Ministry of Health

Document Title	Salmonella Color Ag Detection test (ABCDE or G & VI) SOP.
Document Type	Procedure
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:

MAC	MaConkey Agar Plate
ID	Identification
SOP	Standard operating procedure
CPHL	Central Public Health Laboratory

1. **Purpose**

   This document describes the procedure for preliminary identification test of *Salmonella* species such as *paratyphi A, Salmonella paratyphi B,* and *Salmonella typhi*.

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 Typhoidal salmonellosis:

   Also known as typhoid fever, is a bacterial infection caused by the *Salmonella enterica serotype Typhi*. It is a systemic illness that can affect multiple organs and is characterized by symptoms such as fever, headache, abdominal pain, and diarrhea. Typhoid fever is typically transmitted through contaminated food or water and is more common in areas with poor sanitation and hygiene practices. The disease can be treated with antibiotics, but in some cases, it can become chronic and require long-term management. Typhoid fever is a serious condition and can be life-threatening if left untreated or if complications arise. Vaccines are available to prevent typhoid fever, and travelers to high-risk areas are often advised to get vaccinated before their trip.

4. **Procedure**

   4.1.Clinical background:

   Salmonella Ag test is mainly for the diagnosis of Salmonella infections, which are common bacterial infections that affect the intestinal tract. The test is usually performed on bacterial isolates that are suspected to be salmonella based on growth and biochemical characteristics for example, oxidase negative non lactose fermenters. Common sites of isolation is from stool or blood samples.

   Salmonella is a group of bacteria that can cause a variety of illnesses, collectively known as salmonellosis. The most common symptoms of salmonellosis include diarrhea, fever, and abdominal cramps, which usually appear within 12 to 72 hours after infection and last for 4 to 7 days. There are many different species of Salmonella, but some of the most clinically relevant include *Salmonella enterica*, *Salmonella typhi*, and *Salmonella paratyphi*. *S. enterica* is the most common cause of non-typhoidal salmonellosis, which affects the intestinal tract and typically causes self-limiting diarrhea, while *S. typhi* and *S.*

paratyphi are responsible for typhoid fever and paratyphoid fever, respectively, which are systemic infections that can be severe and life-threatening.

4.2.Principle:

The Salmonella test involves using a bacterial growth and reacting it with two test reagents containing suspensions of red, blue, and green latex particles coated with antibodies specific to different Salmonella serogroups. If the sample contains the corresponding antigen, one of the colors in the mixture will clump together, and the antigen's identity is indicated by the color of the clumped particles against a contrasting background. The results are easy to distinguish from negative or non-specific results, which appear as smooth gray-brown suspensions or gray-brown clumps against a cleared background.

4.3.Pre – analytical stage:

4.3.1. Sample:

4.3.1.1 Sample type: Well isolated pure colonies on Solid agar medium.

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**
Latex Reagent **1** latex coated with: • Red latex Salmonella group B. • **Blue** latex Salmonella group C. • **Green** latex Salmonella group D. Latex Reagent **2** latex coated with: • **Red** latex Vi. • **Blue** latex Salmonella groups E and G. • **Green** latex Salmonella group A. Red Positive Control Blue Positive Control Green Positive Control Negative Control – Normal Saline	Sterile loops Wood/plastic sticks disposable sample dispenser white cards test tube micropipette	Safety cabinet class II Incubators Rotator Fridge

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.

4.3.4.4 Record the quality control results in the appropriate QC sheet (see Annexes #1: Quality Control Records sheet)

4.4.Analytical stage:

This test is performed from colonies obtained from purity plate preferably from blood agar plate, as follows:

4.4.1 Dispense about 200 µl of saline into a suspension tube.

4.4.2 Take one or two average-sized colonies of suspected Salmonella from an overnight culture and emulsify them in the saline using the flat end of a sampling stick. Discard the stick properly.

4.4.3 Shake Latex Reagents 1 and 2 vigorously for a few seconds. Use the same stick to place one drop of each reagent in separate circles on a flat reaction card.

4.4.4 Using a disposable sample dispenser, transfer one drop of the bacterial suspension to each circle, being careful not to introduce air bubbles. Discard the dispenser properly.

4.4.5 Use the sampling stick to mix the contents of each circle, spreading it to cover the whole area. Discard the stick properly.

4.4.6 Place the card on a flat-bed rotator and run at 150 ± 5 rpm for 2 minutes. Observe for agglutination without removing the card from the rotator. Compare the result with a 40 µl drop of saline.

4.4.7 Discard the used reaction circles and refrigerate the Latex Reagents at 2 to 8°C.

4.5.Post – analytical stage:

4.5.1. Interpretation / Results / Alerts:

4.5.2.1 Negative:

If neither Latex Reagent causes agglutination and the appearance remains smooth gray-brown throughout the test.

4.5.2.2 Positive:

A color change occurs due to agglutination of one of the colored latex suspensions in the mixture, with a contrasting change in the color of the background. This usually results in single color agglutination in one Latex Reagent, but in a mixed Salmonella culture, two colors may agglutinate in one Latex Reagent or a single color in both Reagents.

4.5.2.3 Non-specific:

All the particles clump together, resulting in gray-brown clumps against a cleared background. This may occur even in the presence of a positive reaction. If there has been a distinct color change in the test, these gray-brown clumps should be ignored. In this case, consider other method of identification.

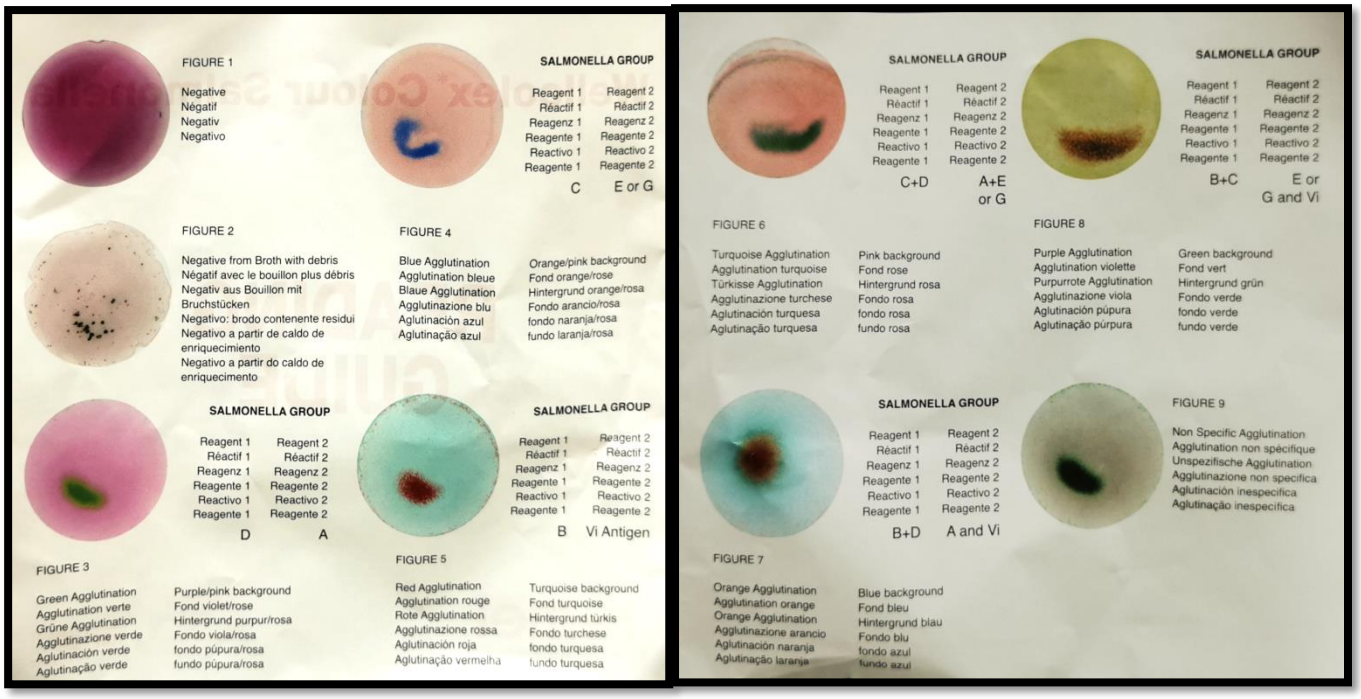
4.5.2.4 Alerts:

Positive results for Salmonella sp. as confirmed by biochemical tests (such as API 20E or automated ID) and Salmonella 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.

4.5.2. Reporting:

Use Colour Salmonella Reading guide:



4.5.3. Limitation:

- The Colour Salmonella test can identify Salmonella isolates to the serogroup level, which is sufficient for most purposes, but further identification through conventional biochemical and serological procedures may be needed.
- False-positive reactions may occur due to shared antigens in other species or genera, such as Citrobacter, but these can be differentiated through additional tests. The test may not detect all serogroups of Salmonella and may not give positive results if the level of Salmonella in the sample is low.
- A negative result does not confirm the absence of Salmonella, and additional testing may be necessary. Additionally, caution should be exercised when interpreting results obtained in tests with certain sub-optimal preparations of Selenite F broth.

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
Wellcolex Colour Salmonella, Package insert	Oxoid, Remel	2003	

8. Annexes #1: Quality Control Records sheet:

Test Name: Salmonella Colour Ag grouping Test								Kit Manufacture Name: ……... Date Kit opened:……..							
Positive Control Lot No.					Saline				Saline				Saline		
Positive Control Expiry Date															
Latex Reagent	Lot No.	Exp. Date	Red	Blue	Green	Negative Control	Red	Blue	Green	Negative Control	Red	Blue	Green	Negative Control	
R1															
R2															
QC Pass/Fail															
Staff name & Initial															
Date															
Remarks															