ARENA BioScien

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PROTEUS (slide test)

Diagnostic reagent set for the in-vitro qualitative screening and semi-quantitative determination of Proteus antibodies present in infected human serum manually.

REF: BS.1/PX01.100.0100	100 tests for OX19	REF: BS.1/OX01.100.0100	100 tests for OX2
REF: BS.1/PK01.100.0100	100 tests for OXK	REF: BS.1/PO01.100.0100	100 tests for OX19+OX2+OXK

CLINICAL SIGNIFICANCE

Proteus spp. are Gram-negative bacteria belonging the Enterobacteriaceae family and are common commensals of the gastrointestinal microbiota.
Proteus spp. bacteria were first described in 1885 by Gustav Hauser, who had revealed their feature of intensive swarming growth. Currently, the genus is divided into Proteus mirabilis, Proteus vulgaris, Proteus penneri, Proteus hauseri, and three unnamed genomospecies and consists of O-antigenic serogroups.
Antigen suspension of Proteus OX19 antigen suspension reacts strongly with sera of patients with typhus group rickettsiae and rocky mountain spotted fever. Proteus OX2 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, killed stained 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 antigen suspension to produce an agglutination reaction. No agglutination indicates the absence of Rickettsial antibodies.

REAGENT COMPOSITION

Reagents:	Composition
Antigen	- OX19 Antigen Suspension - OX2 Antigen Suspension - OXK Antigen Suspension
Positive Control	Poly-specific positive control reactive with antigens.
Negative Control	A buffer solution containing 5mg/ml BSA and 0.1% sodium azide.

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the Proteus reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien Proteus reagent is ready-to-use and is stable until expiration date stated on label when properly stored in an upright position and refrigerated at 2-8°C (do not freeze).

Deterioration

The ***BioScien*** Proteus reagent can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagent be verified with the positive controls.

SPECIMEN COLLECTION AND PRESERVATION

Clean and dry glassware free from detergents must be used for sample collection, freshly collected serum is preferable. Specimen should be free of turbidity and hemolysis. samples can be stored at 2-8°C for 24 hours, or frozen for 8 days should a delay in testing occur.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes
- Centrifuge
- Stop watch
- Variable Micropipettes
- Physiological saline (as negative control)

ASSAY PROCEDURE

Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures. Shake and mix antigens well before dispensing

Qualitative procedure

1. Identify each reaction circle of the slide test to make one positive control for each type, one negative control and the desired number of samples respectively.
2. Place one drop (50 µl) of positive control reagents, one drop (50 µl) of negative control and one drop (50 µl) of patient's serum to be tested onto each reaction circles.
3. Add one drop (50 µl) of Proteus antigen suspension to the reaction circles containing positive controls, negative control and patient's serum.
4. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
5. Rock the slide gently back and forth, and observe for agglutination macroscopically at one minute.

Semi-quantitative method

1. Dispense 80 µl, 40 µl, 20 µl, 10 µl, and 5 µl of patient serum to be tested onto 5 different reaction circles on the slide. The corresponding titers obtained will be 1:20, 1:40, 1:80, 1:160, and 1:320 respectively.
2. Continue with steps 3-5 of the qualitative procedure.

Note: This method is recommended for obtaining quick approximate titers only.

Tube test method

1. Take 8 Test tubes and label them 1 to 8.
2. Pipette 1.9 ml of isotonic saline to tube No.1
3. To each of the remaining tubes (2-7) add 1.0 ml of isotonic saline.
4. 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 37°C (approximately 18 hours).
11. Observe for agglutination macroscopically in each tube of the dilution series.

READING AND INTERPRETATION

Qualitative procedure	
Positive	Agglutination as indication for the presence of Proteus antibody in the patient's serum.
Negative	No agglutination as indication for the absence of Proteus antibody in the patient's serum.
Semi-Quantitative procedure	
Titer	The titer of the patient serum corresponds to the visible agglutination in the test circle with the smallest amount of serum sample.
TUBE TEST procedure	
Positive	The titer of the patient serum is the reciprocal of the last dilution of the serum sample that gives a granular agglutination.
Negative	The appearance of the suspension remains unchanged, which shows a typical swirl when the tube is flicked.

ANALYSIS OF RESULTS

Infection	Vector	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 Aferican tick-bit fever	Tick	+	+	+
Brills, disease Louse	Louse	Usually neg.	Usually neg.	-/+
Trench fever Louse	Louse	-	-	-
Q Fever	Tick	-	-	-

QUALITY CONTROL

The positive controls may included with the test kit to monitor the performance of the reagent. Good physiological saline may be used as a negative control, if the expected results have not been observed, the reagent should not be used. For more information please contact ***BioScien*** technical support.

PERFORMANCE CHARACTERISTICS

Precision (reproducibility and repeatability): Precision of Proteus antigen suspensions is 100% (+/- one double dilution).

Analytical sensitivity: Accurate titer determination of the reference material, under the described assay conditions.

Diagnostic sensitivity: 70 %.

Diagnostic specificity: 70 %.

LIMITATIONS OF PROCEDURE

1. 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.
2. Many serotypes pathogens have common somatic antigens. Agglutination with any of Proteus 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.
3. 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.
4. Only qualified and well trained staff should use the reagents.
5. False positive results are likely if the test is read more than one minute after mixing on the slide test.
6. The level of agglutinins in “normal' human sera can be 1:80 or more, especially with Proteus OXK antigen suspension which may give “normal” titers up to 1:160. Rising or falling titer is more significant than a single elevated titer.

REFERENCES

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3. Huddleson, L.F. andAbell, E. (1928). Rapid macroscopic agglutination for the serum diagnosis of Bang'sAbortion diseases. J, infect, Dis, 42,242.
4. Pedersen. C.E. (1978).j.Amer, Med,Technol., 40,79.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		