniques. At this point it is a "presumptive Salmonella."

The isolation of a Salmonella is always potentially significant.

2.1.5 Predictive values

A negative culture does not exclude the diagnosis of salmonellosis.

2.1.6 Suitable acceptance criteria

- On DCA and XLD plates - non-lactose fermenting, non spreading, H$_2$S producing colonies

- On Bismuth sulphite agar – hydrogen sulphide (H$_2$S) producing colonies

- On MAC non-lactose fermenting colonies which biochemically fit Salmonella species (Ewing,1986)
2.1.7 Suitable internal controls

Properly documented, relevant, quality control programme for each type and batch of medium used. Incorporation of a control *Salmonella* with each batch of cultures is not recommended because of the risk of cross contamination of the test organisms.

2.1.8 Suitable test validation criteria

Isolation of a *Salmonella* species, confirmed by both biochemical and serological parameters, is the gold standard.

2.1.9 Suitable external QC programme

Royal College of Pathologists of Australia Quality Assurance Programs Pty. Limited

2.1.10 Special considerations

A medium other than XLD is required to detect S. Paratyphi A.

Recovery of SPA on XLD - RCPA experience:
- 96:6:3 SPA - 10% of 290 respondents failed to report a salmonellae
- 99:5:1A,B - 14% (41/284) respondents failed to report a salmonellae.
- Issuing laboratory found that on XLD (in house, BBL base) the count of normal flora was 1 x 10^8/L and the SPA was 4 x 10^8/L

Comment: question that some XLD media are slightly inhibitory for the particular strain of SPA used.

2.1.11 References


2.2 Culture for *Salmonella* Typhi
Blood culture is probably the single most useful diagnostic procedure for diagnosis of enteric fever. With few exceptions, a positive blood culture has a high predictive value for current enteric fever. Bone marrow has repeatedly been shown to be the specimen yielding the most positive cultures. Sampling of marrow is not often performed because it requires sterile equipment and skill and is unpleasant for the patients (Forsyth, 1998).

Reference


2.3 Identification of *Salmonella* species

There are two levels of *Salmonella* identification

- Clinical laboratories identify the Salmonella on the basis of biochemical parameters and may perform preliminary serology.
- Reference laboratory includes both state and Australian reference laboratories confirm biochemical identification, complete serological identification, perform any other specific subtyping eg. biotyping, phage typing, molecular finger printing.

2.3.1 Conventional biochemical tests

2.3.1.1 Suitable specimen

A pure culture on solid medium

2.3.1.2 Media

Various specific biochemical substrates.

2.3.1.3 Test sensitivity

 Depends on the biochemical tests performed. Production of lysine decarboxylase and hydrogen sulphide, no fermentation of lactose, no production of urease are good parameters to identify a *Salmonella* species. Most salmonellae are motile (Ewing, 1986).

2.3.1.4 Test specificity

Not all salmonellae exhibit the same biochemical properties. The notable exceptions are:

S. Paratyphi A does not produce H$_2$S or decarboxylate lysine

S. Typhi may only produce small amounts of H$_2$S. H$_2$S production may be slow i.e. > 24 hr. some strains of Typhi may fail to produce detectable H$_2$S in triple sugar iron or Kligler's iron agar
S. enterica subspecies IIIa and IIIb ferment lactose

Salmonellae from dairy factory environments may ferment lactose

Of 11,373 isolates submitted to an enteric reference laboratory as "Salmonella species", 155 (1.36%) isolates were not confirmed as Salmonella (MDU unpublished data).

2.3.1.5 Predictive values

Mathematical data not available

An isolate which biochemically resembles a Salmonella must be confirmed by serotyping.

2.3.1.6 Suitable test acceptance criteria

An isolate which exhibits biochemical characteristics consistent with documented reactions for Salmonella species.

2.3.1.7 Suitable internal controls

Each batch of biochemical substrate tested with both positive and negative control strains. Results of all testing recorded and kept.

2.3.1.8 Suitable validation criteria

Correct biochemical reactions exhibited by a standard Salmonella strain.

2.3.1.9 Suitable external QC program

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2.3.1.10 Special considerations

As indicated in 2.2.1.4 biochemical variants of salmonellae occur. Even if an isolate is not biochemically typical of Salmonella, serology may be performed to confirm any suspicious isolates. Salmonella species can be further subdivided using specific parameters.

2.3.1.11 References


2.3.2 Kits/ automated systems for biochemical identification

Various kits and automated machines exist for the identification of Enterobacteriaceae. Of the numerous kits available in Australia Microbact (Medvet Science Pty. Ltd., Adelaide, Australia, Micro - Id (Organon Teknika N.V. Belgium) and API would be the most commonly used.

MicroScan (Baxter, Baxter Healthcare Corporation, West Sacramento, USA), Vitek
Microbact TM Rapid Salmonella a simple tube test for confirmation of Salmonella spp. (Medvet Science Pty. Ltd. Adelaide, Australia.) based on negative reaction in o-nitrophenyl-B-D-galactoside (ONPG) and positive reaction for caprylate esterase. API BBL Crystal Enteric is a specific kit to identify any suspect Salmonella colonies.

The information stated in sections 2.2.1.1 - 2.2.1.9 also applies to both kits and automated identification systems. Comparison of sensitivity, specificity and predictive values of each kit/system as well as between systems is far too complex to discuss in this document.

2.3.2.10 Special considerations

For accurate results it is imperative to follow the manufacturers procedure exactly. Re sensitivity - Api 20E, Vitek GNI card, MicroScan and Becton Dickinson Cobas versus conventional biochemicals were compared for their abilities to identify accurately 252 strains of biochemically typical and atypical members of the family. The errors were random in all systems, with the exception of two atypical S. Enteritidis strains which were misidentified in all of the systems (O'Hara.C.M., et al., 1993).

3.3.2.11 References


2.3.3 Latex agglutination kit

A latex agglutination kit has been described for screening for salmonella from selenite enrichment broth (Wellcolex Color Salmonella; Murex Diagnostics, Inc., Norcross, GA.). This can also be used to screen individual colonies from primary plates. Suspect colonies may be inoculated into media to biochemically identify Salmonella. This technique only identifies Salmonella serological groups, it does not give a complete serological identification. NB Isolates must be sent to a designated reference centre for complete serological identification.

2.3.4 Molecular identification

Probes for Salmonella are being constantly developed for the detection and identification of salmonellae, particularly for use in the food industry, however, to date they are not used routinely in clinical laboratories.

Clinical laboratories should consider that an isolate is confirmed as a Salmonella when both determination of O serogroup and biochemical identification have been completed. (Murray et al., 1999). Preliminary flagella serology such as a poly "H" screening may also be performed.
2.4 Serological identification of *Salmonella* species

2.4.1 Suitable specimen

Pure bacterial culture.

2.4.2 Materials

Verified polyvalent and monospecific Salmonella somatic and flagella antisera. Many Enteric (salmonella) reference laboratories raise their own antisera in rabbits. Prior to use each serum is titred for the specific antigen and for cross reactions to any other *Salmonella* antigens. It is imperative to do this on all sera used in the reference section. All testing/titres must be fully documented.

Commercial antisera are available, however for a large volume turnover reference laboratory they are not satisfactory as they are expensive and sometimes the titres and cross reactions are not documented.

2.4.3 Test sensitivity

Unless an isolate is rough, most *Salmonella* isolates will serotype. Of 1,976 *Salmonella* isolates submitted to an enteric reference for serotyping 37 (1.87%) were designated "rough" and therefore unable to be completely typed (MDU unpublished data).

For non motile salmonellae only the somatic antigens can be determined.

2.4.4 Test specificity

The mathematical data not available.

2.4.5 Predictive values

Failure to completely serotype a strain does not mean it cannot be serotyped. Using a different batch of antisera, using a younger subculture or another isolate from the patient may achieve serotyping of the *Salmonella* infecting the patient. Occasionally a culture will not give an acceptable serotype. This may indicate that the culture is mixed. Individual colonies must be tested.

2.4.6 Suitable test acceptance criteria

Identification of both the somatic and flagella antigens which corresponds with, a documented serotype (Popoff and Le Minor,1997), and the same serotype has been previously isolated from a similar source. Any very unusual serotype must be verified by the Institute Pasteur (WHO designated International Reference Laboratory for Salmonella Serotyping) before a final report on the serotype is issued.

2.4.7 Suitable internal controls

In each batch of serotyping incorporate a *Salmonella* type strain to be serotyped.
2.4.8 Suitable validation criteria

Unequivocal demonstration of somatic and flagella antigens of recognised serotypes.

2.4.9 Suitable External QC program

None in Australia

2.4.10 Special considerations

Because members of the genus Enterobacteriaceae are closely related there is antigenic cross over. Therefore it is imperative that both flagella and somatic antigens are identified, the strain has been confirmed biochemically as a Salmonella and only properly quality controlled antisera are used for serotyping.

2.4.11 References


2.5 Further subtyping

Phage typing is a technique routinely used, in specified Enteric reference laboratories, to further subdivide particular significant serovars e.g. Typhi, Paratyphi A, Paratyphi B, Typhimurium, Enteritidis, Virchow, Hadar and Bovismorbificans. Because the same typing phages and the same techniques are used for the specified phage typing schemes internationally, results between countries are comparable. Various molecular techniques, such as plasmid profile, ribotyping, IS200 insertions, and pulsed field gel electrophoresis are performed in particular instances.

2.6 Rapid methods

Commercial rapid diagnostic tests are available for the testing of foods, but currently these techniques are not being used in the clinical diagnostic laboratories.

2.7 Serodiagnosis

Widal - the most commonly used method for the serodiagnosis of Salmonella enteric fever, measures agglutinating antibodies to the O and H antigens of Salmonella Typhi

Other serodiagnostic techniques have been developed for the detection of antibodies to various antigens e.g., outer membrane proteins lipopolysaccharide, flagellin protein, however these are not routinely used or recommended for the diagnosis of salmonellosis (Murray et.al., 1999). Only the Widal test will be discussed.

2.7.1 Suitable specimen
Appropriately stored, sterile serum (acute and convalescent phase).

2.7.2 Test sensitivity

The Widal test is only moderately sensitive - 30% of proven culture-proven cases have negative results (http://www.merck.com/pubs/mmanual/section13/chapter157/157d.htm)

2.7.3 Test specificity

Normal serum may agglutinate the test suspensions in low dilutions, and no diagnostic significance can be attached to such reactions. Non-specific antigens, such as fimbrial antigens, may be present in test suspensions and react with an agglutinin in human serum.

Infections, such as septicaemia, with other enteric organisms including salmonellae may produce a low titre response to the antigens, particularly "O" antigens, used in the Widal test (Cruickshank, 1969). Agglutination with the Widal antigens is not specific, for example S.Typhi "O" antigen will agglutinate with antibodies to any group D Salmonella, and Shigella "H" antigen will agglutinate with antibodies produced against any organism possessing an H = d.

Low titres may be exhibited by patients previously vaccinated with TAB.

Cirrhosis is associated with non-specific antibody production. (http://www.merck.com/pubs/mmanual/section13/chapter157/157d.htm)

2.7.4 Predictive values

A fourfold rise in O and H antibody titres in paired serum acquired two weeks apart suggests S.Typhi infection.

2.7.5 Suitable test acceptance criteria

All test antigens must react to titre with the control sera and the antigen controls negative.

2.7.6 Suitable internal controls

Standardised antigen suspensions and relevant antisera with documented titres.

2.7.7 Suitable validation criteria

Isolation of a Salmonella Typhi.

2.7.8 Suitable external QC program

None. RCPA 89:4: 7A,B sent out in 1989 no longer done as test not performed by many laboratories in Australia.

2.7.9 Special considerations

Infections, such as septicaemia, with other enteric organisms including other salmonellae may produce low titres to the antigens used in the Widal. Previous typhoid or paratyphoid
vaccination may affect the titres.

2.7.10 References


3. PHLN laboratory definition

3.1 Condition Typhoid fever

3.1.1 Tests

Definitive criteria – isolation of Salmonella Typhi

3.2 Condition Paratyphoid fever

3.2.1 Tests

Definitive criteria – isolation of Salmonella Paratyphi A or Salmonella Paratyphi B

3.3 Condition Salmonellosis

3.3.1 Tests

Definitive criteria – isolation of a Salmonella species - not S. Typhi, S. Paratyphi A or S. Paratyphi B.