PHLN
Public Health Laboratory Network

Shigellosis (*Shigella*)

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *shigella*.

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1. Introduction

Shigella causes bloody diarrhoea (dysentery) and non-bloody diarrhoea. Shigellosis often begins with watery diarrhoea accompanied by fever and abdominal cramps but may progress to classical dysentery with scant stools containing blood mucus and pus. All four species of *Shigella* are capable of causing dysentery, but *Sh. dysenteriae* 1 has been associated with a particularly severe form of the illness thought to be related to its production of Shiga toxin.

In 1998, 527cases of shigellosis were notified to the National Enteric Pathogens Surveillance Scheme (1999). The dominant types were *Sh. sonnei* and *Sh. flexneri* 2a.

2. Laboratory diagnosis/tests

2.1 Culture for *Shigella* species

While shigellae are classed as non-fastidious organisms, because of the nature of the specimens for culture, *Shigella* detection is still highly dependent on employing appropriate culture media.

2.1.1 Media

For optimal isolation of *Shigella*, two different selective media should be used:
a general - purpose plating medium of low selectivity (e.g., MacConkey-MAC) and
a more selective agar medium (e.g. xylose lysine desoxycholate agar-XLD).
Salmonella -shigella agar (SS) should be used with caution because it inhibits some strains of Sh.dysenteriae There is no reliable effective enrichment medium for all types of *Shigella*, but gram-negative (GN) broth and Selenite broth are frequently used (Murray et.al. 1999).

2.1.2 Suitable specimens

Faeces/rectal swab.
Faeces collected in an appropriate sterile container, transported to the laboratory ASAP (if longer than 2h keep at 4° C), may be stored at 4° C for 24 hours before culture.
Rectal swabs put into a tube transport medium containing modified Stuart's medium, transported to the laboratory ASAP. May be stored at RT for 24 hours before culture.

2.1.3 Test sensitivity

No mathematical data available.
Depends on the quality of the specimen, the type of media chosen, the number of organisms and the time of collection after the onset of diarrhoea. Antibiotic treatment may inhibit growth of shigellae

2.1.4 Test specificity

No mathematical data available.
The isolation of a *Shigella* like organism, confirmed by specific biochemical and preliminary serological techniques. At this point it is a "presumptive *Shigella*"
The isolation of a *Shigella* species is significant.

2.1.5 Predictive values

A negative culture does not exclude the diagnosis of shigellosis.

2.1.6 Suitable acceptance criteria.

On MAC, XLD or SS a non-lactose fermenting, non-spreading colonies which biochemically fit a *Shigella* species.

2.1.7 Suitable internal controls

Properly documented, relevant, quality control program for each type and batch of medium used.
Use of a control *Shigella* 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 *Shigella* 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. Ltd. (RCPA)

2.1.10 Special considerations

Based on DNA-DNA hybridization studies the genera *Escherichia* and *Shigella* are very closely related are essentially the same genus. For practical and historical reasons both groups are still treated separately. It is essential therefore, before a final designation of Shigella is made the organism must conform to both recognised biochemical and serological parameters. Preferably, the isolate identity should be confirmed by a designated enteric reference laboratory.

2.1.11 References

Ewing, W.H. 1986 Edwards and Ewing's Identification of Enterobacteriaceae. 4Th Edition, Elsevier Publishing Company, New York.
Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Yolken, R.H. 1999. Manual of Clinical Microbiology, ASM Press, Washington, D.C.

2.2 Identification of *Shigella* species

There are two levels of *Shigella* identification

A. Diagnostic laboratories - identify the *Shigella* using biochemical parameters and may perform preliminary serology.
B. Reference laboratories - both the state and Australian reference laboratories.

2.2.1 Conventional biochemical tests

2.2.1.1 *Suitable specimen*

A pure culture on solid medium.

2.2.1.2 Media

Various specific biochemical substrates

2.2.1.3 Test sensitivity

Depends on the biochemical tests performed. "Any strain that blackens triple sugar iron, is urease positive, or is able to grow on Simmons' citrate or decarboxylates lysine, produces phenylpyruvic acid from phenylalanine, or is motile does not belong to the genus *Shigella"* (Ewing, 1986). Christensen's citrate sodium acetate and sodium mucate media are of considerable value in the differentiation of *Shigella* from *Escherichia* (Ewing, 1986). (Particularly from anaerogenic *E. coli*)

2.2.1.4 Test specificity

Of 139 isolates submitted to an enteric laboratory as "probable *Shigella* species" eleven (7.9%) were not confirmed as a *Shigella* species (MDU unpublished data). Not all shigellae exhibit the same biochemical properties. The notable exception is *Sh.sonnei* which is the only *Shigella* serotype to ferment lactose. A few particular types produce gas in mannitol.

2.2.1.5 Predictive values

A negative culture does not preclude a diagnosis of shigellosis.

2.2.1.6 Suitable test criteria

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

2.2.1.7 Suitable internal controls

Each batch of biochemical substrate tested with positive and negative control strains.
Results of all testing recorded and the records maintained.

2.2.1.8 Suitable validation criteria

Correct biochemical reactions exhibited by a standard *Shigella* strain.

2.2.1.9 Suitable external QC program

RCPA Quality Assurance Programs Pty. Ltd.

2.2.1.10 Special consideration

As indicated in 2.2.1.4 biochemical variants of shigellae occur. Even if an isolate is not biochemically typical of *Shigella,* serology may be performed on suspicious isolates. The major biochemical problem is the differentiation of *Shigella* from inactive or atypical *E. coli.* An isolate which biochemically resembles a *Shigella* must be confirmed by serotyping.

2.2.1.11 References

Ewing, W.H. 1986. Edwards and Ewing's Identification of Enterobacteriaceae. 4th Edition, Elsevier Publishing Company, New York.
2.2.2 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 (bioMerieux, Marcy- l'Etoile, France), MicroStation (Biolog, Heyward, USA) and Cobas (Becton Dickinson Instrument Systems, Sparks, USA) are the major systems.

The information stated in sections 2.2.1.1 - 2.2.1.9 also applies to both kits and automated identification systems.

2.2.2.10 Special considerations

For accurate results it is imperative to follow the manufactures procedure exactly.

2.2.3 Latex agglutination kit

A latex agglutination kit has been described for screening for shigellae from selenite enrichment broth (Wellcolex Color Shigella; 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 *Shigella.* This technique only identifies *Shigella* serological groups; it does not give a complete serological identification.

NB Isolates must be sent to a designated reference centre for complete serological identification.
"A preliminary report of suspect *Shigella* infection may be issued if biochemical or serologic screening tests are positive "(Murray et. al., 1999)

2.2.4 Molecular identification

Probes for *Shigella* have been developed for the detection and identification of shigellae, however, to date they are not used routinely in clinical laboratories.

2.3 Serological identification of *Shigella* species

2.3.1 Suitable specimen

Pure bacterial culture.

2.3.2 Materials

Verified polyvalent and monovalent somatic *Shigella* 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 *Shigella* antigens. It is imperative to do this on all sera used in the reference section. All testing/titres must be fully documented. The production of *Shigella* antisera is more complex compared to the production of *Salmonella* antisera. Commercial antisera are available, but it is recognised that not all brands of antisera are reliable. For reference laboratories, which test large numbers of isolates, commercial antisera are expensive and not as useful because of cross-reactions and lack of information about the antigen titres.

2.3.3 Test sensitivity

Unless an isolate is rough, most *Shigella* isolates will serotype.

2.3.4 Test specificity

No data available.

2.3.5 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 *Shigella* infecting the patient. Occasionally a culture will not give a satisfactory serotype. This may indicate that the culture is mixed.

2.3.6 Suitable test acceptance criteria

Agglutination, to the specified titre, with specific *Shigella* antisera.

2.3.7 Suitable internal controls

Regular serotyping of a *Shigella* type strain.

2.3.8 Suitable validation criteria

Unequivocal demonstration of a recognised serotype.

2.3.9 Suitable External QC program

None

2.3.10 Special considerations

Because members of the genus Enterobacteriaceae are closely related there is antigenic cross over. Therefore it is imperative that been confirmed biochemically as a *Shigella* and only properly quality controlled antisera is used for serotyping. Very occassionally an isolate will be biochemically indistinguishable from *Shigella* species but no agglutination with the standard antisera is observed.

This isolate may represent a new serotype. The isolate must be sent to the WHO designated Shigella Reference Centre at CDC, Atlanta, USA. for verification.

2.3.11 References

Ewing, W.H. 1986 Edwards and Ewing's Identification of Enterobacteriaceae. 4th Edition Elsevier Publishing Company, New York.

2.4 Further subtyping

Biotyping and colicin typing (particularly for *Sh.sonnei*), plasmid profiling and various other molecular typing techniques may be used for the subtyping of shigellae, however to date they are not in use routinely.

2.5 Rapid methods

Commercial rapid diagnostic tests have been developed, but currently these techniques are not being used in the clinical diagnostic laboratories.

2.6 Serodiagnosis

"Several groups have developed serodiagnostic assays based on several antigens possessed by *Shigella.* These assays are practical only in research settings for seroepidemiology surveys and are not currently used for the diagnosis of infection in individual patients who are acutely ill" (Murray et .al., 1999).

3. PHLN laboratory definition

3.1 Condition Shigellosis

3.1.1 Tests

Definitive Criteria: Isolation of a *Shigella* species.