# PROTEUS OXK, OX2, OX19

## DIAGNOSTIC KIT FOR DETERMINATION OF PROTEUS OXK, OX2, OX19 ANTIBODIES FOR SLIDE AND TUBE TEST

# GENUINE BIOSYSTEM

**INTRODUCTION**

Rickettisae is an arthropod associated infection of ticks, mites, lice, or fleas. The disease is frequently associated with vertebrates; including humans as accidental hosts. Rickettsia conorii causes Mediterranean Spotted Fever in humans and is contracted by contact with infected brown dog ticks. Other Rickettsia include Rickettsia prowazekii, which causes typhus, Rickettsia rickettsii, which causes Rocky Mountain Spotted Fever and Rickettsia akari, which causes rickettsialpox. PROTEUS OXK, PROTEUS OX19, PROTEUS OX2 antigen suspensions are employed for the Weil-Felix test. The Weil-Felix test is based on the principle that some strains of Proteus share common somatic constituents with certain species of Rickettsia. Sera from patients infected with Rickettsiawill therefore produce agglutination with Proteus antigen suspensions. Antigen suspension of PROTEUS OX19 react strongly with sera of patients with typhus group rickettsiae and rocky mountain spotted fever. PROTEUS OX2 antigen suspension reacts strongly with sera of patients with spotted fever infections, while the PROTEUS OXK antigen suspension reacts strongly with sera of patients infected with scrub typhus.

**METHOD PRINCIPLE**

The smooth, attenuated stained PROTEUS antigen suspensions are mixed with the patient's serum. Specific antibodies to PROTEUS Antigens if present in the patient serum will react with the antigen suspension to produce an agglutination reaction. No agglutination indicates the absence of specific antibodies to PROTEUS antigens.

**REAGENTS**

**Package**

**PROTEUS OXK, Ox2, Ox19 1 x 5 ml or 1 x 10 ml**

**Working reagent preparation and stability**

1. Store the reagent at 2-8°C. DO NOT FREEZE.
2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial labels.

**SPECIMEN**

Store the reagent at 2-8°C. Do not freeze. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. Avoid exposure to elevated temperatures and air, as the reagents is highly sensitive to denaturation and drying.

**ADDITIONAL MATERIAL REQUIRED**

**Slide Test Method:** Stop watch, Positive control, Isotonic saline and Glass slide with clear/white background, appropriate Pipettes / Micropipettes,Mixing sticks & a High intensity direct light source.

**Quantitative Method:** Timer, Test tubes (12 mm x 75 mm), Test tube rack, appropriate Pipettes / Micropipettes, Isotonic saline/0.25% phenol saline, Incubator (37° C)

**NOTES:**

1. In vitro diagnostic regent for laboratory and professional use only. Not for medicinal use.
2. The reagent contains 0.01% thimerosal as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
3. Performance of the reagent must be verified with positive and negative controls and it is recommended that controls be run with each test series.
4. The reagent can be damaged due to microbial contamination or an exposure to extreme temperature.
5. Shake the reagent vials well before use to dispense the antigen suspension uniformly and improve test readability.
6. Only a clean and dry glass slides/tubes must be used. Clean the glass slides/tubes with distilled water and dry.
7. It is necessary to use the calibrated dropper provided in the reagent vial to dispense a reagent drop.
8. PROTEUS antigen suspensions are not from human sources, hence contamination due to HBsAg and HIV is practically excluded.
9. Do not use damaged or leaked reagents.

**SAMPLE COLLECTION AND STORAGE**

1. No special preparation of patient is required prior to sample collection by approved techniques. Do not use hemolysed and turbid serum samples.
2. Clean and dry glassware free from detergents must be used for sample collection.
3. Do not heat inactive in the serum.
4. Though freshly collected serum is preferred, samples can be stored at 2-8 °C, for 24 hours, or frozen for 8 days should a delay in testing occur.

**PROCEDURE - SLIDE METHOD**

Bring reagent and samples to room temperature before testing.Shake and mix the PROTEUS antigen suspensions well before dispensing. The procedure for PROTEUS OXK, Ox2, OX19 is identical.

**QUALITATIVE METHOD**

1. Place one drop of the test sample, positive and negative controls onto separate reaction circles of the glass slide using a sample dispensing pipette.
2. Add one drop of saline onto the next reaction circle of the glass slide.
3. Add one drop of patient serum to be tested on the next reaction circle of glass slide.
4. Add one drop of the appropriate PROTEUS antigen suspensions in each of the above circles (controls and patient sample which dispensed)
5. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
6. Gently rock the slide back and froth, observe for agglutination macroscopically at 1 minute against the white background.

**QUANTITATIVE METHOD**

1. Using saline prepare serial dilutions of the test sample positive in the qualitative method 1:2,1:4,1:8,1:16,, 1:32, 1:64, 1:128 and so on.
2. Perform the qualitative test procedure using each dilution as test specimen.
3. The titre is reported s the reciprocal of the highest dilution which shows as positive test result.

**PROCEDURE - TEST TUBE METHOD**

1. Take 8 test tubes and label them 1 to 8.
2. Pipette 1.9 ml of isotonic saline or preferably 0.25% phenol saline to tube no.1
3. To each of the remaining tubes (2-7) add 1.0 ml of isotonic saline of preferably 0.25% phenol saline.
4. To the tube No.1, add 0.1 ml of serum sample to be tested. Mix well.
5. Transfer 1.0 ml of the diluted serum from tube no.1 to tube no.2 and mix well.

6. Transfer 1.0 ml of the diluted serum from tube no.2 to tube no.3 and mix well. Continue this serial dilution till tube no.7.
7. Discard 1.0 ml of the diluted serum from tube no.7.
8. Pipette 1.0 ml of isotonic saline in tube no.8, which serves as a negative control.
9. To all the tubes, add 1 drop of appropriate PROTEUS antigen suspensions and mix well.
10. Cover the tubes and incubate at 37º C for 24 hours.
11. Observe for agglutination macroscopically in each tube of the dilution series.

**INTERPRETATION OF TEST RESULTS.**

**Quantitative Method**

1. Large and Medium agglutinates against white background : **Reactive**
2. Small black agglutinates against white background: **Weakly Reactive**
3. No agglutinates, even grey Background: **Non- Reactive**

Agglutination is a positive test result and indicates the presence of PROTEUS antibodies in the test sample.

No agglutination is a negative test result and indicates the absence of PROTEUS antibodies in the test sample.

**Quantitative Method:**

The titre of PROTEUS antibodies is the highest dilution of the test sample giving a positive test result.

**REMARKS:**
1. Positive results obtained in the slide test should be confirmed with the tube test to establish whether the titres are diagnostically significant or not.
2. Patients occasionally fail to develop any antibodies.
3. Weil-Felix reaction may vary widely from case to case of spotted fever and therefore may be of little help in either detecting the disease or differentiating if from murine typhus.
4. The test is not a substitute for culture. An appropriate attempt should be made to recover and identify the etiologic organism.
TM 5. The level of agglutinins in “normal' human sera can be 1:80 or more, especially with PROGEN OXK antigen suspension which may give “normal” titres up to 1:160. Arising or falling titre is more significant than a single elevated titre.
6. Agglutinins tend to fall rapidly within few months of recovery from an infection and therefore a high titre is useful indication of recent infection.
TM 7. Many serotypes pathogens have common somatic antigens. Agglutination with any of PROGEN antigen suspensions by the patient's serum cannot therefore be taken as a proof of infection by that particular organism but possibility of infection by an organism of similar antigenic constitution should be considered when reporting results.
8. Positive reactions due to previous vaccinations, anamnestic response, antibiotic therapy, narcotic addiction, other diseases such as malaria, infectious mononucleosis, typhoid, Brucellosis, tuberculosis, liver disease and autoagglutinations as well as urinary infection by Proteus, may affect the test results and therefore the results must be judged in the context of the clinical findings.
9. It is recommended to test the suspension as described with known positive and negative control serum with each run of test samples.
10. False positive results are likely if the test is read more than one minute after mixing on the slide test.
11. Any deviation in test procedure could lead to variable results.
12. Since techniques and standardization vary from lab to lab one tube difference in tube titres can be expected.
13. Use a separate disposable tip for each sample to prevent cross contamination.
14. Turbid and contaminated sera should not be used for testing.
15. After usage the antigen suspension should be immediately recapped and replaced at 2-8 ºC.
16. Reagent vials that have leakage/ breakage problem should be discarded.
17. Only qualified and well trained staff should use the reagents.
18. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.
19. The performance of the antigen suspension should be validated occasionally using positive control. Good physiological saline may be used as a negative control.

**PERFORMANCE CHARACTERISTICS**

1. The level of agglutinins in “normal' human sera can be 1:80 or more, especially with PROTEUS OXK antigen suspension which may give “normal” titres up to 1:160.
2. Positive reactions due to previous vaccinations, anamnestic response, antibiotic therapy, narcotic addiction, other diseases such as malaria, infectious mononucleosis, typhoid, brucellosis, tuberculosis, liver disease and auto agglutinations as well as urinary infection by Proteus, may affect the test results and therefore the results must be judged in the context of the clinical findings.
3. It is recommended to test the suspension as described with known positive and negative control serum with each run of test samples.
4. False results may be obtained if the reagents are not allowed to reach room temperature (22 to 30 C) before use. False positive reactions are also likely if the test is read beyond one minute after mixing.
5. A great number of false positive reactions have been reported in healthy individuals with Proteus antigens especially in slide agglutination tests. A titre of less than 1:160 should not be considered significant.

**LITERATURE**

1). Felix, A.(1942). Brit. Med.J., 11, 597-600. (2) J. G. Collee, J. P. Duguid, A. G. Fraser, Practical Medical Microbiology, 14th Ed.: 573-588. (3).