TPHA Screen
For the Detection of Antibodies to
Treponema pallidum

Intended Use:
TPHA Screen is intended for use in the qualitative screening of blood donors for the

detection of IgG and IgM antibodies to Treponema pallidum in human serum and

EDTA plasma by passive hemagglutination using the Galileo and/or Galileo Neo,

Automated Blood Bank System.

Summary of the Test:

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 spirochete Treponema pallidum, and is usually
acquired by sexual contact, although the disease may be transmitted by

transfusion of infected blood. Intrauterine infection can also occur. 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
Screen, which is based on Treponema Pallidum Hemagglutination Assay (TPHA)
methodology, is one such test.

Principle of the Test:

TPHA Screen uses TPHA Screen Test Cells which are preserved avian
erythrocytes coated with antigens of T. pallidum (Nichols strain). TPHA Screen
Test Cells bind with specific antibody present in the sample 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 and negative
reactions are shown by the settling of the cells to a button.

TPHA Screen Positive Control and TPHA Screen Negative Control are used with each
plate run of the TPHA Screen assay to assess the validity of test results of the run.
TPHA Screen Positive Control should produce similar positive results from run to run.
TPHA Screen Negative Control should produce negative results with each run.
Deviations from the expected control results indicate a run failure and all tests
and controls should be repeated.

TPHA Screen Diluent is an isotonic saline solution used to dilute sample plasma or
serum, and control material, as part of TPHA Screen automated methods.

Reagents:

TPHA Screen Test Cells: Reagent vials contain preserved chicken erythrocytes
coated with antigens of T. pallidum. The reagent is prepared as a dilute suspension in

a buffered solution containing stabilizers, non-specific sorbent and preservatives.
Sodium azide is added as a preservative (at less than 0.1% w/v). Gentamicin sulfate
(0.021 mg/mL) is added as a preservative. Store at 1-10°C. Ready for use as
supplied.

Adjunct Reagents to TPHA Screen Test Cells:
(Purchased separately)

TPHA Screen Diluent: An isotonic saline solution containing absorbents. Sodium
azide is added as a preservative (at less than 0.1% w/v). Store at 1-10°C. Ready for
use as supplied.

TPHA Screen Positive Control: Contains antibodies to T. pallidum. Sodium azide is
added as a preservative (at less than 0.1% w/v). Store at 1-10°C. Ready for use as
supplied.

TPHA Screen Negative Control: Contains no antibodies to T. pallidum. Sodium
azide is added as a preservative (at less than 0.1% w/v). Store at 1-10°C. Ready for
use as supplied.

Precautions:

Do not store frozen or expose to temperatures greater than 30°C. Do not use beyond
the expiration date.

Discard the TPHA Screen Test Cells and TPHA Screen Diluent reagents if they
become turbid or change color. Turbidity may be an indication of microbial
contamination. Do not use turbidly contaminated reagents.

TPHA Screen Test Cells should not be used if the red blood cells darken or if
hemolysis is exhibited. Hemolysis of TPHA Screen Test Cells is indicative of
deterioration.

Note: TPHA Screen Test Cells are erythrocytes that have been subjected to mild

treatment with chemicals so that they adsorb soluble antigens onto their surface.
This process is called lysis. As a result of the lysis process, the normal coloration of
TPHA Screen Test Cells when suspended in the vial is reddish brown.

TPHA Screen Positive Control and TPHA Screen Negative Control reagents are
derived from human serum or plasma. Marked turbidity may indicate reagent
deterioration or contamination. Do not use if markedly turbid.

Do not use leaking vials. Do not use unlabelled vials.

TPHA Screen Test Cells are to be used as test cells with TPHA Screen only.

TPHA Screen Test Cells contain bovine serum albumin and it is sourced from donor
animals of United States origin that have been inspected and certified by US
Veterinary Service inspectors to be disease-free. This ruminant-derived product is
designed to have low-TSE (Transmissible Spongiform Encephalopathy) risk.

TPHA Screen Test Cells reagent contains processed avian erythrocytes. The avian
erythrocytes are collected in the United Kingdom from flocks of chickens that are free
of Avian Influenza. The avian erythrocytes are treated with formalin at a temperature
and time which have been deemed by the United Kingdom government Department
for Environment, Food and Rural Affairs to reduce the risk of remaining infectious virus
particles to a negligible level.

Sodium azide is added as a preservative (at less than 0.1% w/v). Waste fluids arising
from the use of the TPHA Screen must be flushed with large quantities of water to
avoid accumulation of potentially explosive compounds in laboratory plumbing.
Handle and dispose of reagent as if potentially infectious.

CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED WAS FOUND NEGATIVE WHEN TESTED IN ACCORDANCE WITH CURRENT FDA REQUIRED TESTS. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS.

TPHA Screen Test Cells should be used not more than 72 hours after a stir ball has
been added to the vial. Longer use of the reagent may compromise the integrity of the
reagent.

The cellular contents of the TPHA Screen Test Cells vial must be completely
resuspended before adding a stir ball. The stir ball is required for use on an
instrument. The outcome of failing to completely resuspend the cellular contents
before the stir ball addition can be erroneous test results.

TPHA Screen Diluent should be used not more than 72 hours after first use of the vial.
Longer use of the diluent may compromise the integrity of this reagent.

TPHA Screen Positive Control and TPHA Screen Negative Control should be used not
more than 240 hours after first use of the vials. Longer use of the controls may
compromise the integrity of these reagents.

Store opened vials of all reagents at 1-10°C when not in use.
The format for the expiration date is expressed as CCYY-MM-DD (year-month-day).

Specimen Collection and Preparation:
Serum or plasma (EDTA anticoagulant) samples, free of red blood cells, can be used for the TPHA Screen.

Draw a blood specimen using an acceptable phlebotomy technique. Testing should be performed as soon as possibly following collection to minimize the chance that falsely positive or falsely negative reactions will occur due to improper storage or contamination of the sample.

Do not use blood samples drawn into tubes with neutral gel separators. The TPHA Screen has not been validated for use with gel separator tubes. Do not use blood samples with obvious signs of microbial contamination such as serum or plasma samples exhibiting flocculation. Samples should not contain particulate matter. Blood samples that exhibit excessive hemolysis, lipemia or icterus should not be tested with TPHA Screen.

Samples that cannot be tested immediately can be stored at 1-10°C for up to 7 days before testing. Samples that require longer storage should be frozen at -20°C or lower. Such frozen samples should be thawed and mixed well prior to testing. Samples must only be frozen and thawed for a maximum of three (3) times prior to testing.

Procedure:

Materials supplied:
TPHA Screen Test Cells in vials

Additional Reagents Required:
1. TPHA Screen Diluent
2. TPHA Screen Positive Control
3. TPHA Screen Negative Control

NOTE: The in-date components (TPHA Screen Test Cells, TPHA Screen Diluent, TPHA Screen Positive Control and TPHA Screen Negative Control) used to perform the TPHA Screen can be used interchangeably with other component lots, provided the components are within their dating periods.

Additional Materials Required:
1. Automation V-Plates (V-bottomed microplates)
2. Phosphate-buffered (approximately 15 mM) isotonic saline, pH 6.5-7.5
3. Blood specimens
4. Immucor Sirtuline
5. Immucor Galileo or Galileo Neo automated instrument

*It is the user's responsibility to validate an accessory device (either listed or otherwise) for its intended use. Validation results should be maintained as part of the laboratory's records for review by regulatory agencies.

Test Method:
1. Bring all reagents and samples to 18-30°C before testing.
2. Prepare serum/plasma samples according to the directions described above.
3. Gently mix the vial of TPHA Screen Test Cells until the red blood cells are thoroughly resuspended. Add a stir bar after mixing the vial contents, but before placing the vial on an instrument.
4. Refer to instructions provided in the instrument operator manual.

Stability of Reaction:
Following the defined incubation period for reaction setting, tests are read immediately. The test wells cannot be re-read following the initial automated read process.

Quality Control:
The performance of the TPHA Screen is evaluated at each test run with TPHA Screen Positive Control and TPHA Screen Negative Control. TPHA Screen Positive Control should produce similar positive results from run to run. TPHA Screen Negative Control should produce negative results with each run. The instrument will automatically determine the validity of the controls at the completion of each plate run. The controls help to determine if technical errors or reagent failures have occurred. Continued failure of the controls to meet the expected results on repeat testing may indicate that TPHA Screen Test Cells or another reagent in the TPHA Screen has deteriorated, or that the test is consistently being performed incorrectly.

Refer to instructions provided in the instrument operator manual.

Interpretation of Results:
Positive (Reactive) Test: agglutination of TPHA Screen Test Cells as demonstrated by full or partial effacement of the red blood cells distributed over the internal wall of the microplate well. A button within this area may occur.

Negative (Non-reactive) Test: settling of TPHA Screen Test Cells to the bottom of the microplate well to form a button.

A sample reported as non-reactive on initial screening is considered to be non-reactive for antibodies to T. pallidum and needs no further testing.

A sample that is reactive on initial screening is considered initially reactive by the test and the sample should be repeated in duplicate. If either duplicate is reactive, the specimen is to be interpreted as repeatedly reactive for antibodies to T. pallidum by the criteria of the test. Initially reactive specimens that are negative in both of the duplicate retests are considered non-reactive by the criteria of the test.

NOTE: The Immucor automated instruments automatically interpret the test results.

Limitations:
The TPHA Screen reagents cannot be used to perform tests by methods other than the automated TPHA Screen method.

Serum samples that have not clotted completely may cause erroneous results to be obtained with TPHA Screen. Such samples may continue clotting after they have been added to the TPHA Screen test.

Do not use blood samples with obvious signs of microbial or chemical contamination. Errenous test results can occur from bacterial or chemical contamination of TPHA Screen reagents, improper storage of the TPHA Screen reagents, or the omission of the TPHA Screen reagents from the assay procedures.

Errenous test results can occur if the assays in which TPHA Screen reagents are employed are not performed correctly.
The TPHA Screen controls are used to determine if technical errors or reagent failures have occurred. The reagents cannot be used to validate negative tests obtained by other methods outside of the prescribed TPHA Screen.

Testing of blood samples that contain antibodies to chicken red blood cell antigens can cause false positive results.

TPHA Screen is for use only in screening blood donors and has not been evaluated for use other than blood donor screening.

Carryover between specimens is a potential source of interference. Carryover was not demonstrated up to a level of 256.

Frozen samples that are thawed prior to testing must only be frozen and thawed for a maximum of three (3) freeze and thaw cycles.

Specific Performance Characteristics:
The performance of TPHA Screen was evaluated at six (6) US blood centers in comparison with a commercially available automated MHA-TP system (PK-TP) on the Galileo and Galileo Neo.

Specimens consisted of either serum or plasma collected with EDTA anticoagulant. Specimens were evaluated within seven days of blood collection and stored at 1-10°C if not tested within 24 hours. A total of 7190 specimens were tested, with 4202 specimens evaluated on the Galileo and 2988 specimens evaluated on the Galileo Neo. Specimens that were repeatedly reactive in either test were confirmed with the Treponema pallidum Particle Agglutination assay (TP-PA).

The results for the Galileo are summarized in the following three (3) tables.

### PK-TP System

<table>
<thead>
<tr>
<th>PK-TP</th>
<th>Reactive</th>
<th>Non-reactive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>243</td>
<td>3</td>
<td>246</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>352</td>
<td>356</td>
<td></td>
</tr>
<tr>
<td>247</td>
<td>355</td>
<td>422</td>
<td></td>
</tr>
</tbody>
</table>

Overall Agreement = 99.8% (95% CI = 99.7% - 99.9%)

Relative Sensitivity = 98.4% (95% CI = 96.9% - 99.6%)

Relative Specificity = 99.9% (95% CI = 99.8% - 99.9%)

The following Galileo table excludes 250 known reactive samples that were tested with TPHA Screen or the Galileo and the PK-TP system.

<table>
<thead>
<tr>
<th>Galileo PK-TP</th>
<th>Number</th>
<th>TP-PA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>3944</td>
</tr>
<tr>
<td>Non-reactive</td>
<td>Reactive</td>
<td>0</td>
</tr>
<tr>
<td>Reactive</td>
<td>Non-reactive</td>
<td>7</td>
</tr>
<tr>
<td>Reactive</td>
<td>Reactive</td>
<td>1</td>
</tr>
</tbody>
</table>

Total          3952  3952

Galileo Reactive Rate | 0.20% | 0.08%

PK-TP Reactive Rate | 0.03% | 0.03%

*Only discordant and reactive samples, after repeat testing was performed, were tested with TP-PA.

The following Galileo table summarizes the 250 known reactive samples that were tested with TPHA Screen on Galileo and the PK-TP system, but are excluded from the Galileo table above.

<table>
<thead>
<tr>
<th>PK-TP System</th>
<th>Reactive</th>
<th>Non-reactive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>243</td>
<td>4*</td>
<td>246</td>
<td></td>
</tr>
<tr>
<td>Non-reactive</td>
<td>1*</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>243</td>
<td>7</td>
<td>250</td>
</tr>
</tbody>
</table>
The results for the Galileo Neo are summarized in the following three tables.

### PK-TP System

<table>
<thead>
<tr>
<th>Galileo Neo</th>
<th>PK-TP</th>
<th>Number</th>
<th>TP-PA*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reactive</td>
<td>Non-reactive</td>
<td>Total</td>
</tr>
<tr>
<td>Reactive</td>
<td>198</td>
<td>8</td>
<td>206</td>
</tr>
<tr>
<td>Non-reactive</td>
<td>2</td>
<td>3000</td>
<td>3002</td>
</tr>
</tbody>
</table>

Overall % Agreement = 99.7% (95% C.I. = 99.4% - 99.9%)
Relative Sensitivity = 99.0% (95% C.I. = 96.4% - 99.9%)
Relative Specificity = 99.7% (95% C.I. = 99.5% - 99.9%)

The following Galileo Neo table excludes 198 known reactive samples that were tested with TPHA Screen on the Galileo Neo and the PK-TP system.

<table>
<thead>
<tr>
<th>Galileo Neo</th>
<th>PK-TP</th>
<th>Number</th>
<th>TP-PA*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reactive</td>
<td>Non-reactive</td>
<td>Total</td>
</tr>
<tr>
<td>Reactive</td>
<td>198</td>
<td>0</td>
<td>198</td>
</tr>
<tr>
<td>Non-reactive</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Reproducibility:
The reproducibility of TPHA Screen was evaluated at four (4) US sites. Three (3) sets of twelve (12) reproducibility samples were provided to each site. Each set consisted of three replicates of four samples representing samples with high-, medium-, low-, and non-reactivity.

Testing was conducted using three (3) lots of each reagent in two separate runs for each lot on each of three (3) days.

### Galileo Panel Member

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Negative</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sites %CV</td>
<td>0</td>
<td>14.64</td>
<td>6.61</td>
<td>3.07</td>
</tr>
<tr>
<td>Lot %CV</td>
<td>0</td>
<td>5.60</td>
<td>1.31</td>
<td>0</td>
</tr>
<tr>
<td>Day %CV</td>
<td>0</td>
<td>12.69</td>
<td>2.45</td>
<td>2.43</td>
</tr>
<tr>
<td>Run %CV</td>
<td>0</td>
<td>5.50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Repeatability %CV</td>
<td>0</td>
<td>18.81</td>
<td>4.54</td>
<td>6.73</td>
</tr>
<tr>
<td>Reproducibility %CV</td>
<td>0</td>
<td>28.12</td>
<td>8.49</td>
<td>7.79</td>
</tr>
<tr>
<td>Mean Reaction Score</td>
<td>0</td>
<td>57.02</td>
<td>81.36</td>
<td>93.58</td>
</tr>
<tr>
<td>Range of Scores</td>
<td>0</td>
<td>31.0 - 96.9</td>
<td>81.7 - 96.1</td>
<td>33.5 - 99.9</td>
</tr>
</tbody>
</table>

The low sample was negative 1/162 times.
All other samples remained in the same status 162/162 times.

### Staged Specimen Evaluation:
The performance of TPHA Screen was evaluated using 31 specimens from syphilitic patients who had previously been characterized as primary, secondary, and latent, both treated and untreated. The specimens were tested using TPHA Screen on Galileo and Galileo Neo and were confirmed with the Treponema pallidum Particle Agglutination assay (TP-PA). The results of this staged syphilis specimen testing are summarized in the following table.

<table>
<thead>
<tr>
<th>Disease Stage</th>
<th>% Reactive By Galileo</th>
<th>% Reactive By Galileo Neo</th>
<th>% Reactive By TP-PA</th>
<th>Number of Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Treated</td>
<td>100% (5/5)</td>
<td>100% (5/5)</td>
<td>100% (5/5)</td>
<td>5</td>
</tr>
<tr>
<td>Primary Untreated</td>
<td>100% (8/8)</td>
<td>100% (8/8)</td>
<td>100% (8/8)</td>
<td>8</td>
</tr>
<tr>
<td>Secondary Treated</td>
<td>100% (11/11)</td>
<td>100% (11/11)</td>
<td>100% (11/11)</td>
<td>11</td>
</tr>
<tr>
<td>Secondary Untreated</td>
<td>100% (4/4)</td>
<td>100% (4/4)</td>
<td>100% (4/4)</td>
<td>4</td>
</tr>
<tr>
<td>Latent Treated</td>
<td>100% (1/1)</td>
<td>100% (1/1)</td>
<td>100% (1/1)</td>
<td>1</td>
</tr>
<tr>
<td>Latent Untreated</td>
<td>100% (2/2)</td>
<td>100% (2/2)</td>
<td>100% (2/2)</td>
<td>2</td>
</tr>
<tr>
<td>All Disease Stages</td>
<td>100% (31/31)</td>
<td>100% (31/31)</td>
<td>100% (31/31)</td>
<td>31</td>
</tr>
</tbody>
</table>

All results for staged specimen testing demonstrated 100% agreement.

### Disease States:
The performance of TPHA Screen was evaluated using 522 specimens from individuals who were medically diagnosed with disease states other than syphilis and also had no known history of, and demonstrated no serological evidence for, syphilis. This group included specimens representing disease states other than syphilis and/or characteristics known to cause false reactive results in other serological tests for syphilis. The results are summarized in the following table.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of Specimens</th>
<th>Reactive Galileo</th>
<th>Reactive Galileo Neo</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (characterized) (Alanine transaminase)</td>
<td>94</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>HTLV</td>
<td>29</td>
<td>0</td>
<td>1*</td>
</tr>
<tr>
<td>Systemic Lupus (SLE)</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HBsAb</td>
<td>76</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cardiolipin IgA</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>134</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Drug user</td>
<td>33</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HCV</td>
<td>89</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HIV</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HSV-2</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lyme Disease</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: *One specimen in the HTLV group demonstrated weak positive reactivity on the Galileo Neo platform while demonstrating a negative result on the Galileo platform.

Interfering Substances:
The performance of TPHA Screen was evaluated to determine the effect of the presence of interfering substances. This evaluation included specimens representing the conditions of icterus, icterus, and hemolysis. This group of specimens also included examples of both syphilis reactive and non-reactive and they were tested using TPHA Screen on both Galileo and Galileo Neo. This evaluation demonstrated that the presence of interfering substances in a specimen does not affect the ability of the Galileo or Galileo Neo to interpret TPHA Screen reactions. The results are summarized below.

The interfering substances studied were phospholipid, hemoglobin and bilirubin. EDTA plasma and serum were set up as control specimens. Six (6) control positive EDTA plasma specimens were created by diluting a positive reactive specimen with non-reactive EDTA plasma specimens to create reactive specimens. Sets of both reactive and non-reactive specimens were spiked with interfering substances, as described below.

The testing of the six reactive EDTA plasma specimens, sixteen (16) non-reactive EDTA plasma specimens and ten (10) non-reactive serum specimens that were spiked to contain 1000 mg/dl of phospholipid, 1000 mg/dl of hemoglobin and 20 mg/dl of bilirubin demonstrated 100% agreement between the spiked icteric, hemolyzed and icteric specimen results and the corresponding unspiked specimen aliquots.

The performance of this product is dependent on adhering to the recommended methods found in this insert.

For additional information or for technical support, contact Immucor at 855-IMMUCOR (466-8267).

Bibliography: