HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank (Serology)		
Document:	Internal Policy and Procedure		
Title:	Widal Test		
Applies To:	All Laboratory Staff		
Preparation Date:	January 06, 2025	**Index No:**	LB-IPP-176
Approval Date:	January 20, 2025	**Version :**	2
Effective Date:	February 20, 2025	**Replacement No.:**	LB-IPP-176(1)
Review Date:	February 20, 2028	**No. of Pages:**	03

1. PURPOSE:

1.1 The purpose of this document is to outline the standard operating procedure (SOP) for properly processing, examining, and reporting results for WIDAL test.

2. DEFINITONS:

2.1 Widal test is a diagnostic test for the in- vitro detection and quantitative estimation of specific antibodies to Salmonella organisms present in serum. It is used to detect, identify and quantitate specific antibodies in serum samples from patients suffering from (suspected) enteric fever, or pyrexia of unknown origin.

3. POLICY:

3.1 The Widal test is one method that may be used to help make a presumptive diagnosis of enteric fever, also known as typhoid fever.

4. PROCEDURE:

4.1 **Principle of WIDAL test:**

4.1.1 The Widal Test, an assay for Salmonella antibodies in which Salmonella organisms are agglutinated by patient sera, is performed by mixing serum dilutions with killed Salmonella suspensions harbouring known O (Somatic) and H (Flagellar) antigens. The assay endpoint is the highest dilution of patient serum that causes visible agglutination of the Salmonella suspension.

4.2 **Specimen Requirements:**

4.2.1 Fresh serum samples should be collected in the manner routinely used for any clinical laboratory test.

4.2.2 Sample collected in plain tube (Yellow/ Red) only – 3 ml.

4.2.3 Sample must be at room temperature upon performing the test.

4.2.4 Sample is stable for no longer than 72 hours after collection if stored at 2- 8° C and for longer period if frozen.

4.3 **Procedure:**

4.3.1 Slide agglutination method (Qualitative):

4.3.1.1 Bring reagents and specimens to room temperature. The sensitivity of the test may be reduced at low temperature.

4.3.1.2 Place 50 uL of undiluted serum into clean transparent slide test.

4.3.1.3 Swirl the antigen vial gently before using. Add 1 drop of antigen to each circle next to the sample to be tested.

4.3.1.4 Mix well using applicator sticks and spread over the entire area enclosed by the circle.

4.3.1.5 Place the slid on the mechanical rotator at 80-100 r.p.m. and read within 1 min.

4.3.2 Slid agglutination method (titration):

4.3.2.1 Bring reagents and specimens to room temperature. The sensitivity of the test may be reduced at low temp.

4.3.2.2 Place 20 uL, 10 uL, 5 uL of undiluted serum into clean transparent slide test.

4.3.2.3 Shake the antigen well & add 1 drop (50 ul) to each circle next to the sample to be tested.
Mix well using applicator sticks starting with the highest dilution up to the lowest.
Place the slid on the mechanical rotator at 80-100 r.p.m. within 1 min.
Read results immediately.

4.4 **Reading:**

4.4.1 Examine macroscopically the presence or absence of clumps within 1 min.

4.4.2 Negative: No agglutination
POSITIVE= degree of agglutination: 1/80, 1/160, and 1/320

4.5 **Interpretation:**

4.5.1 Titre in excess of 1:80 are usually significant & may reflect infection, but low titters can be found in positive patients.

4.5.2 Rise in titters on repetition of test after a few days will confirm the diagnosis of enteric fever.

4.5.3 Calculation:

4.5.3.1 Titers ≥ 1/80 (O antibodies) and ≥ 1/160 (H antibodies) indicates recent infection

4.6 **Results reporting:**

4.6.1 Qualitative Test:

4.6.1.1 Negative results are released as negative.

4.6.1.2 Positive results are indicated by observable agglutination in the reaction mixture.

4.6.2 Semi- Quantitative Test:

4.6.2.1 Record the last dilution of serum with visible agglutination:

Dilution	1/80	1/160	1/320
Serum	20ul	10ul	5ul
Reagent	50ul	50ul	50ul

4.7 **Quality Control:**

4.7.1 The positive control should show agglutination within 1 min.

4.7.2 The negative control should show NO agglutination within 1 min.

4.7.3 Otherwise repeat the test.

5. MATERIAL AND EQUIPMENT:

5.1 Bacterial antigens
5.2 Positive control
5.3 Negative control
5.4 Mechanical rotator adjustable to 80-100 r.p.m.
5.5 Heater at 37°c
5.6 Kit insert

6. RESPONSIBILITIES:

6.1 All technician assigned in Serology lab
6.2 The final report must be signed by section supervisor and approved by lab pathologist

7. APPENDICES:

7.1 N/A

8. REFERENCES:

8.1 CRESCENT Febrile Antigens kit Pamphlet
8.2 Medical Laboratory Technology Methods & Interpretations 4th ed by Ramnik Saad

9. APPROVALS:

	Name	Title	Signature	Date
Prepared by:	Dr. Fatma Hassan Ahmed	Clinical Pathologist		January 06, 2025
Reviewed by:	Dr. Kawther M. Abdou	Consultant & Lab. Medical Director		January 08, 2025
Reviewed by:	Ms. Noora Melfi Alanizi	Laboratory & Blood Bank Director		January 09, 2025
Reviewed by:	Mr. Abdulelah Ayed Al Mutairi	QM&PS Director		January 12, 2025
Reviewed by:	Dr. Tamer Mohamed Naguib	Medical Director		January 14, 2025
Approved by:	Mr. Fahad Hazam Alshammari	Hospital Director		January 20, 2025