Practical Immunology

Lab 3

Widal Test

Widal Test

Widal test: is a serological method used to diagnose **enteric** or **typhoid fever** that is cause by the infection with pathogenic microorganism like ***Salmonella typhi*** and/or ***Salmonella paratyphi***.

This test was first used by a French physician called Fernand **Widal in 1896**.

Principle of Widal test:

The test is based upon a visible to the naked eye agglutination (clumps) reaction between antibodies of patient serum and antigens **specifically** prepared from ***Salmonella sp.*** (kit)

Clinical Manifestation of Typhoid Fever

1. High fever
2. Bowel disturbance (Diarrhea or Constipation)
3. Weakness
4. Gastroenteritis
5. Headache
6. Loss of appetite
7. Stomach pains

Mode of Transmission

1. Ingestion of contaminated food or water
2. Rarely, from person to person- fecal- oral route.
3. Food handlers/ Carries.

Serodiagnosis of Typhoid

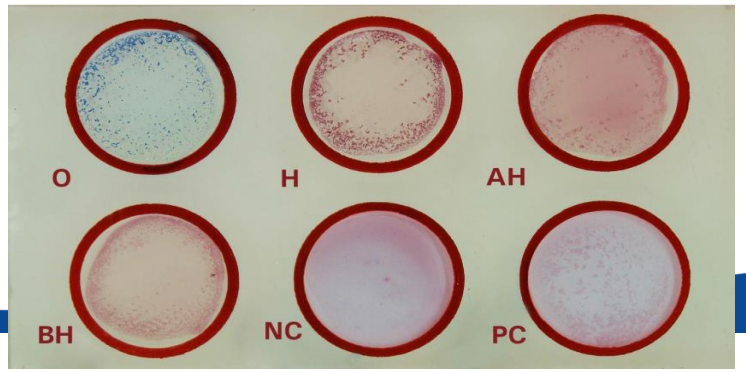
No.	Methods	Time Consuming
1	Widal test by Rapid Slide (Screening) test	1 min
2	Widal test by Tube Agglutination test	2-4 hours
3	Typhidot Tests (IgG/IgM rapid test) by Chromatography	15 minutes
4	Tubex TF	10 minutes
5	ELISA (Enzyme-Linked ImmunoSorbent Assay) (IgG/ IgM)	45 minutes – 2 hours
6	ECL (ElectroChemiluminescent immunoassays) (IgG/ IgM)	45 minutes – 2 hours
7	PCR (Polymerase Chain Reaction)	7-10 days

1. Slide Agglutination Test

A. Qualitative slide test

Procedure:

1. Bring all reagents to room temperature and mix well.
2. Add **80 µl** of serum onto each reaction circle labeled as O, H, BO, BH according to given antigen solution.
3. Add 1 drop of positive control onto the circle marked as PC and 1 drop of negative control onto the reaction circle marked as NC.
4. Add 1 drop of antigen solutions of *Salmonella typhi* 'O', *Salmonella typhi* 'H', *Salmonella paratyphi* 'BO' and *Salmonella paratyphi* 'BH' to circles labeled as O, H, BO, BH respectively in which test samples has been added.
5. Add 1 drop of O antigen to each of the control circles.
6. Mix well using a stick and rotate the slide gently.
7. Observe for agglutination.
   - Positive Test: Agglutination within a minute
   - Negative Test: No agglutination



B. Semi-quantitative slide test

This is performed for the samples which showed positive agglutination during qualitative slide test.

Procedure:

1. Using a pipette, dispense **80 µl, 40 µl, 20 µl, 10 µl and 5 µl** of patient serum onto the five reaction circles.
2. Add a drop of the antigen, which showed positive agglutination with the test sample in the qualitative method, to each circle.
3. Mix well using a stick and rotate the slide gently for one minute.
4. Observe for agglutination.

B. Semi-quantitative slide test

Interpretation:

The antibody titre of the test sample is its highest dilution that gives a **visible agglutination**.

- 80 µl = 1:20
- 40 µl = 1:40
- 20 µl = 1:80
- 10 µl = 1:160 and
- 05 µl = 1:320 titre.

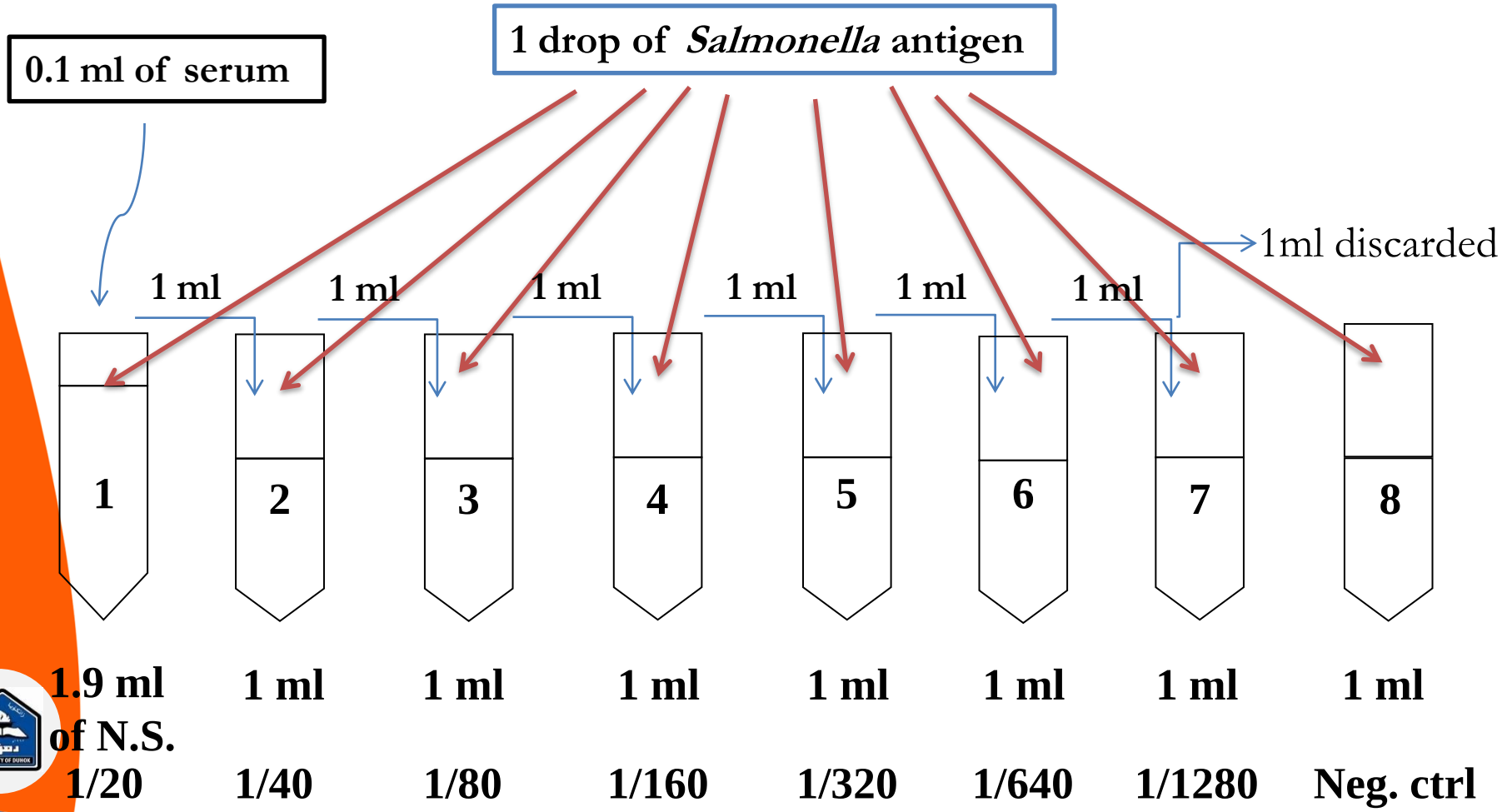
In this way, the **semi-quantitative slide test** provides an approximation to the expected results from tube agglutination test.

2. Tube agglutination test

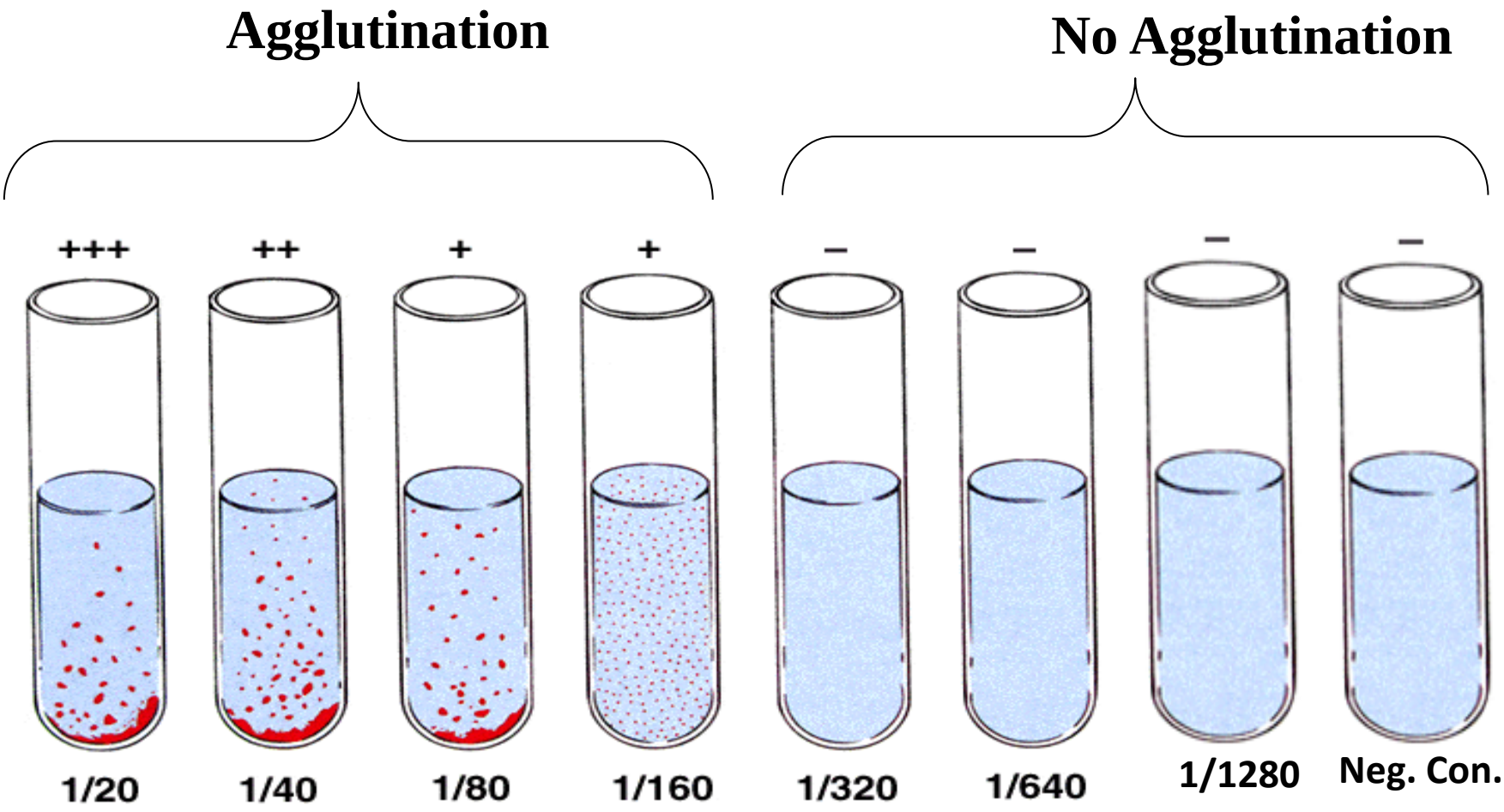
Procedure:

1. Label up 8 small plastic tubes in a rack. Using a pipette, dispense **1.9 ml** of saline into first tube, and **1.0 ml** into the remaining seven tubes.
2. Using a pipette, dispense **0.1 ml** of the patient's serum into the first tube, and mix well.
3. Make serial 2-fold dilutions of patient's serum by transferring **1.0 ml** from each tube into the second one, and mix well.
4. Continue the method of 2-fold dilutions up to the seventh tube, discard **1.0 ml** from the seventh tube. The eighth tube will contain only saline as a control.
5. Shake the reagent bottle and add 1 drop of the appropriate antigen suspension into each tube and mix well.
6. Incubate the tubes at 37° C for 2 - 4 hours.

2. Tube agglutination test



2. Tube agglutination test



In this case, the titre is **1/160**

2. Tube agglutination test

Observation and Interpretation:

Do not shake tubes before reading the results

Control tube (Tube No. 8): no agglutination **(-)**

Lowest titer tube (Tube No. 1): absolutely agglutination **(+++)**

Agglutination titer (Tube No. 4): the **highest dilution** of serum which appears bacteria agglutination.

Limitations of Widal test

1. Low specificity
2. Less sensitive
3. Confusing and difficult for the diagnosis of typhoid fever.

Why?

✓ **In spite of several limitation many physician depend on Widal Test.**

False positive reactions with Widal test

1. Previous immunization with *Salmonella* antigen.
2. Cross-reaction with non-typhoidal *Salmonella*.
3. Variability and poorly standardized commercial antigen preparation (kit).

False negative reactions with Widal test

1. First week of disease
2. Early treatment
3. Agammaglobulinemia
4. Prozone effect
5. An inadequate inoculum of bacterial antigen in host to induce antibody production.

Interpretation of Widal test

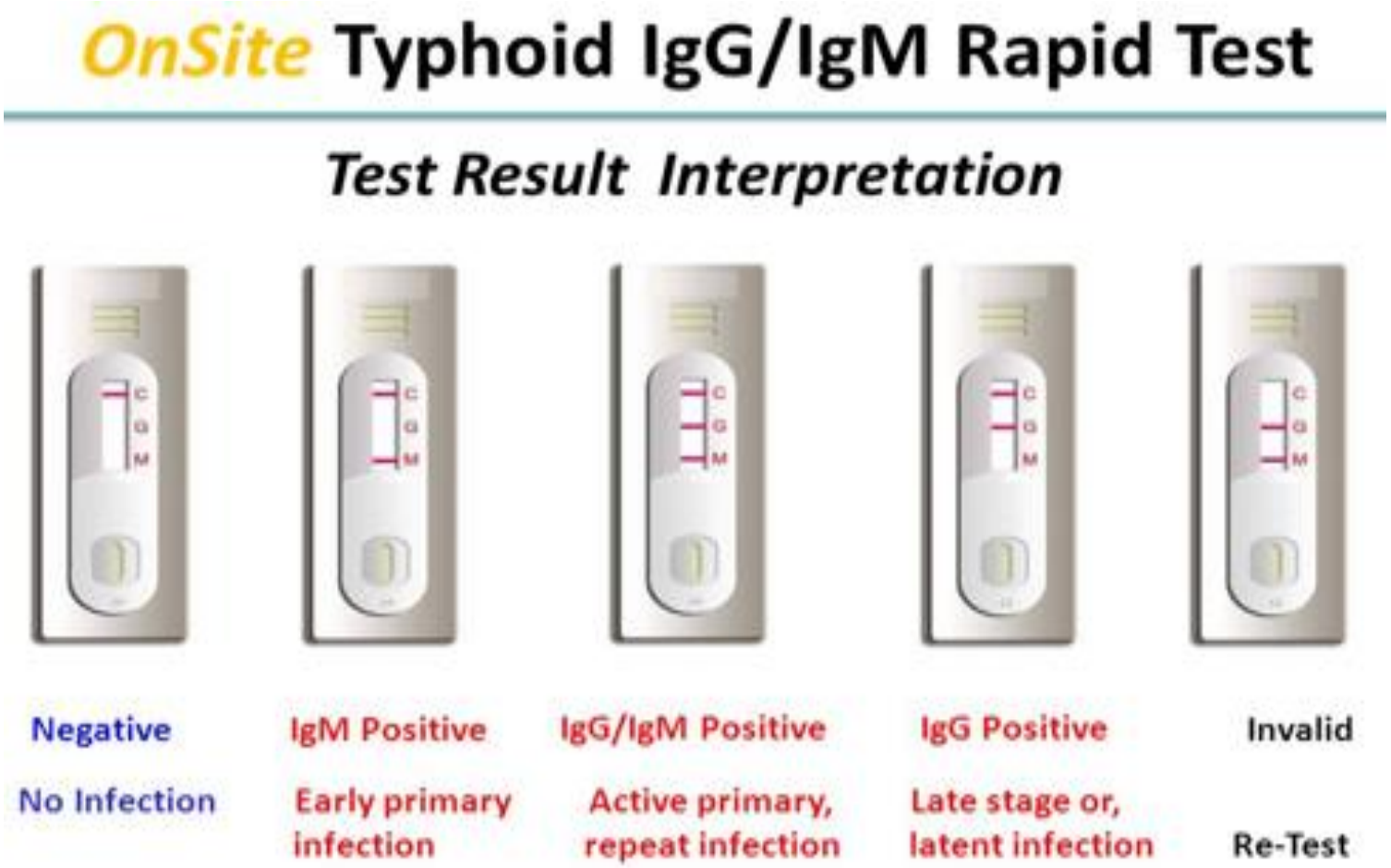
- Generally, the results of Widal test both qualitative, semi-quantitative and quantitative are inconclusive and cannot differentiate between **recent** and **past** infections.

Salmonella typhi O (+ve mean recent (acute) infection)

Salmonella typhi H (+ve mean old (chronic) infection)

Salmonella paratyphi BO, BH (+ve mean carrier can infect other)

Immunochromatography strip test for Typhoid fever

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