

# Agglutination Slid Tests for Febrile disease

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# Febrile Tests

*Brucella*

Undulating  
fever

**Culture &  
serology**

*Salmonella*

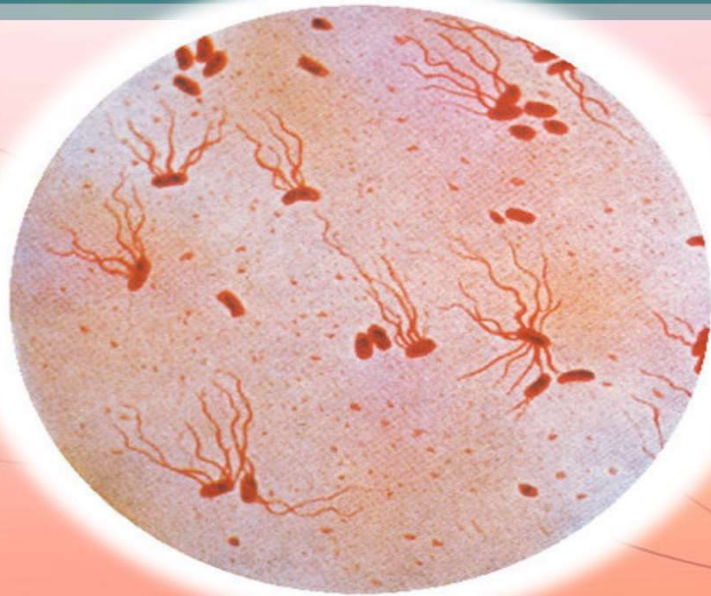
Typhoid &  
paratyphoid  
fever

**Widal test**

# Widal test



# *Salmonella*




- ***Salmonella*** is a Gram-negative facultative rod-shaped bacterium belonging to family ***Enterobacteriaceae***
- ***Salmonellae*** live in the intestinal tracts of warm and cold blooded animals.
- The main cause of Typhoid fever
- Also implicated in food infection and food poisoning.

# Types of *Salmonella*

- Typhoidal *Salmonellosis*:
- *S. Typhi*.....(8-14 )days
- *S. Paratyphi*....(A,B &C).....(1-10 )days
- There are human pathogen
- They enter via oral rout or usually with contamination drink & food.
- When bacteria ingested they transfer to blood stream cause:
  - ✓ High fever, Stomach ache
  - ✓ headache, loss of appetite & rash.
- Complications:
  - ✓ infection of brain
  - ✓ Gastrointestinal bleeding
  - ✓ Elderly immunosupressed.....high risk

# Non Typhoidal

- *S. enteritidis*
- *S. typhimurium*
- *Cause Gastroenteritis disease*
- ✓ **Incubation ...6-72 h.**
- **Symptoms ...2-7 days**
- ✓ *Fever, abdominal pain, diarrhoea, nausea & vomiting.*

- 
- In humans, *Salmonella* are the cause of two diseases called **salmonellosis**:
    - **Enteric fever (typhoid)**, resulting from bacterial invasion of the bloodstream.
    - **Acute gastroenteritis**, resulting from a food borne infection/intoxication.



# *Salmonella*

□ There are two species of Salmonella :

1. *Salmonella typhi*

2. *Salmonella paratyphi*

*S. paratyphi* A

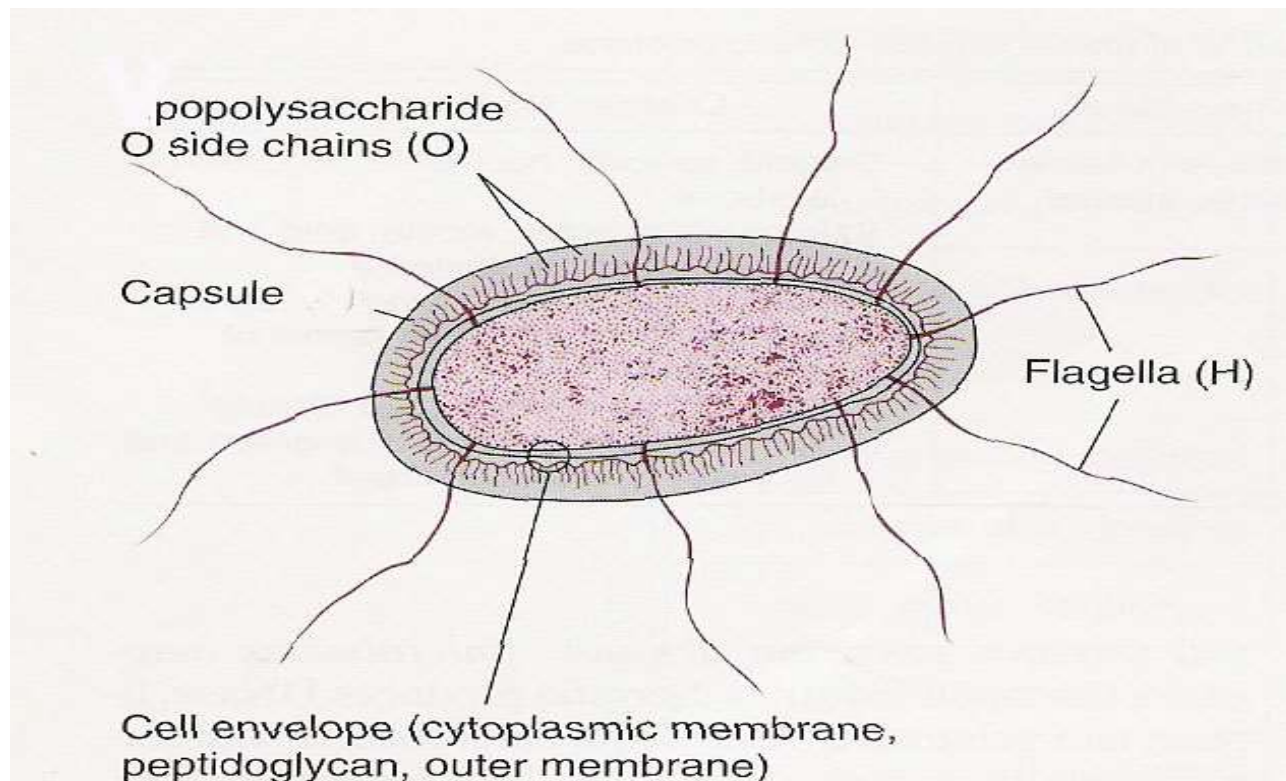
*S. paratyphi* B

*S. paratyphi* C



## *Antigenic structure of Salmonella*

1. H( flagella) antigens
2. O (somatic) antigens
3. Vi (Virulence) capsular polysaccharide antigens



❑ Both typhi and paratyphi have two types of antigen:

∞ Somatic ( O ) antigen, and that is thermostable.

∞ Flagellar( H ) antigen, and that is thermolabile.

• Salmonella can be isolated from GIT,

• urine

• blood

• bile

• bone marrow

• sputum

• food products, and milk.

❑ The confirmatory test for Salmonella is stool culture at the first week of infection.

# *Widal test*

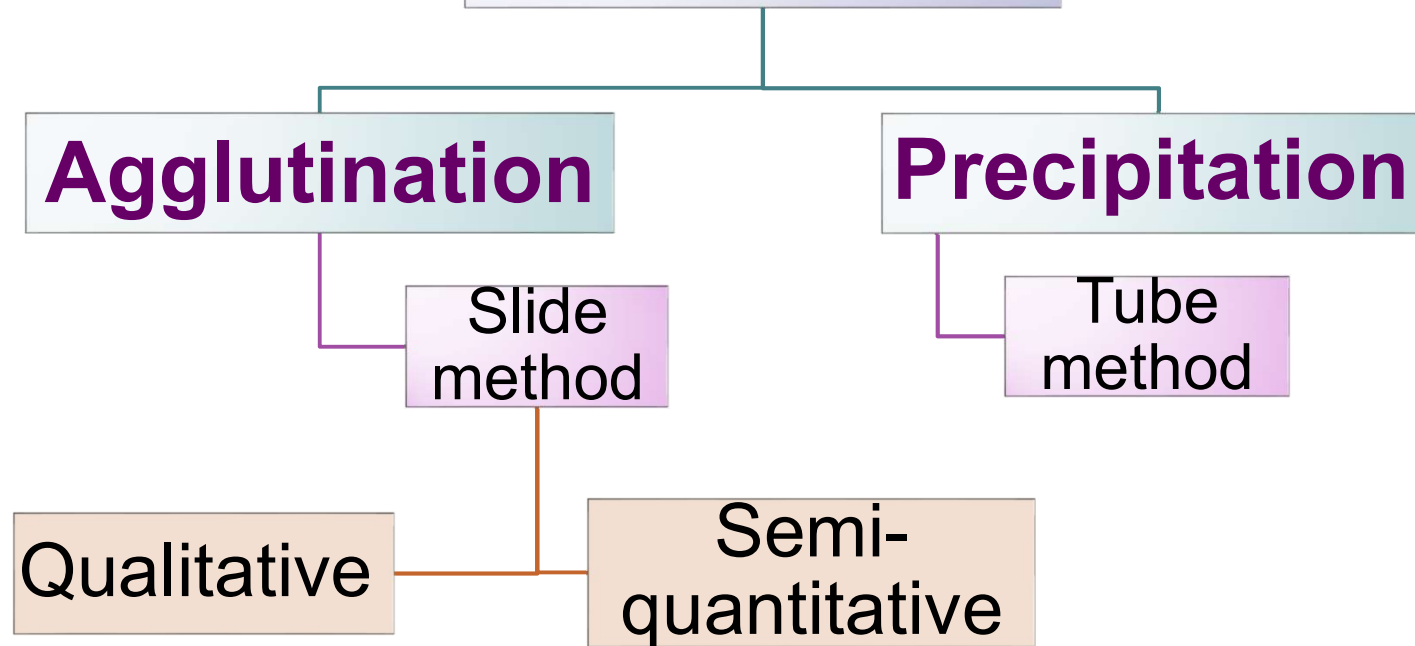
- ❑ Widal test defined as a test involving agglutination of typhoid bacilli when they are mixed with serum containing typhoid antibodies from a person having typhoid fever; used to detect the presence of *Salmonella typhi* and *S. paratyphi*.
- ❑ Use to diagnose the typhoid fever that caused by *Salmonella*.
- ❑ In diagnosis of typhoid fever, patient serum is tested for salmonella O and H antibodies against Ag suspension.
- ❑ The test is done after 2-3 weeks of infection or after 10 days of fever.



## **Principle of test :**

- Patients suffering from enteric fever would possess antibodies in their sera which can react and agglutinate serial doubling dilutions of killed colored Salmonella antigens in a tube agglutination test.

# *Widal test*



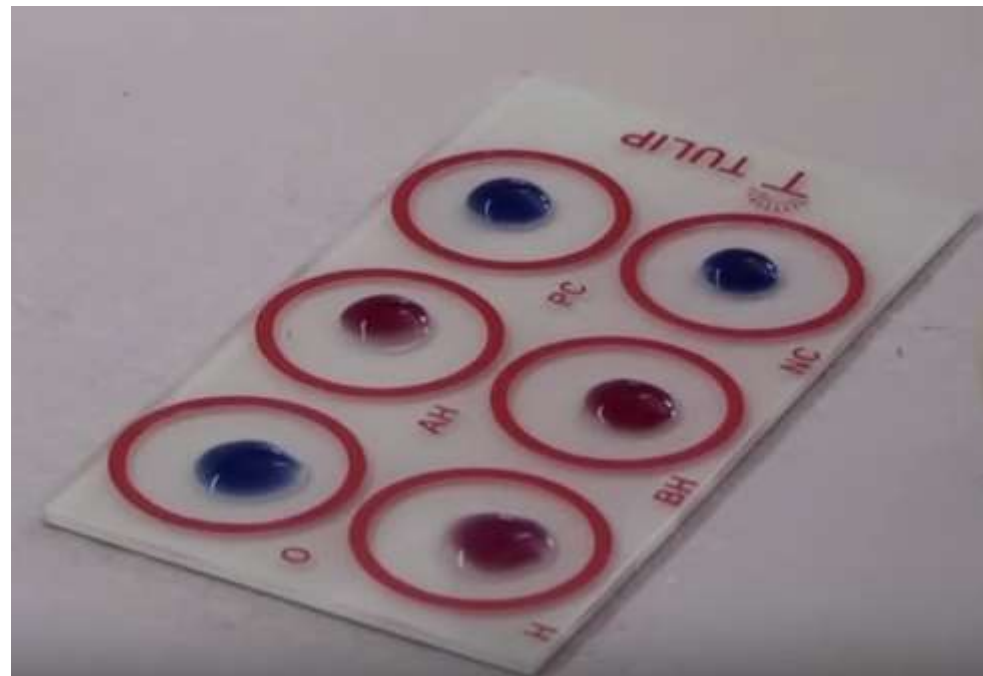
# Agglutination procedures

## Slide method

### 1<sup>st</sup> : Qualitative test :

- One drop each of patients' serum samples for the six antigens are placed on the circled card and one drop of each of the six Salmonella antigens are added separately and gently rotated for one minute.
- Positive & negative controls are also placed on their specific circles and mixed with drops of Widal TEST antigen suspension 'O'.

- Appearance of agglutination gives qualitative results.
- To know the titer for each of the antigens, the test is repeated with dilutions of serum.

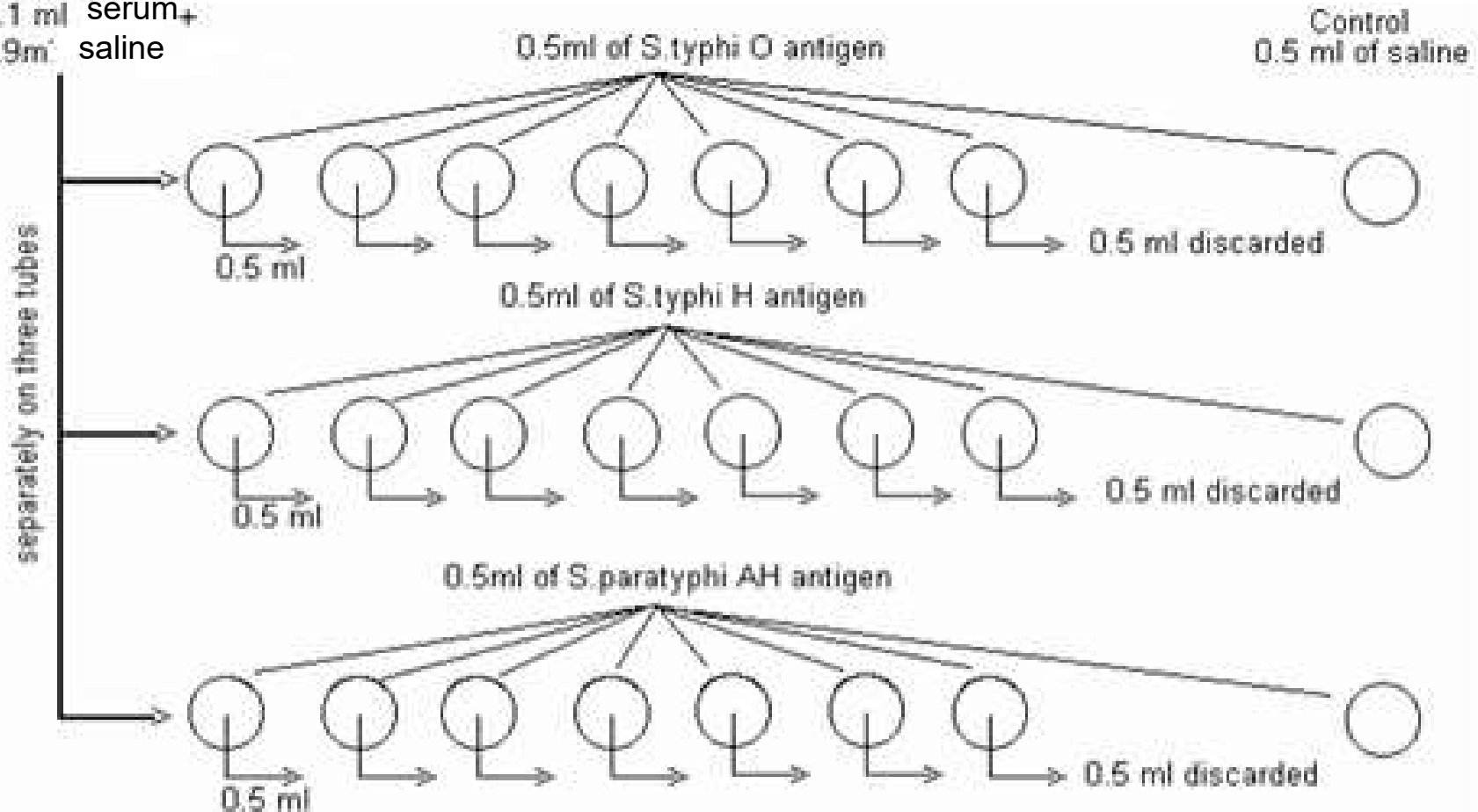




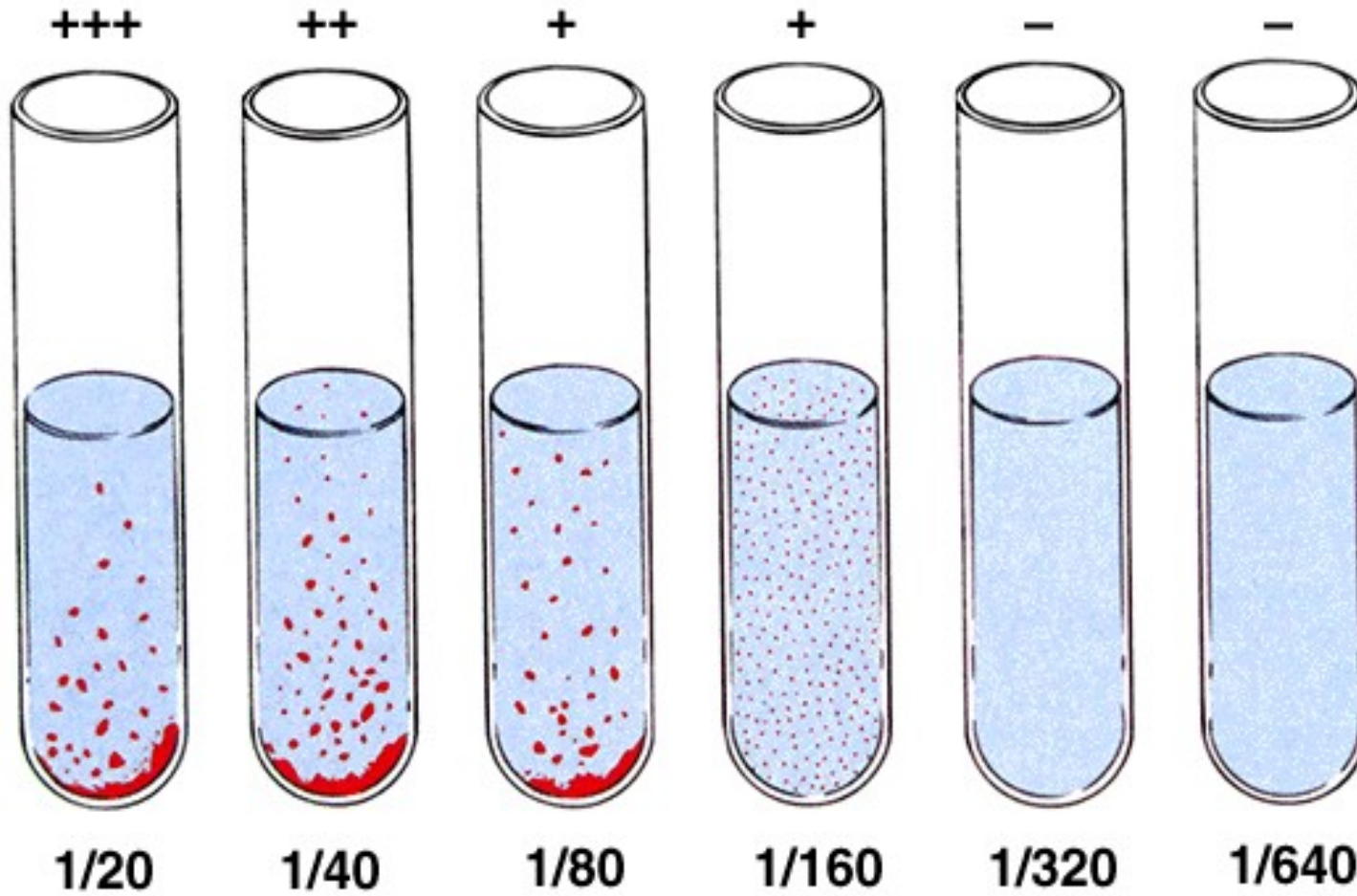
# 2<sup>nd</sup> Semi - Quantitative test

**Procedure:**

0.1 ml serum +  
0.9 ml saline

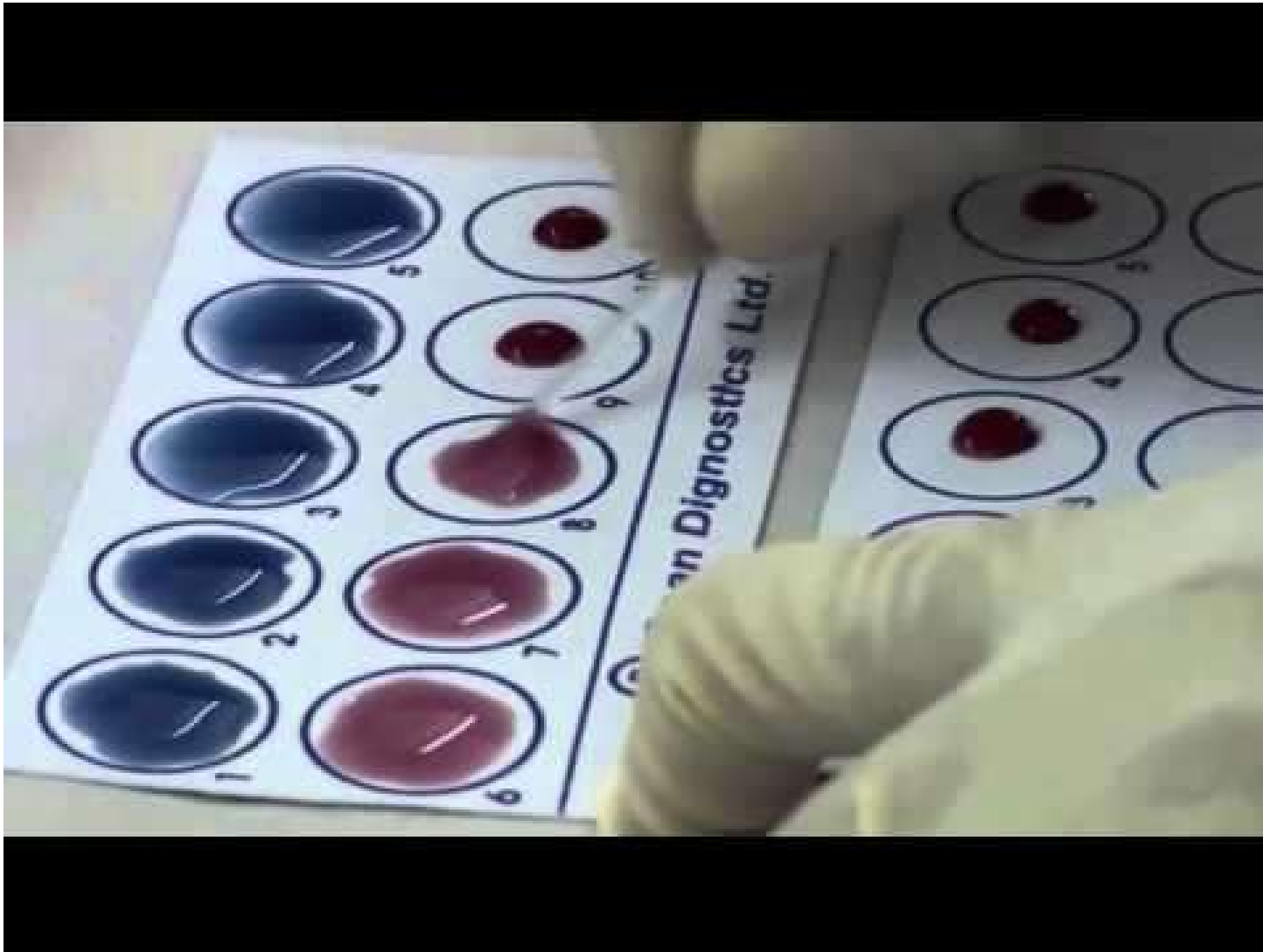


Initial dilution	1 in 10	1 in 20	1 in 40	1 in 80	1 in 160	1 in 320	1 in 640
Final dilution (after adding antigen)	1 in 20	1 in 40	1 in 80	1 in 160	1 in 320	1 in 640	1 in 1280



Here, Titer = 160

# another method for Semi - Quantitative test



# Precipitation procedures

Step



20 ul

serum +

180 ul

saline

1 : 10



8 tubes

and

10 ul

serum +

190 ul

saline

1 : 20



8 tubes

Step



100 ul

sample +

900 ul

**Typhi O**  $1/10 * 1/10$   
**reagent** = 1/100

and

100 ul

sample +

900 ul

**Typhi O**  $1/10 * 1/20$   
**reagent** = 1/200

## Step



Repeat step 2 for the remainder of *Salmonella* Ags

- Typhi H
- paratyphi A . H
- paratyphi B . H
- paratyphi C . H
- paratyphi A . O
- paratyphi B . O
- paratyphi C . O

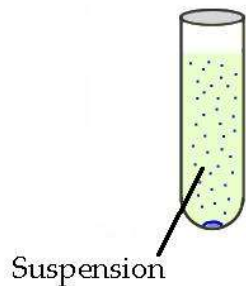
## Step

④

We finally get 16 tubes, Centrifuge them at 3,000 rpm for 5 min.

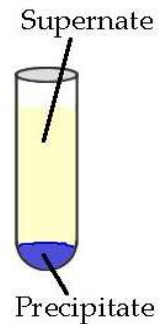


tap the bottom of the tube 2 times



If the ppt is resuspended,  
the the results considered  
**positive** (Ag-Ab rxn)

If the ppt still at the  
bottom, the results  
considered **negative**



## *Other Methods:*

### ➤ **Typhiod Rapid IgM-Assay**

- Detects specific IgM and IgG antibodies to S. Typhi

### ➤ **RT-PCR**

- The PCR technology has an unparalleled sensitivity and specificity for the diagnosis of typhoid

I thank  
you!

