

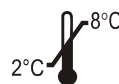
For research use only

Standard AB T. Pallidum

Performance panel of human sera containing anti- *Treponema pallidum* antibodies and containing no anti- *Treponema pallidum* antibodies

REF A-091

16 positive members
8 negative members



Store at 2–8°C.












Lyophilized sera are
stable until the expiry
date printed on the label

Instruction Manual

Version: 26, March 2007

1 Symbols used on labels

	Shelf life
	Date of manufacture
	Temperature limitation for storage conditions (store at 2 – 8 °C)
	Manufacturer
	Attention, see instructions for use
	Caution, see accompanying documents
	Catalogue number
	Lot number
	For research use only

2 Intended use

Standard AB T. Pallidum is a performance panel of human sera containing anti-*Treponema pallidum* antibodies and containing no anti-*Treponema pallidum* antibodies. Standard AB T. Pallidum is designed for use in control Enzyme Immunoassays to test anti- *Treponema pallidum* ELISA or immunoblot analysis performance, sensitivity and specificity. This panel is intended:

- For sensitivity and specificity evaluation of anti-*Treponema pallidum* ELISA or immunoblot analysis during an assay development and before the product release – carried out by a manufacturer of test kits.
- For sensitivity and specificity evaluation of anti-*Treponema pallidum* test kits before the patients blood screening – commonly carried out by a customer of test kits.
- To determine whether the kits' characteristics fulfill the approved requirements – carried out by regulatory agencies.

Note! Each taken separately performance panel of sera is recognized as the Russian National Immunobiological Standard and has specific branch standard number (Standard AB T. Pallidum is currently in the process of registration). The intended use and results interpretation are given in this Instruction Manual as it is approved by the Ministry of Health of the Russian Federation for Russian National Immunobiological Standards' use on the territory of Russia.

International users can apply these panels:

- To evaluate and validate new test methods and equipment
- To provide comprehensive data for comparative analysis
- To evaluate the compliance of the kits characteristics to the Russian Regulations requirements
- To use as an auxiliary material either by a manufacturer or a customer of test kits in their investigations and/or R&D
- To train the laboratory personnel

International users who applied these panels as an auxiliary materials in their investigations and/or R&D interpret the results according to their specific task.

3 Introduction

Treponema pallidum is the causative agent of syphilis. It is a spirochete, a helical to sinusoidal bacterium with outer and cytoplasmic membranes, a thin peptidoglycan layer, and periplasmic flagella (12.1, 12.2). Mechanisms of *T. pallidum* pathogenesis are poorly understood. No known virulence factors have been identified, and the outer membrane is mostly lipid with a paucity of proteins (12.3). Syphilis is characterized by multiple clinical stages and long periods of latent, asymptomatic infection (12.4). There are a lot of diagnostic tests for syphilis, and no vaccine against *T. pallidum* is available. Early diagnosis of invasive infection increases the likelihood of successful therapy (12.5).

Serological tests are good as diagnostic tool at the early stage of disease (12.6,12.7).

ELISA is a widespread test to determinate *Treponema pallidum* infection (12.8). Testing the work standards of clinical diagnostic laboratories dealing with ELISA diagnostics should be regularly provided.

It is necessary to perform the incoming control of quality of ELISA test kits used for diagnostics of infectious diseases in the clinical laboratory before the screening of patients' sera and in the QC Department of manufacturer before the launching of every lot of test kits on the market. Appropriate reference materials should be used in such tests.

Performance Panels are panels of human sera intended for use in control serological assays. The sera members of Performance Panels represent the full reactivity range of the assay, including negative reactivity. Performance Panels are used to challenge test kits performance, sensitivity, and specificity and can be used to evaluate and validate new test methods, new equipment, demonstrate lab proficiency, and train laboratory personnel.

These panels of sera are developed and manufactured under the requirements specification of Russian National Control and Research Agency - Tarasevich State Institute of Standardization and Control of Medical Biological Preparations (Tarasevich SISC).

The panel Standard AB *T. Pallidum* is awaiting Tarasevich SISC approval and Russian Branch Standard number assignment.

4 Characteristics of the sera

Standard AB *T. Pallidum* is a panel containing 16 positive and 8 negative members spanning the dynamic range of Anti-*T. Pallidum* assays. Sera provided possess the following characteristics:

- ❑ Anti-*T. Pallidum* positive sera are collected from individuals with syphilis, negative sera are collected from healthy individuals.
- ❑ Various concentrations of anti-*T. Pallidum* antibodies in different members of the panel are achieved by selection of native sera from the blood collection.
- ❑ Panel is thoroughly characterized by a variety of commercially available ELISA test kits. Comprehensive datasheet includes comparative optical density and S/CO reactivity coefficient data (S/CO is the ratio of serum optical density value over cut-off value) and results of investigation of sera in complex of serologic reactions.
- ❑ Sera have passed the corresponding control and have shown absence of HBsAg and antibodies to HIV-1,2 and HCV.
- ❑ Sera are inactivated. Procedure of inactivation has been performed during 3 hours at 56°C.
- ❑ Specimens are supplied as powders (lyophilized from 200µl of sera liquid).

5 Precautions and warnings

It is necessary to keep all precautions accepted at work with potentially infectious material during performance of the analysis. Handle as if capable of transmitting infectious agents:

- ❑ Use rubber gloves for all the manipulations;

- ❑ Use approved labware, precise pipettes and sterile tips working with panel. All procedures should be carried out under the aseptic conditions.
- ❑ Treat all the waste material with 70% (v/v) ethanol or 6% (w/w) hydrogen peroxide followed by exposure at 20 – 25 °C for at least 2 hours.
- ❑ Immediately clean up any spillage containing potentially infectious agents with disinfectants. Dispose of the cleaning material by an accepted method.

6 Preparation for use

- 6.1 Inspect the contents of the box: check the bottles and labels for integrity. If label damage and/or loss is discovered, vial should not be used. Dispose the material if a bottle is broken.
- 6.2 Register the characteristics of the panel (lot No, shelf life etc.), the amount of the sera to be used, and the goal of the work you are planning to perform in the laboratory journal.
- 6.3 Open the bottle carefully to avoid the overshoot of materials.
- 6.4 Add the 200µl of distilled water into each vial with serum powder. Mix thoroughly. Seal the vials and keep them for 15 min at room temperature (15 – 30 °C). Reconstituted sera are stable for 1 month at 2 – 8 °C and for 6 months at -20 °C. Three freeze-thaw cycles of reconstituted sera are admissible.

7 ELISA assay procedure

- 7.1 Prepare the working dilutions of standard sera in accordance with the instruction manual of the test kit being validated.

Note! *When challenging performance, sensitivity and specificity of test kit for the detection of anti-T. Pallidum antibodies it is necessary to use all the vials supplied in the panel.*

- 7.2 Add the necessary amount of each standard serum in working dilution to 3 wells of the plate as test samples. Add test kit control sera (positive and negative controls) to the assigned wells of the plate. Reserve one or two wells for the conjugate control.

- 7.3 Carry out the assay according to the test kit instruction manual.

8 Quality control

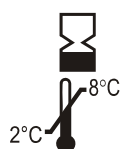
- 8.1 The results are considered significant only if optical density (OD) in the conjugate control well, mean OD value in negative control wells and OD value in positive control well are within the limits stipulated in test kit instruction manual. Calculate the cut-off value using the formula given in test kit instruction manual.
- 8.2 The test kit sensitivity determined on the positive sera supplied in Standard AB T. Pallidum should be 100%. It means that OD values in all the wells where positive sera (## 1 - 16) were tested should be equal to cut-off value or higher. If at least one false-negative result is obtained all the kits from the evaluated lot should be rejected.
- 8.3 The test kit specificity determined on the negative sera supplied in Standard AB T. Pallidum should be 100%. It means that OD values in all the wells where negative sera (## 17 - 24) were tested should be less than cut-off value. If at least one false-positive result is obtained all the kits from the evaluated lot should be rejected.

8.4 When validating test systems based on other methods of immunological analysis please refer to the corresponding instruction manual for the proper amounts of standard sera and assay protocol.

9 The form of manufacturing

Standard AB T. Pallidum panel is manufactured as a set of 24 vials with lyophilized sera packed in a rigid box. Instruction manual, panel certificate of analysis and comprehensive datasheet are provided inside the box. The box is covered with a shrinkable film.

10 Shelf life, storage and transportation conditions



Shelf life of Standard AB T. Pallidum panel is 2 years.

Store at 2 – 8 °C in the dry dark place. Transport at 2 – 8 °C. It is allowed to store the panel at the room temperature for up to 5 days during transportation.

11 Ordering information and related MBU products

CAT. #	PRODUCT NAME	DESCRIPTION	PACK SIZE
A-090	Control AB T. Pallidum	4 human sera containing anti-Treponema antibodies and 2 human sera containing no anti- Treponema antibodies for testing the work standards of clinical diagnostic laboratories dealing with serodiagnostics of Treponema pallidum infection	4 (+) x 1000µl * 2 (-) x1000µl *
A-091	Standard AB T. Pallidum	16 Human sera containing anti-Treponema antibodies and 8 human sera containing no containing anti-Treponema antibodies for specificity and sensitivity evaluation of test kits for detection of anti-Treponema pallidum antibodies	16 (+) x 200µl *; 8 (-) x 200µl *
A-092	External run control AB T.Pallidum	10 equal members lyophilized from human serum containing antibodies to Treponema pallidum for day-to-day testing the performance of ELISA test kits for anti-Treponema antibodies detection inside clinical laboratory	10 (+) x 200µl *

* The volumes of sera indicated in "µl" mean volumes before lyophilization.

12 References

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- 12.3 Blanco DR, Miller JN, Lovett MA (1997) Surface antigens of the Syphilis Spirocheate and their potential as virulence determinants.** Emery Infect Dis. 3(1):11-20.
- 12.4 Reynolds D, Evangelista F, Ward B, Notenboom R, Young E, D'Cunha CO (1998) Syphilis in an urban community.** Can J Public Health. 89(4):248-52.

- 12.5 Rawstron SA, Vetrano J, Tannis G, Bromberg K (1997)** *Congenital syphilis: Detection of Treponema pallidum in stillborns.* Clin Infect Dis. 24:24-7.
- 12.6 Lukehart SA (1986)** *Identification and characterization of Treponema Pallidum antigens by monoclonal antibodies.* In: Monoclonal Antibodies. Academic Press, p. 1.
- 12.7 Dorfman DH, Glaser JH (1990)** *Congenital syphilis presenting in infants after the newborn period.* N Engl J Med. 323:1299-302.
- 12.8 Farshy CE, Hunter EF, Helsel LO, Larsen SA (1985)** *Four-step Enzyme-linked Immunosorbent Assay for Detection of Treponema Pallidum Antibody.* J Clin. Microbiol. 21(2):387-9.

For technical assistance please contact manufacturer:



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