TPHA PK

Cat. No. 60124
Product Description TPHA PK 2000 tests

Kits for the qualitative detection of antibody to Treponema pallidum in human serum or plasma by passive haemagglutination on the PK7200 or PK7300 analyser.

CLINICAL BACKGROUND
Syphilis is a chronic infection that progresses through distinct stages of infection: primary, secondary, tertiary, and quaternary. These stages produce diverse clinical symptoms, typically producing initial sores known as chancres then syphilitic rash followed by long periods of dormancy. Untreated infection may eventually result in cardiovascular problems and neurosyphilis. The infection is caused by the spirochaete Treponema pallidum, and is usually acquired by sexual contact, although the disease may be transmitted by transfusion of infected blood. Intrauterine infection also occurs. The organism has proved virtually impossible to culture in artificial media, and diagnosis of the infection usually depends on the demonstration of antibodies in the blood, which appear soon after initial infection.

Tests for syphilis fall into four categories: direct microscopic examination; treponemal antibody tests; non-treponemal antibody tests; and direct antigen tests. Because of the long periods of dormancy and the non-specific nature of non-treponemal tests, methods that detect specific anti-treponemal antibodies in blood specimens have become increasingly popular for screening. TPHA is one such test.

INTENDED USE
These kits are intended for use by appropriately trained and qualified personnel for the detection of antibodies to Treponema pallidum in human serum and plasma.

PRINCIPLE OF THE TEST
The Lab21 TPHA kits use preserved avian erythrocytes coated with antigens of T. pallidum (Nichols strain), which will bind with specific antibody present in patient's serum or plasma. The cells are suspended in a medium containing components to eliminate non-specific reactions. Positive reactions are shown by agglutination of the cells, negative reactions by the settling of the cells to a button or small ring.

KIT CONTENTS

<table>
<thead>
<tr>
<th>Name</th>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1: Test Cells</td>
<td>Preserved chicken erythrocytes coated with antigens of T. pallidum</td>
<td>80ml</td>
</tr>
<tr>
<td>R2: Diluent</td>
<td>Saline solution containing absorbers</td>
<td>600ml</td>
</tr>
<tr>
<td>R3: Positive Control</td>
<td>Human serum</td>
<td>5ml</td>
</tr>
<tr>
<td>R4: Negative Control</td>
<td>Human serum</td>
<td>5ml</td>
</tr>
</tbody>
</table>

Directions for use
Reagents are sufficient for 2000 tests.

WARNINGS AND PRECAUTIONS
For in-vitro diagnostic use only
All reagents contain sodium azide (< 0.1% w/v). Waste fluids arising from use of the kit must be flushed with large quantities of water to avoid accumulation of potentially explosive compounds in laboratory plumbing.
The control materials supplied are derived from human serum. They have been tested at donor level and found negative for Hepatitis B and C, and for HIV 1 and 2. However, they should be treated as if capable of transmitting disease.
Specimens of human serum and plasma should be treated as microbiologically hazardous, and handled in accordance with the applicable regulations.
Do not use the kit after its expiry date.
Do not combine or interchange reagents from kits with different lot numbers.

STORAGE
Store at 2–8°C when not in use. Store bottles upright. Do not freeze.
Shelf life is valid until date stated on kit label.

EQUIPMENT REQUIRED
PK7200 or PK7300 Analyser
P3-Microtitration plates: Not provided with reagents

SPECIMENS
Serum or plasma specimens should be free of blood cells and of obvious microbial contamination. They may be stored at 2-8°C for up to 7 days before testing. Specimens needing longer storage should be frozen at -20°C or lower. Frozen specimens should be thawed and well mixed before testing.

TEST PROCEDURE
Bring test reagents to room temperature.
Connect sample diluent dispenser to diluent bottle.
Re-suspend test cells by thoroughly mixing and add to the reagent reservoir.
Ensure suspension throughout processing.

Note: Any surplus test cells should be decanted into a bottle and stored at 4°C for future use.
It is recommended that the positive and negative control provided with each set of reagents should be included at the beginning and the end of each run.

INSTRUMENT SETTINGS FOR PK7200
Please note these settings are for guidance. Variances between test centres may require that they be adjusted slightly. Please consult Lab21 for advice.

1. REAGENT PARAMETERS

| 1. REAGENT NAME | : TPHA |
| 2. DILUENT NAME | : TPHA DILUENT |
| 3. DISPENSING CONDITION |
| 1st REAGENT VOLUME | : 35µl |
| SAMPLE | : 1 (PLASMA) |
| DILUENT | : TPHA DILUENT |
| SAMPLE/DILUENT RATIO | : 160/1000 |
| DILUTED SAMPLE VOLUME | : 15µl |
| 7 PLATE WELL | : 16µm |
| 8 REACTION TIME | : 60 min. |
| REACTION TEMP | : 18-37°C |

4. THRESHOLD

| 2 DYNAMIC RANGE SET |
| P DYNAMIC RANGE | : 1-99 |
| C DYNAMIC RANGE | : 1-99 |
| LIA DYNAMIC RANGE | : 0-999 |

3 THRESHOLD SET

| SPC THRESHOLD | : Low 16 High 16 |
| P/C THRESHOLD | : (+) 41 (-) 26 |
| LIA THRESHOLD | : (+) 240 (-) 100 |

4 LIMIT SET

| BG/C LIMIT | : Low |
| LIA SELECTION | : 5 |

2. TEST PARAMETERS

| 1. TEST NAME | : TPHA |
| 2 REAGENT SELECTION | : TPHA |
| DECISION LOGIC NUMBER | : 2 |
| 4 Decision Logic Set |
| 1: POS + |
| 2: NEG - |

3 PANEL NAME |
| : XXX |

4 PANEL SET

| 2 CH REAGENT No. |
| TPHA No. |
| CHANNEL No. |
| DILUENT No. |
| TPHA No. |
| DILUENT No. |
| SAMPLE VOLUME | : 40µl |
| DILUENT VOLUME | : 250µl |

7. SAVE AND LOAD ALL PARAMETERS
IN VITRO DIAGNOSTIC MEDICAL DEVICE

Please note these settings are for guidance. Variances between test centres may require that they be adjusted slightly. Please consult Lab21 for advice.

REAGENT NAME: TPHA
DILUENT NAME: TPHA DILUENT
REAGENT PARAMETERS:
REAGENT VOLUME: 35µl
SAMPLE: PLASMA
DILUENT: TPHA DILUENT
SAMPLE/DILUENT RATIO: 7:2
DILUTED SAMPLE VOLUME: 15µl
PLATE WELL: 16µm
REACTION TIME: 60 min.
REACTION TEMP: 18-37°C
THRESHOLDS:
DYNAMIC RANGE SET:
P DYNAMIC RANGE: 0-99
C DYNAMIC RANGE: 0-99
LIA DYNAMIC RANGE: 0-999
THRESHOLD SET:
SPC THRESHOLD: Low 16 High 16
P/C THRESHOLD: (+) 41 (-) 26
LIA THRESHOLD: (+) 240 (-) 100
LIMIT SET:
BG/C LIMIT: Low
LIA SELECTION: 5
LIH THRESHOLD: 0

INTERPRETATION AND ASSAY VALIDATION

Results are determined by the threshold parameters defined in the PK7200 or PK7300 manual.

<table>
<thead>
<tr>
<th>SPC</th>
<th>LIA</th>
<th>P/C</th>
<th>Interpretation</th>
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<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Positive</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Negative</td>
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<td>+</td>
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<td>+</td>
<td></td>
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<td>+</td>
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<td>+, -</td>
<td>+, -</td>
<td>Indeterminate</td>
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<td>+, -</td>
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<tr>
<td>?</td>
<td>-</td>
<td>+, -</td>
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The positive control provided should give a strong positive pattern. The negative control provided should give a clearly negative result. Positive results should be repeated and if repeat reactive confirmed by a reference method e.g. FTA - ABS.

TROUBLESHOOTING

The most common cause of atypical reactions resulting in an unexpectedly high initial reactive rate is improper cleaning of the plate. Consult instrument manufacturer for recommended method of plate cleaning. Inaccurate delivery of test reagents may lead to incorrect results. It is essential that the PK7200 or PK7300 is properly maintained on a daily basis.

Technical problems should be directed in the first instance to the local distributor.

PERFORMANCE CHARACTERISTICS

Sensitivity
Three studies on 312 specimens from known cases of syphilitic plasma showed 99.7% sensitivity (95% confidence limits 97.74-100%).

Analytical sensitivity
This kit has been shown to be capable of detecting 0.05 IU of anti-treponemal antibody absorption test, the microhemagglutination assay for Treponema pallidum antibodies, and the hemagglutination treponemal test for syphilis. J. Clin. Microbiol., 1981;23:441–445

Specificity
Three studies on 1199 random negative donor specimens showed 100% specificity (95% confidence limits 98.04-100%).

Precision and Accuracy

For N = 10 assays of a positive sample CV = 8.1% Accuracy = -2.5%

BIBLIOGRAPHY

1. Rathlev T. Haemagglutination tests utilizing antigens from pathogenic and apathogenic Treponema pallidum WHO/VDT/RES 1965:77:65
10. Dauguet G.L. Diagnostic Biologique de la Syphilis. Technique et Biologie, 1995; 120-5-30

KEY TO SYMBOLS

IVD  In Vitro Diagnostic Medical Device
Manufactured by
Temperature limitation
Use by
Batch code
Consult instructions for use
Control
Positive control
Control
Negative control
Test Cells
Test Cells
Diluent
Diluent

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