INTRODUCTION:
Brucellosis is a worldwide zoonosis caused by bacteria of the genus Brucella, and represents an important public health problem in many countries. The high variety of clinical manifestations of the human brucellosis make the diagnosis difficult. Direct agglutination, Rose Bengal test, Coombs test and ELISA are the most widely used techniques for the serological diagnosis of brucellosis. The Rose Bengal is a fast, simple and sensitive assay used as screening test. It should be always confirmed by other bacteriological and serological test (BRUCELLACAPT® and ELISA).

PRINCIPLE OF THE TEST:
It is a card test for the detection of agglutinating antibodies by using inactivated Brucella cells, stained with Rose Bengal and suspended in an acid buffer. The acid pH of the suspension prevents the non-specific agglutination of the bacteria, increasing the specificity of the test. Agglutinating IgM, IgG and IgA antibodies contribute to the reactions.

KIT FEATURES:
All reagents are supplied ready to use. Sample predilution is not necessary. Antigen is supplied in a dropper for an easier dispensing. The results are immediately obtained.

KIT CONTENTS:
- VIRCELL ROSE BENGAL SUSPENSION: 5 ml of an acid suspension of inactivated Brucella stained with Rose Bengal, containing phenol.
- VIRCELL ROSE BENGAL POSITIVE CONTROL: 1 ml of positive control, containing phenol.
- VIRCELL ROSE BENGAL NEGATIVE CONTROL: 1 ml of negative control, containing phenol.

STORAGE REQUIREMENTS:
Store at 2-8°C and check expiration date.

STABILITY AND HANDLING OF REAGENTS:
Handle reagents in aseptic conditions to avoid microbial contaminations. Use only the amount of bacterial suspension solutions required for the test. Do not return the excess solution into the bottles.

RECOMMENDATIONS AND PRECAUTIONS:
1. For in vitro diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Do not use in the event of damage to the package.
5. Never pipette by mouth.
6. The controls in this kit include substances of animal and human origin. Although the human serum controls of this kit have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. Bacterial suspension contains inactivated Brucella abortus antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that these or other infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.
7. Bacterial suspension contains phenol (concentration <1%). Avoid contact with skin, eyes and mucosae.
8. The glass elements contained in kits could cause physical damage in the event of break. Handle with care.
9. Use only protocols described in this insert. Incubation times other than specified may give erroneous results.
10. Do not leave the reagents at room temperature longer than absolutely necessary.
11. It is necessary to shake the bacterial suspension immediately before use.

SPECIMEN COLLECTION AND HANDLING:
Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum samples are to be refrigerated (2-8°C) upon collection or frozen (-20°C) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated sera. Samples containing particles should be clarified by centrifugation. Do not inactivate the sera before to use.

ASSAY PROCEDURE:
1.-Bring all reagent to room temperature.
2.-Carefully shake the antigen suspension.
3.-Dispense 40 µl of each sample and controls onto the individual circles of the cards.
4.-Add onto each circle, close to the sample or control being analyzed, one drop of the Rose Bengal stained Brucella suspension.
5.-Mix both drops with the aid of a mixing rod until it covers all the circle surface.
6.-Carefully shake the card by hand or on a rocking shaker (100 r.p.m.) for 4 min. Read the presence or absence of agglutination after this time.

INTERNAL QUALITY CONTROL:
Each batch is subjected to internal Q.C. testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available. The control material is traceable to reference sera panels internally validated.

FOR IN VITRO DIAGNOSTIC USE
Manufacturer: VIRCELL, S.L. Pza. Dominguez Ortiz 1. Polígono Industrial Dos de Octubre. 18320 Santa Fe *GRANADA* SPAIN* Tel. +34.958.441264* Fax +34.958.510712
http://www.vircell.com
VALIDATION PROTOCOL FOR USERS:
Positive and negative controls must be run with each test run. It allows the validation of the assay and kit.
Positive control must present an evident agglutination. Negative control must present absence of agglutination.

INTERPRETATION OF RESULTS:
The presence of agglutination implies the presence of anti-Brucella antibodies in the sample. The absence of agglutination indicates a negative reaction and implies the absence of agglutinating anti-Brucella antibodies in the sample.

LIMITATIONS:
1.- This kit is intended to be used with human serum.
2.- The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting and timing of the incubation steps are essential for accurate results.
3.- The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by isolation techniques.
4.- This test will not indicate the site of infection. It is not intended to replace isolation.
5.- Lack of a detectable antibody level does not exclude the possibility of infection.
6.- False positives may be obtained in: (i) healthy people living in endemic areas who have been in contact with Brucella; (ii) people who passed the infection and are free of symptoms and signs at the time when the test is carried out; (iii) people who have suffered infections by bacteria sharing common epitopes in the lipopolysaccharide with Brucella.
7.- In patients with localized brucellosis, false negative results may arise due to the absence of agglutinating antibodies. In these cases, an alternative serological technique such as BRUCELLACAP®, which allows the detection of both agglutinating and non-agglutinating antibodies, should be used.

PERFORMANCE
SENSITIVITY AND SPECIFICITY:
147 serum samples were assayed with ROSE BENGAL against another commercial available Rose Bengal card kit obtaining a sensitivity of 99% and a specificity of 97.6%.

INTRA-ASSAY PRECISION:
3 sera (2 positive and 1 negative) were individually titrated in groups of 5 in a single assay performed by the same operator in essentially unchanged conditions. Titer shifts of no more than one dilution were observed.

INTER-ASSAY PRECISION:
3 sera (2 positive and 1 negative) were individually titrated on 5 different conditions in an assay in which the operator or the test day were different. Titer shifts of no more than one dilution were observed.

CROSS REACTIVITY AND INTERFERENCES:
16 samples known to be positive for other members of the syndromic group (Salmonella typhi O, Salmonella typhi H, cytomegalovirus, Toxoplasma, Epstein-Barr virus) were assayed. 3 samples known to be positive for antinuclear antibodies were also assayed. The results of the test demonstrated the specific reaction of the kit with no cross-reaction or interferences with the referred specimens.

SYMBOLS USED IN LABELS:

LITERATURE: