Rose Bengal Test
Determination of anti-Brucella antibodies

Brucellae Characteristics:

- Brucellae are Gram-negative coccobacilli; lacking a capsule, flagella, endospores, and native plasmids; aerobic, but may need added CO_2. Oxidase and catalase tests are positive for most members of the genus *Brucella*. The outer cell membrane closely resembles that of other Gram-negative bacilli with a dominant lipopolysaccharide component.

Classification and Antigenic Types:

- Three species ***B. melitensis*** (goat and sheep), ***B. abortus*** (cattle), ***B. suis*** (swine) are important human pathogens; ***B canis*** (dogs) is of lesser importance. Species are differentiated by production of urease and H_2S, dye sensitivity and cell wall antigens (A and M antigens). The major species are divided into multiple biovars.

Clinical Manifestations:

- Bacteria of the genus *Brucella* is caused Brucellosis disease, primarily in domestic, feral and some wild animals and most are also pathogenic for humans. Brucellosis also called (**Malta fever**, **Mediterranean fever, Gibraltar fever and undulant fever)** is an acute febrile disease caused by bacteria of the genus ***Brucella*** by ingestion of unsterilized milk or meat from infected animals or close contact with their secretions.

Symptoms

- The incubation period is often difficult to determine but is usually from 2 to 4 weeks. The symptoms of brucellosis are nonspecific.

Signs and symptoms

- **are include:** Fever, Chills, Sweats, Fatigue, Headache, Anorexia, Weight loss, Depression, Joint, muscle and back pain.
- The leukocyte count tends to be normal or reduced, with a relative lymphocytosis. On physical examination, **splenomegaly** may be the only finding.
- May produce an **undulant fever** in which, the intensity of fever and symptoms recur and recede at about 10 day intervals.
- Hypersensitivity reactions may occur in individuals who are exposed to infective material after previous, even subclinical infection.

Pathogenesis:

- Brucellae are facultative intracellular parasites, Portals of entry are the mouth, conjunctivae, respiratory tract and abraded skin. Organisms spread, possibly in mononuclear phagocytes, to reticuloendothelial sites. Small granulomas reveal a mononuclear response; hypersensitivity is a major factor.

Epidemiology:

- Brucellosis is a true zoonosis in that virtually all human infections are acquired from animals through handling of infected animals or consuming contaminated milk or dairy products. Exposure is frequently occupational. The disease is common in the Mediterranean and Arabian Gulf regions, Latin America, Africa, and parts of Asia.

Control

- The disease is controlled by the routine practice of pasteurizing milk and milk products, as well as by comprehensive campaigns to eradicate the disease by destroying domestic animals which exhibit positive serologic reactions to brucellae. Vaccines providing some protection to cattle, sheep and goats are available.

Treatments:

- Treatments for 3 to 6 weeks with Doxycycline either alone or with rifampin or gentamicin for patients older than 8 years; doxycycline is replaced with trimethoprim-sulfamethoxazole for children younger than 8 years or for pregnant women.

Diagnosis

There are two main methods use for diagnosis (In humans, the definitive diagnosis is by culture or serology):Physical findings are not very specific.

- **Blood cultures**

Brucella is isolated from a blood culture medium, they are slow-growing(can take up to 6 weeks to confirm presence). Microscopic examination of stained smears (Gram staining method) can be useful for a presumptive diagnosis.

Serum agglutination

Most serological studies for diagnosis of Brucellosis are based on antibody detection These include: Rose Bengal test, Complement fixation test and ELISA.

Principle of Rose-Bengal test:

- The Rose Bengal is a slide agglutination test for the quantitative and semi-quantitative detection of antibodies (anti-Brucella) in human. The stained bacterial suspension agglutinates when mixed with samples containing specific IgG or IgM antibodies present in the patient sample.

Procedures: Direct slide agglutination test (Rose Bengal test).

1. Bring the test reagents and samples to room temperature (Note 1).
2. Resuspend the antigen vial gently. Aspirate dropper several times to obtain a thorough mixing.
3. Place 1 drop (50 µL) of the serum under test into one of the circles on the card. Dispense 1 drop of positive control serum and 1 drop of negative control serum into two additional circles.
4. Add 1 drop of Rose Bengal Antigen to each circle next to the sample to be tested.
5. Mix the contents of each circle with a disposable stirrer while spreading over the entire area enclosed by the ring. Use separate stirrers for each mixture.
6. Rotate the slide slowly either by hand or by means of a mechanical rotator (100 rpm.) for a period of 4 minutes (Note 2).
7. Observe immediately under a suitable light source for any degree of agglutination.

Reading

- **Nonreactive:** Smooth suspension with no visible agglutination, as shown by negative control.
- **Reactive:** Any degree of agglutination visible macroscopically. All reactive specimens should be further tested to clarify the situation.