

# Agglutination Slid Tests for Febrile disease

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# Febrile Tests

*Brucella*

Undulating  
fever

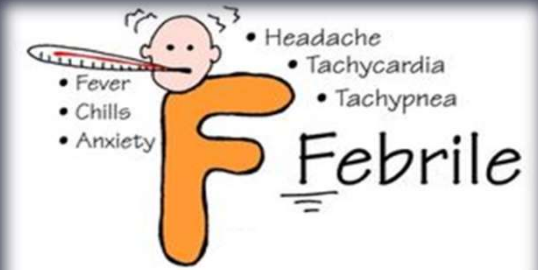
**Culture &  
serology**

*Salmonella*

Typhoid &  
paratyphoid  
fever

**Widal test**

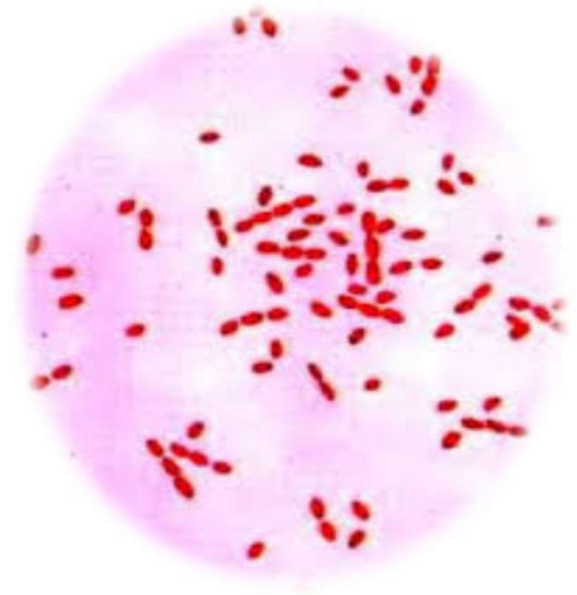
# Brucella



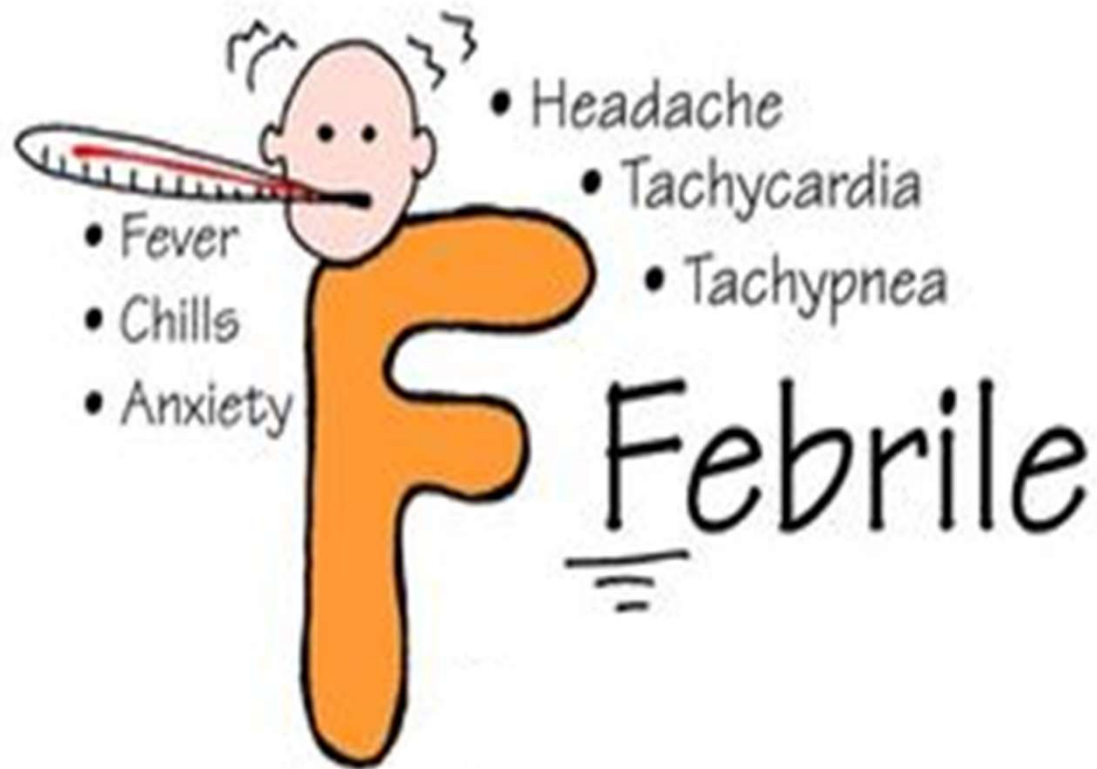
***And other  
organisms cause  
febrile reaction***

# *Brucella*

- Gram negative small coccobacilli
- Non-motile
- Non-capsulated
- Fastidious ; need special media with CO<sub>2</sub> and anaerobic condition called *Castañeda medium*.



- Cause brucellosis disease (*undulating fever, Malta fever*).



## Species of Brucella :

- *Brucella melitensis*....goats
- *Brucella abortus*....cattle
- *Brucella suis*...swine
- *Brucella canis*....dogs

# Mode of transmission

- Ingestion
  - Raw milk, unpasteurized dairy products
  - Rarely through undercooked meat
- Mucous membrane or abraded skin contact with infected tissues
  - Animal abortion products
    - Vaginal discharge, aborted fetuses, placentas

# Diagnosis

## 1. Serological test

- The objective of this test is to look for antibodies against Brucella, usually IgG is tested as IgM appear & disappear quickly.
- The serum agglutination test is the simplest and most widely used testing method.
- CDC utilizes a test called the Brucella microagglutination test (BMAT), a modified version of the serum (tube) agglutination test (SAT), that can detect antibodies to Brucella species. This test done after 2 weeks (10 days) of fever.



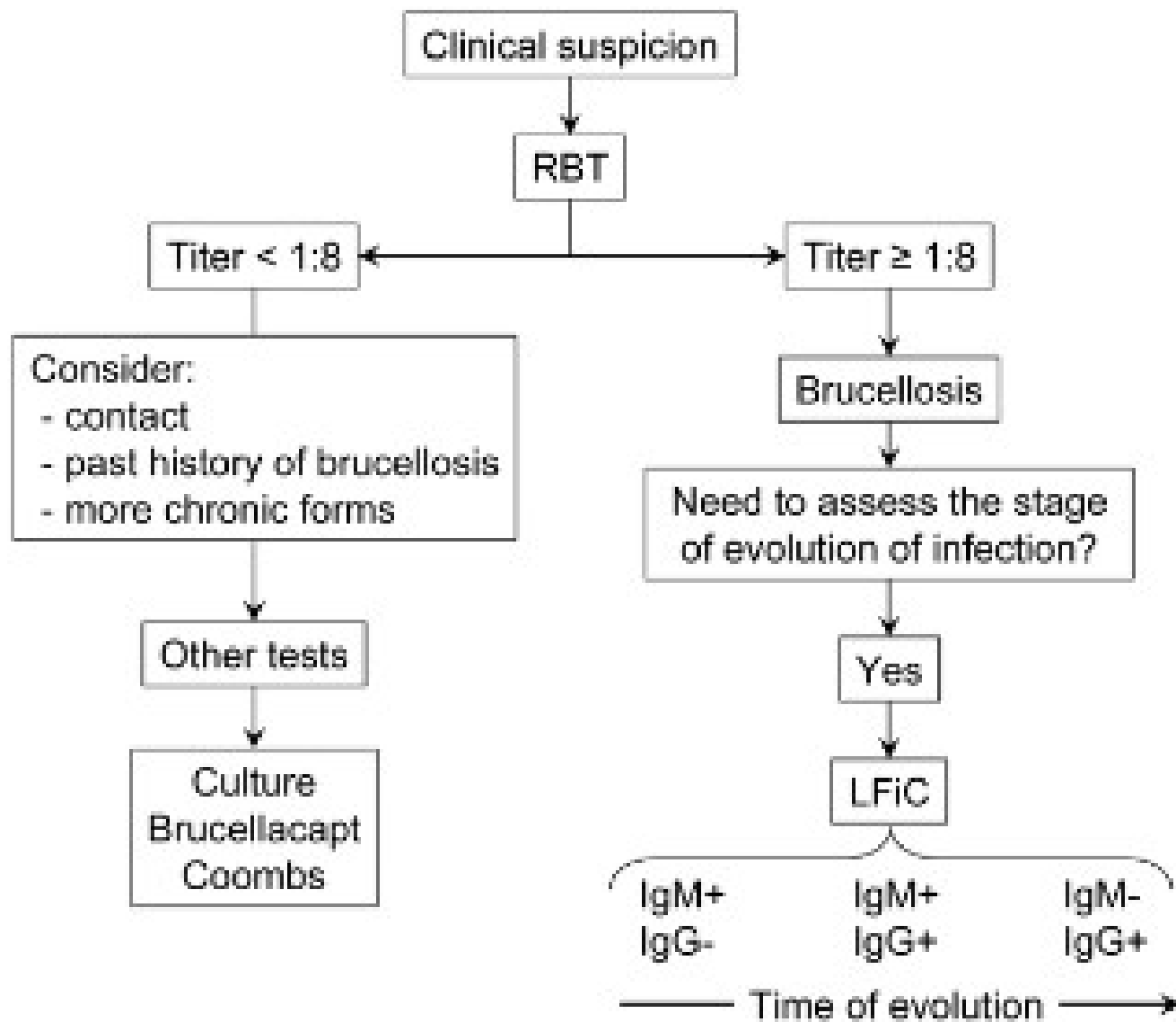
- *Brucella* microagglutination test (BMAT), a modified version of the serum (tube) agglutination test (SAT), that can detect antibodies to *Brucella* species - *abortus*, *melitensis* or *suis*.
- There is no serological test available to detect antibodies to *B. canis*.
- For a diagnosis to be made using serology, **two serum samples are required:**
- **The first serum sample should be** taken when a person is acutely ill ( $\leq 7$  days after symptom onset).
- **The second serum sample** should be drawn 2-4 weeks later to check for a rise in antibodies (a fourfold or greater rise in antibodies would mean an individual is positive for brucellosis).
- If submission of paired sera is not possible, a probable diagnosis can be made with a single serum sample.

## False Positives and Other Concerns About Reliability

- There are a few reasons why diagnosing an active *Brucella* infection can be challenging.
  1. Some other types of bacteria can cause a false positive, which means testing positive for the presence of *Brucella* when it's not present.
  2. Some immunizations can cause a test to be positive when there's no infection.
  3. A positive test doesn't always mean you have a current infection. It could mean you were exposed to *Brucella* at some point in the past. It might also mean you have an immunity against this type of bacteria.
  4. If you were recently exposed to the *Brucella* antigen, there may be too few antibodies to be detected by the test.
  5. More tests or follow-up testing may be needed to confirm or rule out brucellosis.

# The Rose Bengal test (RBT)

- The Rose Bengal test (RBT) is a simple, rapid slide-type agglutination assay performed with a stained *B. abortus* suspension at pH 3.6–3.7 and plain serum.
- It is often used as a **screening test** in human brucellosis and would be optimal for small laboratories with limited means.
- False-negative reactions occur especially in the **early stages of acute infection.**



# Procedure of Rose Bengal Plate Test

- Test Serum (0.03 ml) is mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameter.
- The mixture is agitated gently for 4 minutes at ambient temperature, and then observed for agglutination.
- Any visible reaction is considered to be positive.
- The test is very sensitive, especially in vaccinated animals, and positive samples should be retested by a confirmatory test such as the CF test or ELISA .
- False-negative reactions may occur and can be detected by retesting animals at intervals over a period of at least 3 months.

## 2. Blood Culture :

“ the confirmatory test ”

- Done in the first week of infection.
- Brucella is isolated from a blood culture on Castañeda medium.
- Prolonged incubation (up to 6 week) may be required as they are slow-growing, but by modern automated machines, the cultures often show +ve results within seven days.
- On gram stain they appear as dense clumps as Gram-negative
- coccobacilli and are rather difficult to see.



## 3. Animal inoculation test.



4- More tests or follow-up testing may be needed to confirm or rule out brucellosis, as the following :

1. **X-rays.** X-rays can reveal changes in your bones and joints.
2. **Computerized tomography (CT) scan or magnetic resonance imaging (MRI).** These imaging tests help identify inflammation or abscesses in the brain or other tissues.
3. **Cerebrospinal fluid culture.** This checks a small sample of the fluid that surrounds your brain and spinal cord for infections such as meningitis and encephalitis.
4. **Echocardiography.** This test uses sound waves to create images of your heart to check for signs of infection or damage to your heart.

# Test & Results

- An agglutination test is done by mixing 50ul of sample with 1 drop of reagent.
- A **normal (negative) result** shows no antibodies to Brucella.
- **Titer about 1:80 or more indicate Brucellosis** (serial dilutions).
- However, during the **first few days to weeks** of exposure to antigen, they may be very little antibody production & as brucellosis progresses, more antibodies will be present.
- If you suspects brucellosis, **you may need to repeat the test every 10 days or 2 weeks after the first test to notify this rise.**



Brucella antibody 'IgG' have long lifespan (1-2) years.

### Remember

- Prozone phenomena is the presence of high antibody titer that lead to Ag block and hence, false negative results are obtained.
- Dilution will resolve this problem.
- This phenomena appears obviously in Brucella serology test.

I thank  
you!

