KING ABULAZIZ UNIVERSITY
FACULTY OF MEDICINE & ALLIED HEALTH
MEDICAL TECHNOLOGY PROGRAM

Laboratory Procedure Handout

Stained Brucella Suspensions

Agglutination Reaction

INTRODUCTION

Brucella is a genus of **fastidious**, **gram-negative**, **nonmotile**, **small coccobacilli** (**rods**) resemble Haemophilus in appearance, **with complex growth requirements**. **Growth** is often **promoted** by **elevated carbon dioxide concentration**. The **several species**, **obtained** from **different animal reservoirs**, have only minor cultural and antigenic differences. The **M antigen** is **more prominent** in **Brucella melitensis** and the **A antigen** in **Brucella abortus**. **All species** are **obligatory mammalian pathogens**, able to **grow** in **phagocytes** and **causing chronic infection**. **Brucella abortus** is a brucella that **causes abortion** in **cattle**. **It** may be **shed** in **milk** by **apparently healthy animals** and is thereby **transmitted** to **man**, in whom it **causes** an **infection** that may be **acute**, **relapsing**, **chronic**, or **subclinical**. **Brucella melitensis** is a brucella **obtained** from the **milk** and **urine** of **apparently healthy goats**. **It causes Malta fever**. **Brucella neotomae** is **obtained** from the **desert wood rat**. **Brucella ovis** is **obtained** from sheep. **Brucella suis** is **obtained** from **pigs**. It occasionally infect humans.

Brucella **causes brucellosis**, **or undulant fever**, **it** is an **infection** that **causes fever**. **Brucella** is **transmited via animals (zoonosis)**, **specially cattle**, and **grows well** in **phagocytes**. **Animal tissue** or **unpasteurized milk** is the **usual means by which humans** are **infected**. **Cases are rare**, **often go undetected**, and **develop into chronic** and **recurrent episodes of the systemic disease**. **Culture isolation** is **difficult so that diagnosis is usually based upon laboratory serology**. But antibody does not confer immunity. Immunity to brucella is conferred by activated macrophages and lymphokines derived from them. **Brucella antibodies cross-react with *Vibrio cholerae* and *Francisella tularensis***. **Direct agglutination assay** is a **commonly used serologic test for Brucella infection**.

Stained brucella suspensions are **used** to **detect**, **identify** and **quantitate specific antibodies in sera**. **They are standardized, smooth suspension of killed bacteria which have been stained to facilitate reading of agglutination tests**. **Stained brucella suspensions** are **used primarily** in the **investigation of pyrexia, and are suitable for used in rapid slide tests, standard Widal tests anti-human globulin (Coombs's test) and complement fixation test**. **Serological tests are based on the fact that antibodies in serum, produced in response to exposure to bacterial antigens, will react with bacterial suspensions which carry homologous antigens**. In the **diagnosis of febrile disease**, **such tests should be used in parallel with appropriate cultural techniques for** the **isolation** and **identification** of the **causative organism**.

PRINCIPLE

Stained, **standardized**, **smooth suspensions** of **killed bacteria** (**brucella**) are **agglutinated** when **mixed** with **samples containing specific antibodies to brucella**.

REAGENTS

1. **Stained Brucella suspensions**, **contains** approximately **10^{10} bacteria/ml** and **contain 0.25% formalin and 0.01% thiomersal as preservative**.
2. **Positive serum "control", Which is known to have antibodies against brucella**
3. **Negative serum "control", Which is known to have NO antibodies to brucella.**

SPECIMENS

Use **serum** (**clotted blood**) **or plasma** which should be **fresh** (**stored for max 8 days at +2 to +8 C**) **or stored in the deep freeze**. Deep frozen samples (**at –25 C or below**) **must** have been **frozen within 24 hours after collection** and **must** be **used within 3 months**; **repeated thawing and freezing is to be avoided**. **Serum samples should be completely coagulated and should contain no particles or traces of fibrin after centrfugation. Highly lipaemic samples or frozen smaples which become turbid after thawing should be clarified by centrifugation (10 min, at approx. 15,000 x g) before use in the test**.

METHODS

A. Rapid Screening Test

1. **Bring the patient specimens are reagents to room temperature**
2. **Place 0.02 ml of undiluted serum in a 3 cm diameter circle on a white tile.**
3. **Add one drop of the appropriate well-shaken suspension using the dropper provided**
4. **Mix by stirring for a few seconds and spread to fill the whole area of the circle on the tile**
5. **Slowly rotate for one minute and observe for agglutination.**

*** If agglutination** is **visible within one minute ,** a significant **titer should be obtained** in a **confirmatory tube test**. The reaction is roughly equivalent to that obtained in a tube agglutination test with a **serum dilution of 1 in 80** . **However**, **care should be taken in case a prozone (antibody excess) phenomenon is encountered (when a negative reaction will be obtained with a serum which has a high antibody titer**). **From time to time** it is **advisable** to test the **suspension** in a **complete slide titration or tube agglutination test with** a **known positive serum**, for example **Brucella abortus** or **Brucella melitensis antiserum**, and a **negative control serum**. **If** a **suspension agglutinate** with a **known negative serum**, **or fails to react with** a **known positive serum it should be discarded**. A prozone **phenomenon may be encountered**.

B. Rapid Slide Titration

1. **Using a 0.2 ml pipette, deliver 0.08, 0.04, 0.02, 0.01 and 0.005 ml of undiluted serum into a row of 3 cm diameter circles on a white tile.**
2. **Using the dropper provided, add one drop of the appropriate well-shaken suspension to each serum aliquot.**
3. **Mix by stirring for a few seconds with a wooden applicator stick, proceeding from the mixture containing 0.005 ml serum to that containing 0.08 ml serum, spreading the contents to fill the circles.**
4. **Rotate the tile slowly and read agglutination at one minute.**

- The **reactions obtained** are **roughly equivalent to those** which would **occur** in a **tube agglutination test with serum dilutions** of **1:20, 1:40, 1:80, 1:160, 1:320** respectively, although **significant discrepancies** are **sometimes observed**. **It is possible for a serum which is reactive in a tube agglutination fail to react on a slide**. **It is strongly recommended that a tube test should be performed with a sample if results are obtained which do not conform to clinical indications**. **False results may be obtained if** the **reagents** are **not allowed to reach room temperature before use**. Also, **false positive reactions are likely if the test is read more than one minute after mixing.**

C. Tube Agglutination Test

1. **Make one row of serum dilutions for each antigen to be tested as shown in the following "Table" , using saline or 0.25 % phenol saline as diluent. Mix the contents of tube 1 and transfer 1 ml to tube 2. Repeat for each tube, up to but not including tube 8, finally discarding 1 ml from tube 7.**
2. **Add one drop of the appropriate suspension to each tube of a given row using the dropper provided. Do not dilute the suspension before use.**
3. **Mix and incubate at 37 C for 24 hour.**
4. **Examine for agglutination.**

- **In a positive reaction there is obvious granular agglutination. In a negative reaction and in the saline control the appearance of the suspension should be unchanged, and show a typical swirl when the tube is flicked. The titer is the dilution of serum in the last tube showing agglutination. As a positive control, a dilution series of Brucella abortus or Brucella melitensis antiserum may be included. These are not, strictly speaking, standard sera and although titers should approach those given on the bottle labels, the exact titers may not always be obtained. A prozone phenomenon may sometimes be encountered.**

Tube number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Diluent ml	**1.9**	**1.0**	**1.0**	**1.0**	**1.0**	**1.0**	**1.0**	**1.0**
Patient serum	**0.1**	**_____ 1 ml serial dilutions_____**						**0**
Final dilution	**1:20**	**1:40**	**1:80**	**1:160**	**1:320**	**1:640**	**1:1280**	**Control**

INTERPRETATION

Agglutinations are found in a high proportion of normal individuals and titers of less than 1 in 80 are of doubtful significance.

A rising or falling titer is more singificant than a single high titer. It is difficult to differentiate between a Br. Abortus and a Br. Melitensis infection by serological tests, but from the point of view of treatment, this distinction is not necessary.

False positive reactions may occur with sera from patients infected with *Pasteurella tularensis* or vaccinated with *Vibrio cholerae*.

References

1. **MUREX Diagnostic Limited**
2. **J. H. L. PLAYFAIR "Immunology at a Glance".**
3. **AUSTYN & WOOD "Principle of Cellular and Molecular Immunology".**
4. **D. P. STITES etc. "Basic & Clinical Immunology".**

DR. MUSTAFA HASAN LINJAWI