Diagnosis of *Brucellae spp.*

Learning Objectives

- Determine the Sample and biochemical tests for *Brucellae* spp.
- Analyze the diagnosis and other tests Rose Bengal test.

Brucellae

It is the causative agents of **Brucellosis** specially ***Brucellae melitensis*** causes **Brucellosis** (also called "**undulant fever** or **Malta fever**, or **Bangs disease**) in human mainly by ingestion of **contaminated dairy products** or **contact with infected animals.** It's the most common Species that infect human. The brucella bacteria are located intracellular.

Although **each species of brucella** has a **preferred host**, all can infect a wide range of animals and **humans.** The pathogenic species of Brucella that infect human are as following (other species are found only in animals):-

Brucella Species	animal host
Brucellae melitensis	**goats and sheep**
Brucellae suis	**pigs**
Brucellae abortus	**cattle**
Brucellae canis	**dogs**

General Characteristics:

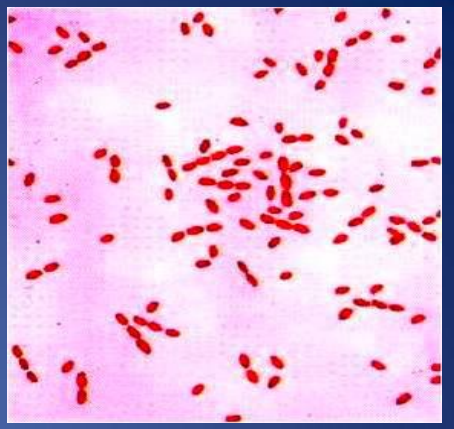
- They are Gram –ve, short and slender coccobacilli, 0.8-1.5 X 0.5-0.7*M. ,* non spore forming, non motile, non capsulated.
- Aerobic, but ***B. abortus*** species prefer CO_2 for growth.
-Grow best on media enriched with animal protein, such as: serum, liver extract agar or serum dextrose agar.

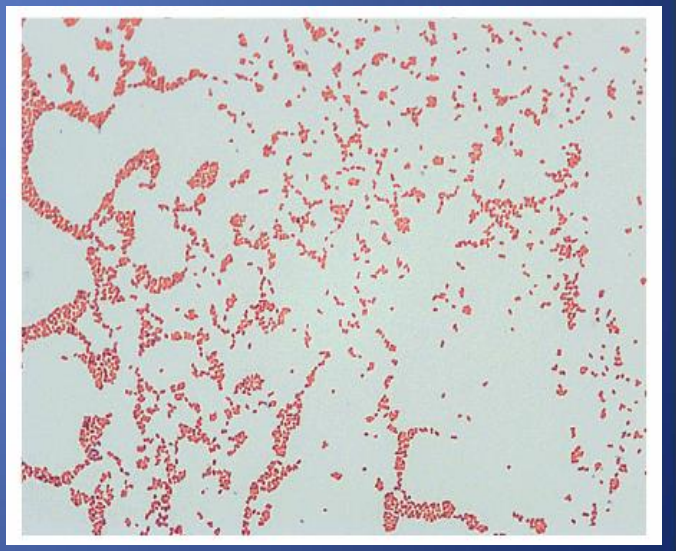
Viability:

* They are killed at 60^{o}C in 10 minutes, and by 1% phenol in 15min.
* They survive in agar at 0^{o}C for 1 month or more.
* Acid protection leads to its death in cheese and butter.

Laboratory Diagnosis:

1. Specimens:
   - A. Blood should be taken for culture.
   - B. Serum (for serology).
   - C. Biopsy from lymph nodes for culture.
2. General Characteristics: Gram –ve, short coccobacilli arranged singly, aerobic, non motile, non capsulated, non spore forming.
3. Culture : Brucella species grow on blood agar, Chocolate agar or supplement media such as Brucella agar.

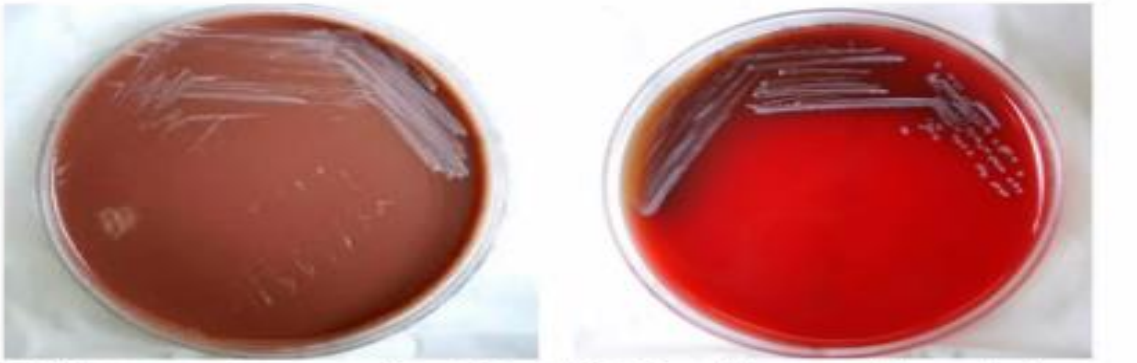




short G-ve bacilli (coccobacillary forms) of Brucella

Brucella (culture)

The media employed currently are serum dextrose agar, serum potato-infusion agar, trypticase soy agar (Castaneda medium), or tryptose agar. (in 10% CO_2)



Fastidious organism; growth on BA and CA, Punctate, grey, shiny, circular, convex colonies at 48 - 72 h at 35/37 °C, Non-hemolytic; non-pigmented

Blood culture:

- It is important in the acute phase of the infection.

It should be carried out repeatedly

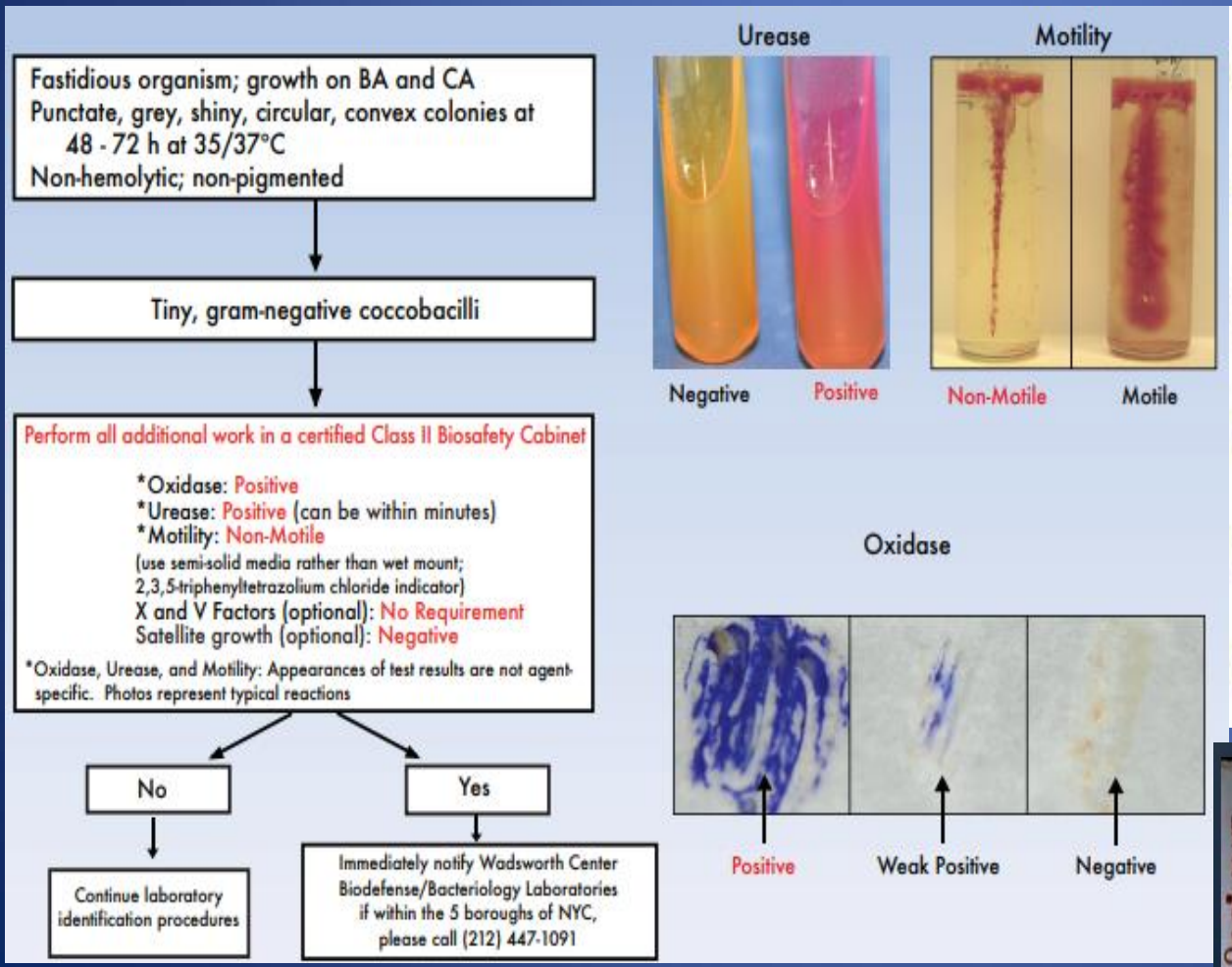
- The confirmatory test is done in the first week of infection.

It can be isolated from a blood culture on Castaneds 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.

- The addition of 5% heated horse or rabbit serum enhance the grow on all media, culture should be incubated in 5-10% CO_2 in humid condition. They are intracellular parasites, there habitats require nutrients such as: vitamins, amino acid, salt, and glucose. They should be observed and subcultured for at least 3 weeks, before being discarded as –ve results.
- On culture the colonies appears small, convex, smooth, translucent non hemolytic, slightly yellow after at least 48hr. of incubation.

4. The biochemical tests: Isolated Brucellae can be identified by biochemical test as followings:

5. Commercially available biochemical tests such as API 20 NE are particularly useful for the rapid and easy identification.



1. Catalase: positive (+ve)
2. Oxidase: positive (+ve)
3. Motility: non-motile
4. Indole, MR, VP, Citrate (IMViC): -, -, -, -
5. Urease test: positive (+ve)
6. Nitrate reduction test: positive (+ve)

6.Serological test : Analysis of a serum sample from patient for a rise in antibody titer to Brucella can be used to make a diagnosis (even if organisms are not isolated). The serum agglutination test that widely used and detects Ab to *Brucellae abortus, B. melitensis* and *B.suis* but not *B.canis*.

- It is +ve in only 30-50% of cases, mainly in the first 2-3 weeks of the disease, and become –ve when patient develop agglutinins, C.F. antibodies, and opsonins.
- It is +ve during relapse, although antibodies are found in high titer.

A titer of 1:160 or greater in the slide agglutination test (Rose Bengal test)is Considered diagnostic.

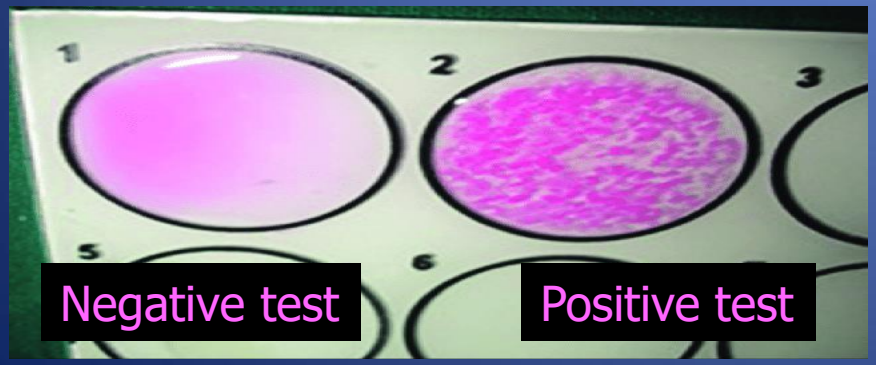
Rose Bengal Test (RBT)

- **Spot test** for brucellae diagnosis(humans and animals).
- Detects specific antibodies (IgM and IgG types).
- Advantages: (rapid, inexpensive, sensitivity and specificity).
- Limitations: low sensitivity particularly in long chronic cases and vaccinations.
- **Procedure**

1.Place a drop (30 µl) of undiluted serum on a slide.

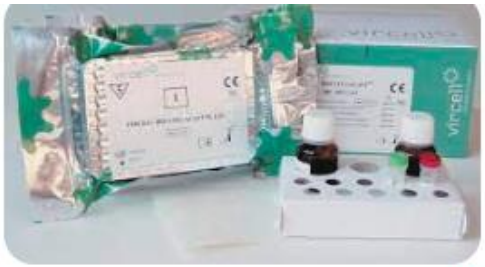
2.Add a drop of the reagent (Rose Bengal Brucella antigen) next to the drop of the serum.

3.Mix both drops by the disposable stirring stick, spreading them over the full surface of the circle. Observe for the result.



BRUCELLACAPT® Test Prensibi

Brucellacapt (Vircell, Granada, Spain) has been introduced as a rapid (18 to 24 hours) and easy serologic test to carry out. The test is based on immunocapture agglutination methodology that can detect, in a single step, the nonagglutinating IgG and IgA antibodies. The sensitivity and specificity are similar to those of the Coombs test.



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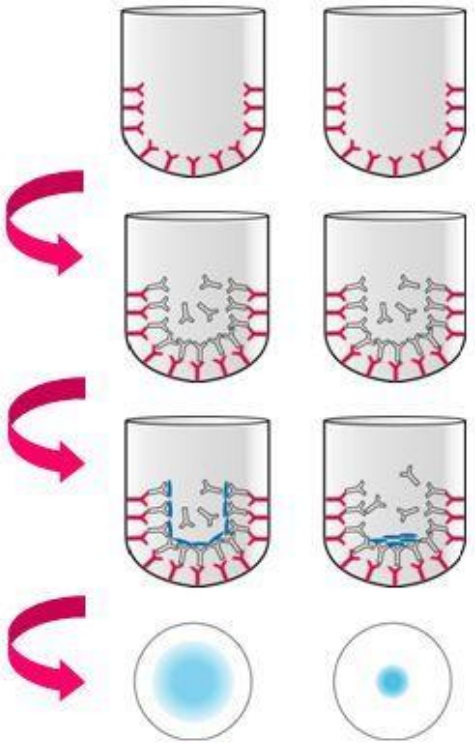

The *Brucella* Coombs gel test (Odak test) is a novel, simple, and rapid agglutination assay that is performed in microcolumns containing a gel matrix and Coombs antibodies. The test uses a centrifugation gel system similar to that employed for blood grouping. The presence of *Brucella* antibodies in the serum sample is revealed by the formation of a pink antigen/antibody complex, which remains trapped in the gel. In the absence of antibodies, the *Brucella* antigen precipitates at the bottom of the gel card system.

Brucella Coombs gel test

Diluent in the amount of 100 µl to the first well and 50 µl to the other wells was put in the dilution plate. 5

perfect agreement. For statistical calculations, 0.05 was accepted as significant. The Minitab (version 14) statistical software package was used for calculations.

Figure 1. Brucella Coombs gel test negative

Figure 2. Brucella Coombs gel test positive with 1/640 titration (two fold dilutions were made starting from the 1/40 dilution in the first well.)

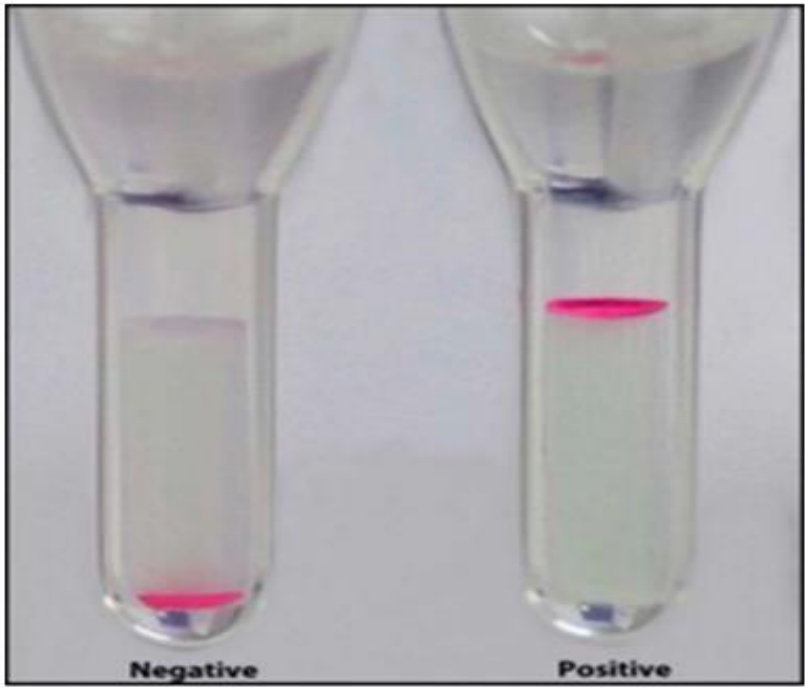

Figure 1. Negative and positive results in the Brucella Coombs gel test (www.toprakmedikal.com/documents/Brucella.ppt).


Thank You!