Medical Laboratory Techniques Department

Lab 13: Rose Bengal test (Brucella test)

Msc. Alaa Khalaf Bediwi

Rose Bengal test (Brucella test)

- Brucella is a facultative, intracellular, Gram-negative bacteria.
- Brucella species are transmitted from infected animals include goats, sheep, cattle, pigs, and dogs to humans, **The most pathogenic species to humans are:**

1- **Brucella melitensis:** Primary host (Goats and Sheep), the most pathogenic in humans.

2- **Brucella abortus:** Primary host (Cattle)

3- **Brucella suis:** Primary host (Swine-Pigs)

4- **Brucella canis:** Primary host (Dogs)

Modes of transmission:

- The consumption of unpasteurized milk and soft cheeses made from the milk of infected animals, especially goats, infected with ***B. melitensis*** and with occupational exposure of laboratory workers, veterinarians, and butchery workers.

Clinical manifestation:

- ✓ High fever
- ✓ Anorexia
- ✓ Arthralgia (Joint Pain)
- ✓ Tiredness
- ✓ Headache
- ✓ Sweating
- ✓ Lymphadenopathy, Hepatomegaly, and Splenomegaly.

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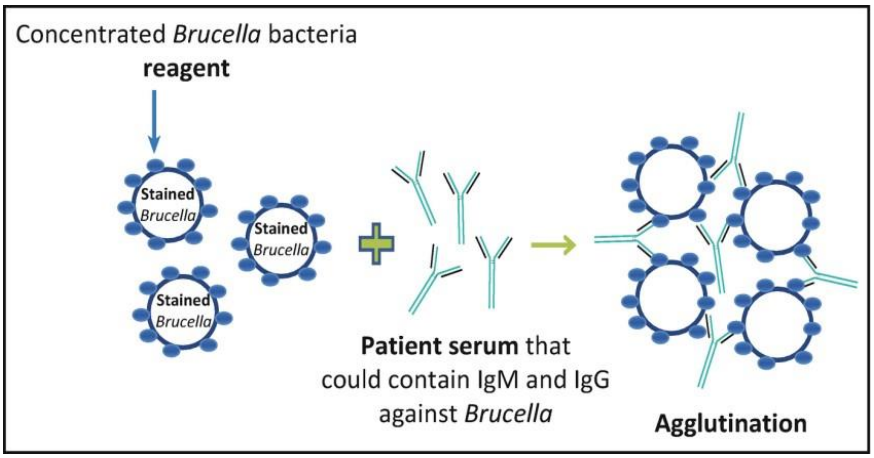
Laboratory Diagnosis (Serological Tests)

Rose Bengal agglutination:

Principle:

Brucella suspended stained with Rose Bengal was used for the direct detection of antibodies in the patient's serum during brucellosis infection.



Kit reagents:

1- **Reagent (antigen):** Brucella suspended with Rose Bengal stained.
2- **Positive control:** Specific antibodies against Brucella.
3- **Negative control:** Non-reactive diluted serum.

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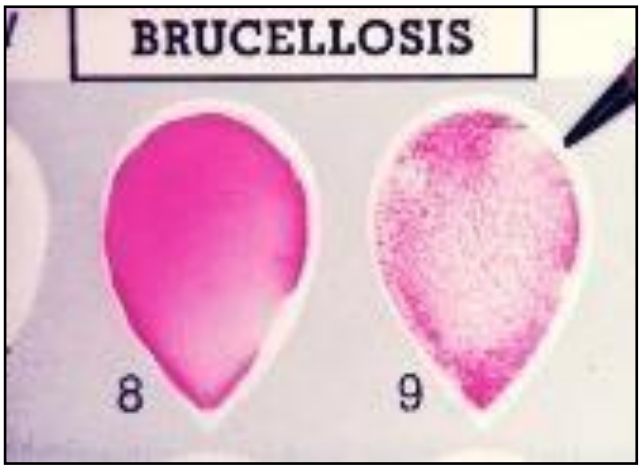
A. Slide agglutination test (Qualitative test):

Procedure:

1- Bring the reagent and sample at room temperature and mix the reagent vial gently before use.
2- Put **1 drop (50µl)** of the patient serum on the card.
3- Add **1 drop** of Rose Bengal antigen to the serum and mix them.
4- Rotate the slide slowly.
5- Observe immediately under a light source for any agglutination.
6- Report the result.



Read result:

- No agglutination = **Negative (-ve)**
- Agglutination = **Positive (+ve)**

✓ If agglutination appear after **15 seconds = +ve (1:640)**
✓ If agglutination appear after **30 seconds = +ve (1:320)**
✓ If agglutination appear after **1 minute = +ve (1:160)**
✓ If agglutination appear after **1.30 minute = +ve (1:80)**

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B. Tube agglutination test (Quantitative test):

- Tube tests are used as quantitative tests to determine the **titer** of antibodies in the samples, by the serial dilution of serum.

For example: If you want to make a serial dilution starting with (**1:20)** and a total volume of **(2 ml)**.

Use this equation: 1/dilution = amount of solute (serum) / total volume

- **1/20 = X / 2 ml**
- **X = 2 /20 = 0.1 mL (100 µl)** amount the serum
- Then add **1.9 ml (1900 µl)** of diluent (distilled water) to obtain a serial dilution with **(1:20)**.

Procedure:

1- Firstly, prepare **(6 or 7)** test tubes.

2- To obtain a serial dilution **(1:20)** with total volume **(2 ml)**.

3- Add **1.9 ml (1900 µl)** distilled water **+ 0.1 mL (100 µl)** serum to the **first tube only**.

4- Add **1 ml (1000 µl)** distilled water into **other tubes**.

5- Take **1 ml** from the **first tube** into the **second tube**, mix, and **repeat up to tube 7**. Then discard the last 1ml from tube 7.

6- When serial dilution is finished, add **50 µl** of a reagent (antigen) to each tube.

7- A positive reaction is the formation of visible clumps (agglutination) after a period of incubation.

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- The **highest dilution** of anti-serum giving agglutination is the **titer** of antibodies.
- The **last tube** that shows an agglutination is called the **end-point** of the test.