National Department of Health Central Public Health Laboratory in conjunction with the Fleming Fund Country Grant (2020-22)

Title: Salmonella serological testing

ID: G_90_SOP_28_A

Developed by: **Diagnostic Micro Development Program** **Date: 2019**

Reviewed by: **C Allen, J Ferguson, V Fabila** **Date: 12/4/22**

Review Period: **2 years**

Authorized by: **W Porau** **Date:**

Changes to the last Authorized Version:

Version	Date	Changes
A	12/4/22	Reflects 28/4/2019 DMDP protocol

1. Purpose and Scope

Confirmatory identification of Salmonella species.
This procedure applies to the clinical microbiology laboratory environment within the CPHL, and Human health Fleming Fund Country Grant partner laboratories.

2. Principle & Clinical application

Salmonellosis is human disease caused by *Salmonella* species. The disease can be mild self-limiting gastroenteritis to severe bacteremia or typhoid fever. Severe disease and bacteremia are primarily associated with three serovars of *Salmonella* enterica subsp. enterica- Choleraesuis, Paratyphi A and Typhi[1], all of which have been reported in Cambodia. Most other strains are associated with gastroenteritis.
This SOP aims to guide identification of *Salmonella* from clinical specimens using *Salmonella* Poly O (A-G) and *Salmonella* Vi antisera.

Salmonella are categorized as typhoidal and nontyphoidal *Salmonella*. Strains of nontyphoidal *Salmonella* usually cause intestinal infections and less commonly cause extra intestinal infections (e.g. bacteremia, urinary tract infection, osteomyelitis, abscess) especially in the immunocompromised. Typhoidal *Salmonella* include *Salmonella* Typhi, Paratyphi A, Paratyphi B and Paratyphi C. *Salmonella* Typhi, Paratyphi A, Paratyphi C and some Paratyphi B are primarily host restricted to humans.
Salmonella Choleraesuis and Dublin are host adapted to cattle and pigs but can cause severe human disease with spread to extra intestinal sites (e.g. blood).[2]

Salmonella is found in nature and occurs in the intestinal tract of many animals, both wild and domestic and are the principal source of nontyphoidal Salmonellosis. Human infections are most commonly caused by ingestion of food, water or milk contaminated by human or animal faeces.

All *Salmonella* serovars belong to two species: S. bongori, which contains 18 serovars, and S. enterica, which contains the remaining 2,300 or more serovars divided among six subspecies. S. enterica subsp. enterica (Subspecies I) are commonly isolated from warm-blooded animals and humans.

Salmonella serotyping is a subtyping method based on immunological characterization of three surface structures:

- O antigen (lipopolysaccharide capsule of the cell wall)
- H antigen (bacterial flagella)
- Vi antigen (capsular polysaccharide present in specific serotypes)

Serotyping is useful in identifying cases and defining outbreaks.

Salmonella O Antisera are used in slide agglutination tests for the identification of *Salmonella* by somatic (O) antigens.

A slide agglutination test for the detection of Vi antigen is useful for the identification of *Salmonella* Typhi.

However, Vi is also occasionally detected in *Salmonella* Paratyphi C, *Salmonella* serotype Dublin and some *Citrobacter* strains.

Salmonella O antigens are somatic (O) heat-stable antigens. Some strains may have O antigen masked by the Vi antigen, a heat-labile envelope antigen, making the O antigen not agglutinate in O antisera. In order to determine the O antigen of these cultures, a suspension of the organism must be boiled to destroy the heat-labile Vi antigen and tested again with O antisera.

3. Responsibilities

Role	**Responsibility**
Medical Lab bench scientist/technician	Setup, reading and data entry
On duty senior scientist	Checking of positive serology results Identify and document isolates that require storage/ referral Addition of interpretative comments on the LIMS for the report

4. **Specimen**
Perform the tests on pure isolated colonies from non-selective media. Perform only when the biochemical test reactions are consistent with the identification of the organism as *Salmonella* species.

It is strongly recommended that samples and reagents should be treated as potentially infectious and be handled following good practice and standard precautions.

5. Equipment & Materials
- *Salmonella* poly O (A-G)
- *Salmonella* Vi
- Glass slides
- Black background
- Saline 0.85%
- Heating block
- Applicator stick
- Plastic/metal loop
- Timer

6. Procedure
Key message prior to performing the test: Bring all materials to room temperature.

Perform *Salmonella* Poly O (A-G) before testing *Salmonella* Vi.

If *Salmonella* Poly O (A-G) is positive, and the isolate appears biochemically as *Salmonella* then the isolate is *Salmonella*.

If *Salmonella* Poly O (A-G) is negative, test *Salmonella* Vi.

If *Salmonella* Poly O (A-G) and *Salmonella* Vi are both negative, the isolate is unlikely to be *Salmonella*.

If *Salmonella* Vi is positive, boil a 2.5 ml saline suspension of the test organism. The saline suspension of the test organism is to be boiled at 100 ◦C for 1 hour to destroy the Vi antigen.

Centrifuge the cooled saline suspension, remove the supernatant and resuspend the deposit in 0.5ml saline.

Retest the saline suspension with *Salmonella* Poly O (A-G). Refer to the diagram below.

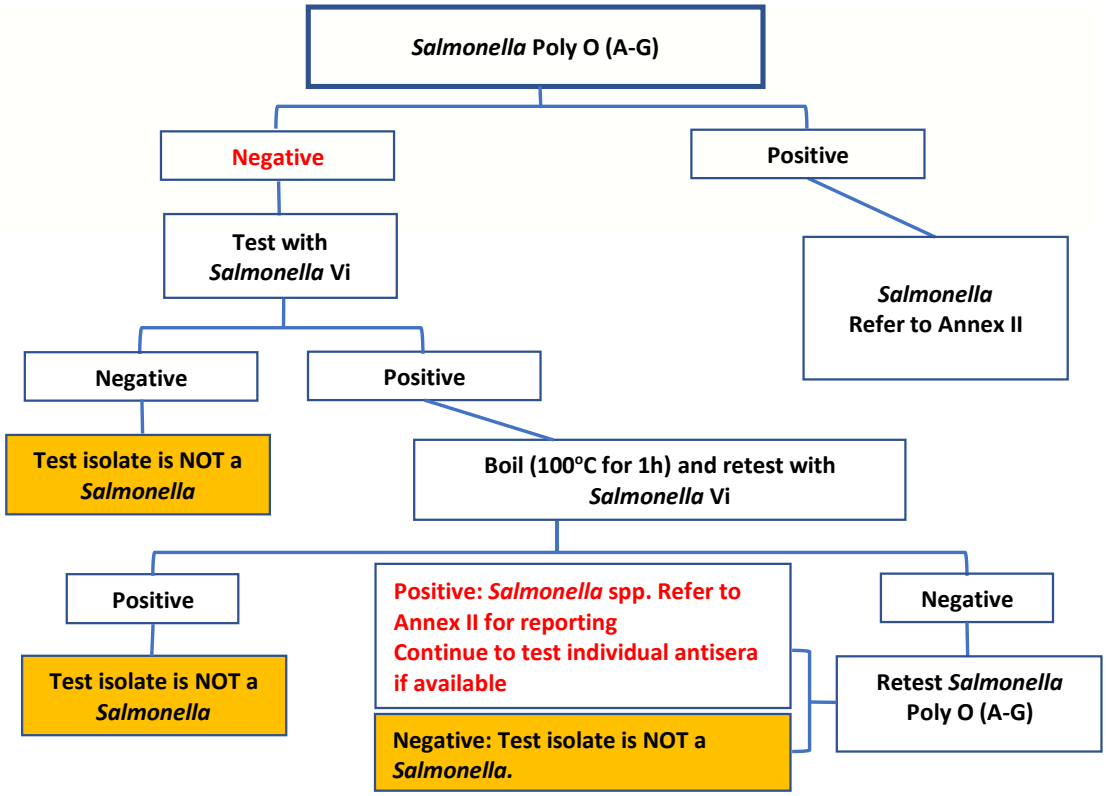
***Salmonella* Poly O (A-G) and *Salmonella* Vi antiserum**
Reagents should be stored at: 2-8 ºC

Warm up reagent in room temperature before testing

Use this procedure to test the isolate with each selected antiserum.
1. Wear proper PPE
2. Label slide as “QC -” and “test”

3. Add one drop (40 µL) of saline on clean slide in each test area
4. From a solid agar medium (nonselective media is preferred), transfer a portion of a loopful of the test isolate to each reaction area above ("QC-" and "test") and mix thoroughly.
5. To the 'test' isolate add one drop of *Salmonella* Poly O (A-G) and mix.
6. To the 'QC -' control add one drop of saline and mix.
7. Rotate the slides for 1 min and read for agglutination over a black background. Results must be read within 1 min.
8. If negative *Salmonella* Poly O (A-G), test with *Salmonella* Vi antisera using the same method.
9. Follow Flow Chart I *Salmonella* serology, below.

Flow Chart I *Salmonella* serology



7. Results recording

Agglutination should be strong and clearly visible within one minute. There should be no agglutination in the negative control.

8. Interpretation

Report result as follows:
If *Salmonella* poly O (A-G) shows strong agglutination and the isolate is *Salmonella* biochemically, the isolate is likely to be *Salmonella*.
Release a presumptive *Salmonella* report.

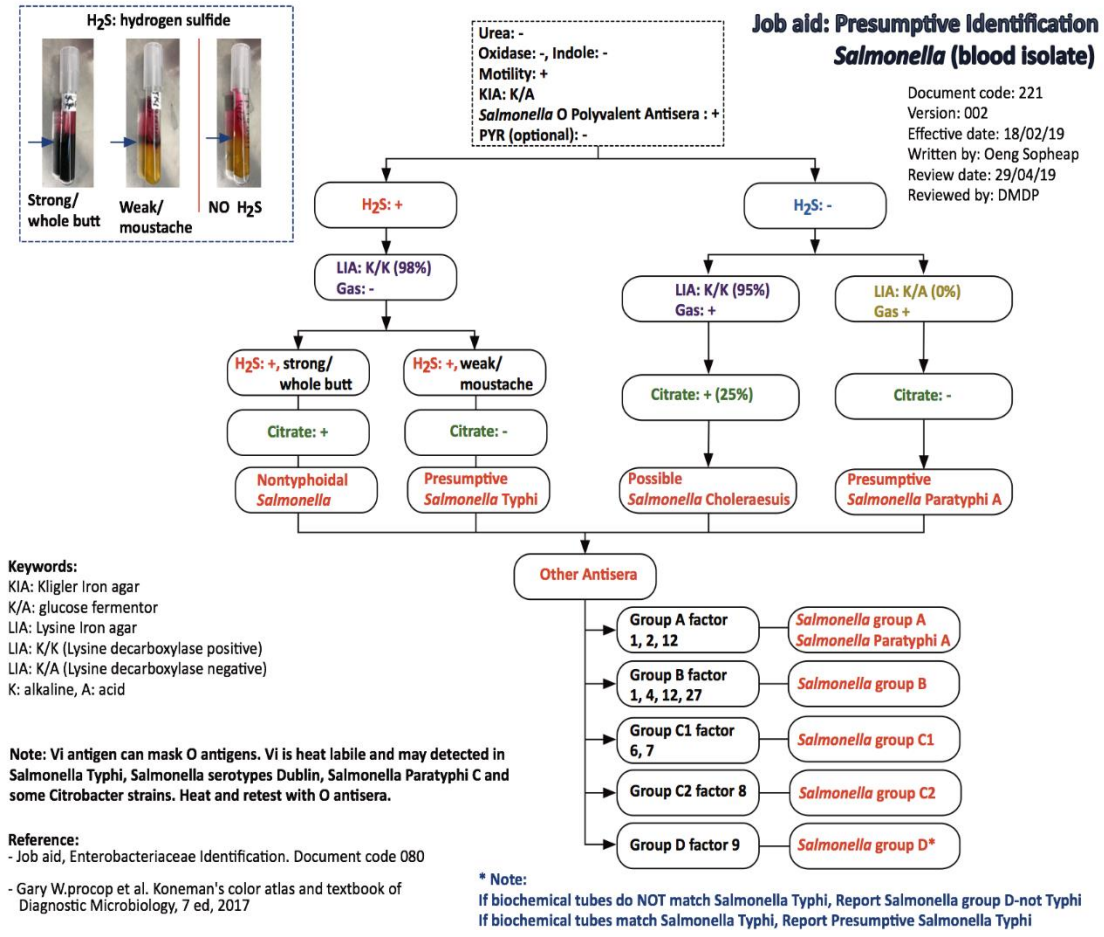
Send the isolate to a CPHL reference laboratory for further confirmation.

There should be no agglutination visible in the negative saline control. If agglutination is present the test is invalid.

Report *Salmonella* only if biochemical test and antisera is positive

Report Presumptive *Salmonella* according to Job Aid

Presumptive Identification of *Salmonella* isolated from blood



Limitations

Cross reactions (with similar antigens) may occur. Therefore, a positive agglutination reaction should be supported by the morphological and biochemical identification of the microorganism.

9. Safety

For other safety aspects, please review this document G_10_Info_3_A Laboratory Biosafety.

10. Quality control

Positive control *Salmonella* Poly O (A-G)

For each vial of *Salmonella* Poly O (A-G) antisera, test an ATCC 14028 *Salmonella* Typhimurium, test one time per vial (when open new reagent kit) as follows:

Dispense 1 drop of saline to an agglutination slide. Take a portion of a colony of ATCC 14028 *Salmonella* Typhimurium with a loop or stick and mix to form a homogenous suspension in the saline.

Add one drop of *Salmonella* Poly O (A-G) antisera and mix.

Rotate the slide and read for agglutination. Results must be read within 1 min.

Record the result on the vial, date and sign.

Positive control *Salmonella* Vi

For each vial of *Salmonella* Vi antisera, test an in-house *Salmonella* Typhi as follows:

Dispense 1 drop of saline to an agglutination slide. Add 1 portion of a colony of an appropriate in-house *Salmonella* Typhi. Mix well.

Add one drop of *Salmonella* Vi and mix well.

Rotate the slide for 1 min and read for agglutination. Results must be read within 1 min.

Record the result on the vial, date and sign.

Negative control *Salmonella* Poly O (A-G) and *Salmonella* Vi antisera

For each test:

Dispense 1 drop of saline on an agglutination slide.

From a solid agar medium, transfer a portion of a loopful of the test isolate to the reaction area above and mix thoroughly.

Add one drop of saline and mix.

Rotate the slides for 1 min and read for agglutination.

There should be no agglutination visible in the negative saline control. If agglutination is present the test is invalid.

11. References

1. BD Difco *Salmonella* O Antisera and *Salmonella* Antiserum Vi package insert http://legacy.bd.com/ds/technicalCenter/inserts/8085889(03).pdf
2. Jorgensen, J. et al. Manual of Clinical Microbiology, 2015. 11th Edition
3. DMDP - Salmonella Antisera Testing Document - Author C. Chanborann- effective 28/04/2019