CE

PROGEN™

PROTEUS ANTIGEN SUSPENSIONS FOR WEIL-FELIX TEST.

INTENDED USE

PROGEN™ antigen suspensions employed in the Weil-Felix test, are used for the diagnosis of Rickettsial infection and differential diagnosis in patients with Febrile fever.

SUMMARY

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.
PROGEN™ OXK, PROGEN™ OX19, PROGEN™ 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 *Rickettsia* will therefore produce agglutination with *Proteus* antigen suspensions.
Antigen suspension of PROGEN™ OX19 react strongly with sera of patients with typhus group rickettsiae and rocky mountain spotted fever. PROGEN™ OX2 antigen suspension reacts strongly with sera of patients with spotted fever infections, while the PROGEN™ OXK antigen suspension reacts strongly with sera of patients infected with scrub typhus.

PRESENTATION

	5 ml	5 ml	5 ml
REF	105810005	105820005	105830005
Reagent	PROGEN™ OXK	PROGEN™ OX19	PROGEN™ OX2
PACKAGE INSERT	1	1	1

REAGENT

PROGEN™ OXK / PROGEN™ OX19 / PROGEN™ OX2 reagents contain ready to use standardized, killed, stained, smooth specific antigen suspensions of *Proteus* OXK/OX19/OX2.

REAGENT STORAGE AND STABILITY

1. Store the reagent at 2-8°C. DO NOT FREEZE. Keep the reagents away from direct sunlight.
2. The shelf life of the reagents is as per the expiry date mentioned on the reagent vial labels. Do not use beyond expiry date.
3. Once opened the shelf life of the reagent vial is as described on the reagent vial label provided it is not contaminated.

PRINCIPLE

The smooth, killed stained PROGEN™ antigen suspensions are mixed with the patient's serum. Antibodies produced due to rickettsial infection if present in the patient serum will react with the stained PROGEN™ antigen suspension to produce an agglutination reaction. No agglutination indicates the absence of Rickettsial antibodies.

NOTE

1. *In vitro* diagnostic reagent for laboratory and professional use only. Not for medicinal use. Keep the reagents away from direct sunlight.
2. The reagent contains 0.1 % sodium azide as preservative. Avoid contact with skin and mucosa. Sodium azide may react with lead and copper in plumbing and form highly explosive metal oxides, on disposal flush with large quantities of water.
3. Only clean and dry glass slides / tubes must be used. Clean the glass slide / tube with distilled water and dry.
4. PROGEN™ antigen suspensions are not from human sources hence contamination due to HBsAg and HIV is practically excluded.

SAMPLE COLLECTION AND STORAGE

1. No special preparation of patient is required prior to sample collection by approved techniques. Do not use haemolysed and turbid serum samples.
2. Blood collected by venipuncture should be allowed to clot naturally. Care should be taken to ensure that the blood sample are fully clotted.
3. Clean and dry glassware, free from detergents must be used for sample collection.
4. Do not heat inactivate the serum.
5. 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.

MATERIAL PROVIDED WITH THE KIT

Reagent Pack

PROGEN™ OXK Antigen Suspension (REF.:105810005), PROGEN™ OX19 Antigen Suspension (REF.:105820005), PROGEN™ OX2 Antigen Suspension (REF.:105830005).

ADDITIONAL MATERIAL REQUIRED

Slide test method: Stop watch, Positive control (available as PROGEN™ Positive Control (REF.:105840001), Physiological saline, glass slide with white background, appropriate pipettes/micropipettes, mixing sticks & a high intensity direct light source.

Quantitative method: Timer, test tubes (12x75 mm), test tube rack, appropriate pipettes/ micropipettes, physiological saline, Incubator (37°C).

TEST PROCEDURE

1. Bring reagents and samples to room temperature before testing.
2. Shake and mix the PROGEN™ antigen suspensions well before dispensing.
3. The test procedures for PROGEN™ OXK / PROGEN™ OX19 / PROGEN™ OX2 are identical.

A. Rapid Slide Screening Test

1. Place one drop of positive control onto a reaction circle of the glass slide.
2. Place one drop of Physiological saline onto the next reaction circle of the glass slide.
3. Place one drop of patient serum to be tested onto the next reaction circle (for PROGEN™ OXK refer note below).
4. Add one drop of appropriate PROGEN™ antigen suspension to the reaction circles containing positive control & physiological saline.
5. Add one drop of appropriate PROGEN™ antigen suspensions to the reaction circles containing the patient serum.
6. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
7. Rock the slide gently back and forth, and observe for agglutination macroscopically at one minute.

Note: The level of agglutinins in normal human normal serum can be 1:80 or more especially with PROGEN™ OXK suspension which may give titres upto 1:160. Therefore it is recommended that in endemic areas for PROGEN™ OXK reagent instead of 50 µl patient serum 5 µl which corresponds to 1:320 titre be used in the rapid slide screening test.

B. Semi-Quantitative Slide Method

1. Using a micropipette place 80 µl, 40 µl, 20 µl, 10 µl, and 5 µl of patient serum to be tested on 5 different reaction circles on the glass slide. The corresponding titres obtained will be 1:20, 1:40, 1:80, 1:160, & 1:320 respectively.
2. Follow step No. 5-7 of rapid slide screening test. This method is recommended for obtaining quick approximate titres only.

C. Quantitative Method

Tube Test Procedure

1. Take appropriate number of sets (as required; one set for each antigen suspension) of 8 Kahn tubes / test tubes and label them 1 to 8.
2. Pipette into tube No. 1 of all sets 1.9 ml of physiological saline.
3. To each of the remaining tubes (2 to 8) add 1 ml of physiological saline.
4. To tube No. 1 of all sets add 0.1 ml of serum sample to be tested and mix well.
5. Transfer 1.0 ml of the diluted serum sample from tube No. 1 to tube No.2 and mix well.
6. Transfer 1.0 ml of the diluted serum sample from tube No. 2 to tube No.3 and mix well. Continue this serial dilution till tube No. 7 in each set.
7. Discard 1.0 ml of the diluted serum from tube No.7 of each set.
8. Now the dilutions of the serum sample achieved from tube No. 1 to 7 respectively in each set is as follows: 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280. Tube No. 8 in all the sets, serves as a negative control.
9. To all the tubes (1 to 8) of each set add one drop of the respective well mixed PROGEN™ antigen suspensions from the reagent vials and mix well.
10. Cover the tubes and incubate at 37°C (approximately 18 hours).
11. Dislodge the sedimented button gently and observe for agglutination.

INTERPRETATION OF RESULTS

Rapid Slide Screening Test

Agglutination obtained within one minute is a positive reaction and indicates the presence of the corresponding antibody in the patient serum.

No agglutination is a negative test result and indicates the absence of the corresponding antibody in the patient serum.

Slide Semi-quantitative Method.

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, and 1:320 respectively. If a positive reaction is observed it is advisable to confirm the result and establish the titre by a tube test. A tube test is indicated when results do not conform to clinical findings. False results may be obtained if the reagents are not allowed to reach room temperature (22- 30^{0}C) before use. False positive reactions are also likely if the test is read beyond one minute after mixing.

Quantitative Method.
In a positive reaction there is obvious granular agglutination, in a negative reaction with physiological saline as negative control, a cell button should be formed.

ANALYSIS OF RESULTS
Agglutination patterns for several rickettsial diseases are shown below:

Infection	Vector	PROGEN™ antigen suspension		
		OX19	OX2	OXK
Epidemic typhus	Louse	+++	+	-
Murine typhus	Flea	+++	+	-
Endemic typhus	Flea	+++	+	-
Rocky Mountain Spotted Fever	Tick	+++	+	-
Tsutsugamushi Fever	Mite	-	-	+++
Scrub typhus Mite	Mite	-	-	+++
Boutonneuse fever	Tick	+	+	+
South African tick-bit fever	Tick	+	+	+
Brills, disease Louse	Louse	Usually neg.	Usually neg.	-/±
Trench fever Louse	Louse	-	-	-
Q Fever	Tick	-	-	-

LIMITATIONS
1. 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.
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 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.
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^{0}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.**

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.
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. A rising 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.
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^{0}C.
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. PROGEN™ antigen suspensions should produce 1+ or greater agglutination at 1: 40 in the slide and tube test when tested with PROGEN™ positive control.
2. The PROGEN™ antigen suspension should show no agglutination with the negative control.
3. Reproducibility of PROGEN™ range is 100% (+/- one double dilution).
4. The generally accepted performance characteristic of this type is 70% sensitivity and specificity.
5. Calibrated to major competitors and in-house standards.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

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). Date on file: Tulip Diagnostics (P) Ltd.

SYMBOL KEYS

Temperature limitation	Manufacturer	Contains sufficient for <n> tests
Use by	Consult Instructions for use	This way up
Date of Manufacture	REF Catalogue Number	PS Production Site
LOT Batch Number/ Lot Number	IVD *In vitro* Diagnostic Medical Device	EC REP Authorised Representative in the European Community

Manufactured by

TULIP DIAGNOSTICS (P) LTD.

Registered Office
GITANJALI, TULIP BLOCK, DR. ANTONIO DO REGO BAGH, ALTO SANTACRUZ, BAMBOLIM COMPLEX P.O., GOA-403 202, INDIA. Website: www.tulipgroup.com

PS **Manufacturing Unit**
PLOT NO. UTILITY VIII, PHASE III B, VERNA IND. ESTATE, VERNA, GOA - 403 722, INDIA.

EC REP
CMC Medical Devices & Drugs S.L., C/ Horacio Lengo No. 18, CP 29006, Malaga, Spain

0581-83/0917/VER-03