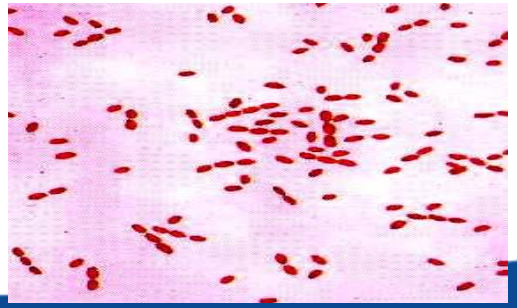
Practical Immunology

Lab 5

Brucellosis

Brucellosis

- **Brucellosis, Undulant fever or Malta fever:** is zoonotic disease caused by *Brucella* spp infecting the domestic animals.
- *Brucella* species are obligate intracellular pathogens (gram-negative cocco-bacilli bacterium) of the lymph nodes, liver, spleen, and bone marrow.
- The disease is named after the discoverer of the bacterium "David Bruce" in 1887. The name "Malta fever" is derived from the geographic endemic region where the fever is originally described.
- Four species can infect human but three species are more important:
  - ***Br. abortus* (Cattle).**
  - ***Br. suis* (Pigs).**
  - ***Br. melitensis* (Goats and sheep).**
  - ***Br. canis* (Dogs)** is of lesser importance.



Clinical Manifestation of Brucellosis

1. High fever
2. Discomfort
3. Anorexia (Malnutrition)
4. Arthralgia
5. Myalgia
6. Fatigue
7. Sweating
8. Headache
9. Weakness
10. Depression

Brucellosis

Rout of Transmission to human:

1. Consuming undercooked meat
2. Consuming raw (unpasteurized) dairy products
3. Contact with infected people (such as butchers)
4. Touching infected animals

Antibody appearance

- Serological tests are useful for diagnosis of **brucellosis** and for cases of **acute** and **chronic brucellosis** by demonstration of specific antibodies in patient's serum.
- **IgM antibodies**, appear in the serum **7–10 days** after infection and **persist** for up to **3 months** after which these **antibodies decline**.
- Then, **IgG antibodies** appear **after 3 weeks** of infection and **persist** for many **months or years**.
- Hence, **in acute brucellosis**, both **IgG** and **IgM** can be demonstrated; **in chronic brucellosis**, only **IgG** can be demonstrated, as **IgM** are **absent**.

Prophylaxis:

1. The disease could be prevented by pasteurization of milk which kill the bacteria.
2. Affected animals are detected and eliminated from the herd.
3. General principles of hygiene are imposed to prevent spread or reintroduction of infection.
4. In labs strict biosafety precautions.

Serological Tests

Serological tests used for the detection of Brucellosis:

No.	Methods
Serology level	
1	Rose Bengal test by Rapid Slide agglutination (screening) test
2	Rose Bengal test by Tube Agglutination test
3	Brucella IgG/IgM by Immunochromatographic assay
4	2 Mercaptoethanol Test
5	ELISA (enzyme-linked immunosorbent assay) (IgG/ IgM)
Molecular methods level	
6	PCR (Polymerase Chain Reaction)

Rose Bengal test

Principle of the test:

- It is a **slide agglutination** test for the **qualitative**, **semi-quantitative** detection of antibodies "**anti-Brucella**" in human and animal serum by using inactivated *Brucella abortus* cells, stained with **Rose Bengal** and suspended in an acid buffer (pH 3.6).

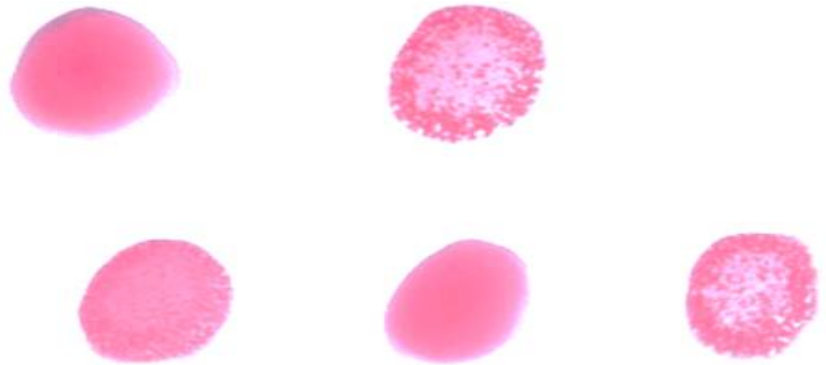
- The stained bacterial suspension agglutinates when mixed with patient serum containing specific IgG or IgM antibodies.

Rose Bengal test

Procedure:

A. Qualitative method

1. Allow the reagents and samples to reach room temperature.
2. Place **50 µl** of serum and one drop each of positive and negative controls into separate circles on the slide test.
3. **One drop** of the Rose Bengal reagent is adding separately.
4. Mix the drops with a stick, the mixtures are agitated gently for **four minutes** at room temperature.
5. Any visible agglutination is considered to be positive.



Rose Bengal test

- If agglutination less than 15 seconds: **1/640**
- Agglutination after 30 seconds: **1/320**
- Agglutination after 1 minute: **1/160**
- Agglutination after 1.30 minute: **1/80**

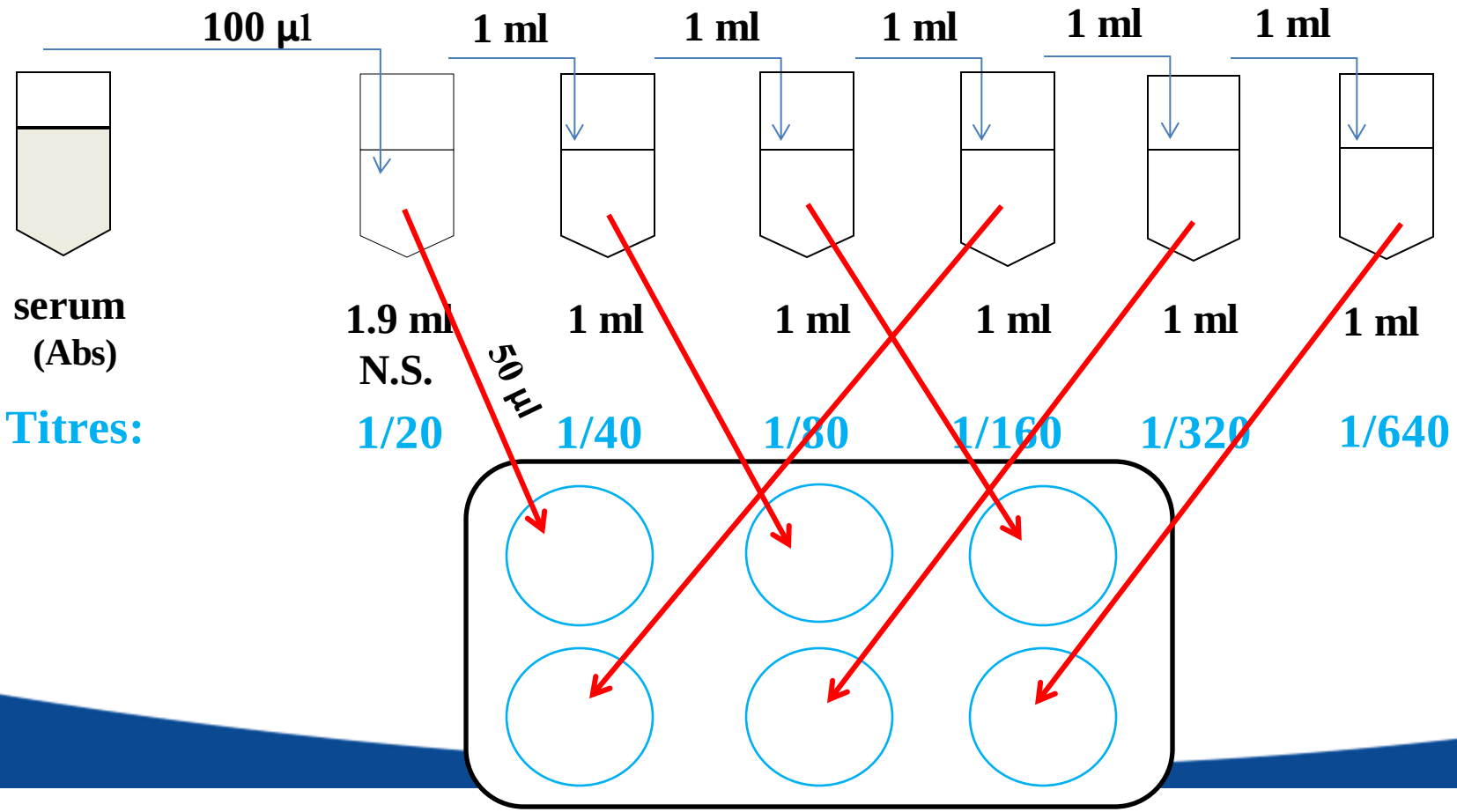
❖ **This test is a screening test only for the detection of Brucella agglutinins. If result is positive it must be confirmed by other serological tests for Brucellosis.**

Rose Bengal test

Procedure:

B. Semi-quantitative method

1. Make serial 2-fold dilutions of the serum in normal saline.
2. Proceed for each dilution as in the qualitative method.



Rose Bengal test

Reading:

- Examine macroscopically the presence or absence of the visible agglutination.
- The presence of agglutination indicates an antibody anti-Brucella concentration equal or greater than **25 IU/ml.**
- The titre in semi-quantitative or quantitative method is defined as the highest dilution showing a positive result.

Limitations of use of Rose Bengal test

False positive results:

- **Cross reactions** between *Brucella* spp antigens and other organisms such as *Yersinia enterocolitica*, *Escherichia coli*, *Salmonella* spp, *Vibrio cholerae*, and *Francisella tularensis.*

False negative results:

- A **prozone** may occasionally occur with the slide procedure (qualitative method). If it suspected, dilute the serum to 1/20 in saline and retest.

Limitations of use of Rose Bengal test

- Low sensitivity particularly in long chronic cases.
- Relatively low specificity in endemic area

2-Mercaptoethanol agglutination test

- This is performed for the samples which showed positive agglutination by Rose Bengal test.
- Addition of mercaptoethanol causes disruption of disulfide bond of IgM; hence only IgG is detected.
- This test is useful for specific detection of IgG antibodies, and titres higher than 1:80 are suggestive of active chronic infection.
- A high IgG antibody titre or a titre that is higher after treatment suggests relapse or persistent infection.

Procedure:

1. One volume of 2-ME plus one volume of patient serum in test tube.
2. Incubate at 37° C for 45 minutes.
3. Do Rose Bengal test.