Ministry of Health

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Directorate/Institution	Diagnostic Laboratories Services at Directorate General of Specialized Medical Care (DGSMC) at Ministry of Health (MOH)
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Acknowledgment

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Acronyms:

BA	Blood agar
MAC	MaConkey
ATCC	American Type Culture Collection
CPHL	Central public health laboratory
H&S	Health and Safety
ID	Identification
IQC	Internal Quality Control
SOP	Standard operating procedure

1. **Purpose**

This document describes the procedure for presumptive identification of *Shigella* species.

2. **Scope**

This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

3. **Definitions:**

3.1 Antisera: Human or animal serum containing one or more antibodies that are specific for one or more antigens.

4. **Procedure**

4.1. Clinical background:

Shigella is a genus of gram-negative, non-motile, rod-shaped bacteria that is a common cause of bacterial diarrhea. *Shigella* is a type of bacteria that can cause an infectious disease known as shigellosis or bacillary dysentery. The clinical symptoms of *Shigella* can vary in severity and may include:

4.1.1 Diarrhea: The most common symptom of shigellosis is diarrhea, which may be watery or bloody. The stool may contain mucus or pus, and frequent bowel movements may occur.

4.1.2 Abdominal pain: Abdominal cramps and pain are common in shigellosis. The pain may be severe and persistent.

4.1.3 Fever: A fever is often present in shigellosis, and it may be high, typically over 101°F (38.3°C).

4.1.4 Nausea and vomiting: Some people with shigellosis may experience nausea and vomiting, especially in the early stages of the illness.

4.1.5 Tenesmus: Tenesmus is a feeling of incomplete evacuation after bowel movement. It is also common in shigellosis.

4.1.6 Dehydration: Dehydration can occur due to the loss of fluids and electrolytes through diarrhea and vomiting. Symptoms of dehydration include dry mouth, thirst, decreased urine output, and fatigue.

4.1.7 Convulsions and seizures: In rare cases, shigellosis may lead to seizures or convulsions.

The symptoms of shigellosis typically appear within 1-3 days after exposure to the bacteria and can last for up to a week or more. Treatment typically involves rehydration and antibiotics, and in severe cases, hospitalization may be necessary.

The genus includes four species: S. dysenteriae, S. flexneri, S. boydii, and S. sonnei. Identification and confirmation of Shigella species is important for appropriate treatment and management of infections.

4.2.Principle:

The traditional method for identification of Shigella species is through culture and microscopy, where the bacteria are grown on selective media and then identified based on their morphological characteristics. However, this method can be time-consuming and may not provide definitive results.

Other method of identification of *Shigella* species involves a process called agglutination testing, which is based on the reaction between antigens and antibodies. In this test, Shigella specific antigens are mixed with corresponding antisera, and if the reaction results in the formation of complexes known as Ag/Ab complexes, it causes a visible clumping or agglutination. This indicates that the organism is likely to be a *Shigella* species. On the other hand, if no clumping is observed, it means that the antigen is not present and the organism is not that particular species of *Shigella*. By testing against all available antisera, it is possible to confirm if the isolate is not a *Shigella* species.

Shigellae are differentiated into four subgroups on the basis of their O (somatic) antigens and further differentiated into serotypes;

- *S. dysenteriae* (Group A) contains 15 distinct antigenic serotypes.
- *S. flexneri* (Group B) contains 6 serotypes (1-6) that can be further divided into subserotypes based on their possession of group factors designated 3,4; 4; 6; 7; and 7,8.
- *S. boydii* (Group C) contains 20 distinct antigenic serotypes.
- *S. sonnei* (Group D) contains only 1 serotype that may occur in two forms, form I (smooth) and form II (rough)

4.3.Pre – analytical stage:

4.3.1. Sample:

4.3.1.1 Sample type: Pure colonies of young growth 18-24 hrs.

4.3.1.2 Amount of sample required, including minimum requirements: 2-3 colonies.

4.3.1.3 Sample stability and storage requirements: to be processed immediately within 18-24 hours.

4.3.1.4 In case of delay, store the sample in 2-8 °C.

4.3.2. Material:

Reagents	Consumables/Supplies	Equipment
➢ *Shigella sonnei* phase 1 & 2 agglutinating Sera. ➢ *Shigella flexneri* polyvalent (1 - 6 & X & Y) agglutinating Sera. ➢ *Shigella dysenteriae* polyvalent (1- 10) agglutinating Sera. ➢ *Shigella boydii* polyvalent (1 - 6) agglutinating Sera. ➢ *Shigella boydii* polyvalent (7 - 11) agglutinating Sera. ➢ *Shigella boydii* polyvalent 3 (12 - 15) agglutinating Sera. ➢ Blood agar ➢ Nutrient agar slant	Microscopic slides Sterile loops Wood Stick Test tube Pipette	Safety cabinet O2 Incubator Refrigerator

4.3.3. Safety precaution:

4.3.3.1 All specimens need to be treated as potentially infectious.

4.3.3.2 Standard procedures for handling of biohazard material must be followed at all times.

4.3.3.3 Universal Precautions must be practiced at all stages of these procedures.

4.3.4. Quality control:

4.3.4.1 Check the expiry dates of all media, reagents and stains before use.

4.3.4.2 All media, reagents, kits, and stains MUST be quality controlled before use.

4.3.4.3 Identification tests should be run with appropriate controls.

- *S. dysenteriae* ATCC 13313.
- *S. flexneri* 2B ATCC 12022.
- *S. boydii (1)* ATCC 9207.
- *S. sonnei* ATCC 25931.

4.3.4.4 Record the quality control results in the appropriate QC sheet.

4.4.Analytical stage:

4.4.1 To perform the agglutination test, take a pure colony of the organism from blood agar preferably and create a light suspension by mixing it with a drop of saline on a glass slide. Make sure that the suspension is evenly mixed before proceeding.

4.4.2 Add one drop of antisera to the suspension. Mix the well using a wooden applicator stick.

4.4.3 Gently rock the slide for 2 minutes while observing for any signs of clumping or agglutination.

4+ All organisms are clumped and the supernatant, or suspending fluid is clear.

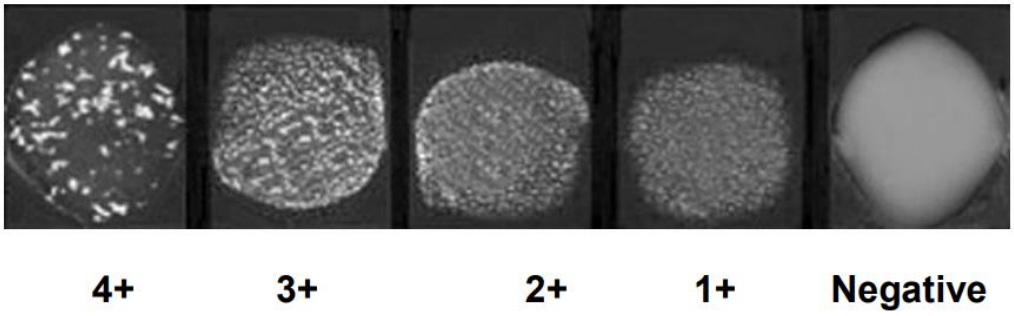
3+ About 75% agglutination, the supernatant fluid is slightly cloudy.

2+ About 50% agglutination, the supernatant fluid is moderately cloudy.

1+ About 25% agglutination, the supernatant fluid is cloudy.

Tr Trace amount of agglutination present.

Negative No agglutination apparent and suspension remains homogenous.



4.5.Post – analytical stage:

4.5.1. Interpretation / Results / Alerts:

- Negative agglutination:
- Positive agglutination:

- After performing the agglutination test, record all the observed reactions for patient and control, making sure to note the specific antisera used and any observed agglutination.
- Once all reactions have been recorded, confirm the results with the by the API 20 E, automated test system or molecular method. This will aid in determining the correct identification of the organism and ensure proper treatment and management of any associated infections.

4.5.2. Reporting:

Positive results for *Shigella sp*. as confirmed by biochemical tests (such as API 20E or automated ID) and *Shigella* antigen tests must be reported and communicated to:

- Microbiologist.
- Treating clinician.
- Hospital infection control.
- Refer the isolate to CPHL for further testing with reflex test as (Salmonella ID, AMST & serotyping). Use Nutrient Agar Slant as a transportation media.

4.5.3. Limitation:

4.5.3.1 Correct interpretation of serological reactions for identifying *Shigella* species requires assessing culture purity, morphological characteristics, and consistent biochemical reactions.

4.5.3.2 However, serological methods alone are not sufficient for identifying the isolate as a Shigella species.

4.5.3.3 It is important to note that external heat sources may compromise test results and cause false-positive reactions. When performing serological procedures, it is important to use smooth culture isolates to avoid auto-agglutination.

4.5.3.4 It is also important to note that commercial antisera, have only been tested using cultures taken directly from agar media, and their effectiveness may vary when used with antigen suspensions or other diluents. Any variations in recommended steps should be tested with known control cultures to verify expected reactions.

5. Responsibilities:

5.1.Responsible staff:

- To ensure the adherence to critical result communication procedure
- To facilitate the alternative channels once needed

5.2.Quality manager /officer

- To follow up the implementation of the procedure
- To monitor regularly communication of critical results and raise non-conformance with corrective action once needed.

5.3.All lab staff:

- To adhere to the procedure.
- To document record and release results as recommended
- To report test failures or incident

6. Document History and Version Control

Version	Description	Review Date
1	Initial Release	May 2026

7. References

Title of book/ journal/ articles/ Website	Author	Year of publication	Page
Shigella infections. The Lancet	Snyder, J. D., & Matson, D. O.	2014	384(9955), 1261-1271
Shigella and enteroinvasive Escherichia coli. In Escherichia coli and Salmonella: Cellular and molecular biology. American Society for Microbiology.	Achtman, M., & Wachsmuth, K.	1991	pp. 1491-1524
Identification of Shigella spp. by PCR and sequencing. Journal of Microbiological Methods	Gharbi, M., & Poirel, L.	2016	128, 1-9
Rapid diagnostic tests for Shigella infections. Current Opinion in Infectious Diseases	O'Hara, C. M., & Kibsey	2012	25(1), 42-47
Shigella Antisera Package inserts			
Laboratory Protocol: "Serotyping of Shigella spp."	van der Ploeg, Claudia A. (INPB) Viñas, María R. (INEI) Terragno, Raquel (INEI) Bruno, Susana B. (INPB) Binsztein, Norma (INEI)	2010	7

8. Annexes: Quality Control Records sheet of Shigella Ag test:

	Manufacture Name:……					
Anti- sera	**A**	**B**	**C**	**D**	**E**	**F**
Lot No.						
Expiry date						
Date opened						
Neg. Control						
Positive Control						
QC Pass/Fail						
Initial & Date						

Anti sera:

A. *Shigella sonnei phase 1 & 2 agglutinating Sera.*
B. *Shigella flexneri polyvalent (1 - 6 & X & Y) agglutinating Sera.*
C. *Shigella dysenteriae polyvalent (1- 10) agglutinating Sera.*
D. *Shigella boydii polyvalent (1 - 6) agglutinating Sera.*
E. *Shigella boydii polyvalent (7 - 11) agglutinating Sera.*
F. *Shigella boydii polyvalent 3 (12* - 15) agglutinating Sera.

Positive controls:

- *S. dysenteriae* ATCC 13313.
- *S. flexneri* 2B ATCC 12022.
- *S. boydii (1)* ATCC 9207.
- *S. sonnei* ATCC 25931.