**XIII. LIMITATIONS**

As with other laboratory tests, the results are to be used as an aid in diagnosis. Positive samples should be verified by confirmatory tests such as MHA-TP and/or FTA-Abs. Except for areas where non-venereal trepanematoses are endemic, positive Trep-Chek results are usually the result of a syphilitic infection.

**XIV. LITERATURE:**


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**WARRANTY**

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TREP-CHEK™ is a trademark of Phoenix Bio-Tech Corp., Ontario, Canada



Patent 5,681,934



*Manufactured By:*

***PHOENIX BIO-TECH CORP.***

CANADA: 1166 South Service Rd. W
Oakville, Ont. L6L 5T7
Tel: (905) 827-9367
Fax: (905) 825-9795
Email: info@phoenixbiotech.com



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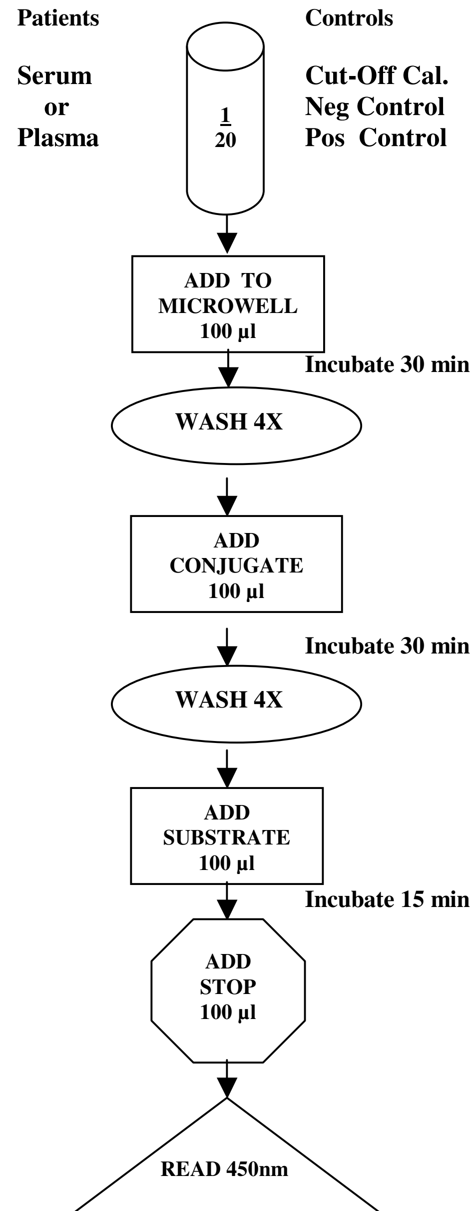
**SUMMARY OF TEST PROCEDURE**




# *PHOENIX BIO-TECH CORP.*

## TREP-CHEK™

### Anti-Treponema EIA

**Catalogue # TP-96**

**For *In Vitro* Use Only**

**I. INTENDED USE**

TREP-CHEK Syphilis EIA is a confirmatory *in vitro diagnostic* enzyme immunoassay for the qualitative detection of *Treponema pallidum* IgG antibodies in human serum or plasma. This product is not cleared by the U.S. Food and Drug Administration (FDA) for use in screening blood or plasma. This test is intended for use by clinical diagnostic laboratories as an aid in diagnosis of syphilis.

**II. CLINICAL RELEVANCE**

In response to *Treponema pallidum* subspecies *pallidum*, the causative agent of syphilis, two types of antibody responses normally result: non-specific (anti-cardiolipin) and specific (anti-treponemal). While non-specific antibodies occur in the majority of infectees, many other conditions can give rise to false positive results, yielding an overall specificity of about 50% in the general population.

One of the major reasons for the continued use of non-specific tests (RPR, VDRL, Wasserman etc.) is based on the observation that in response to appropriate (antibiotic) treatment, a significant decrease in titer usually takes place which is taken as the criterion of adequate treatment.

Treponemal or specific tests are based on the use of treponemal antigens in the assay. Prior to the HIV era, treponemal tests were largely used to confirm positive results obtained by non-specific screen tests. Although older treponemal tests (such as MHA-TP and FTA-Abs) are generally considered reliable for past and current infection (regardless of treatment) their specificity is limited due to the absence of *in vitro* culture techniques for *T. pallidum*. The use of recombinant treponemal antigens in "TREP-CHEK" results in increased sensitivity and specificity. Unless applied during early primary syphilis, treatment does not significantly affect the treponemal antibody status, hence no assumptions about efficacy of treatment or staging of disease can be made.

### III. PRINCIPLE OF THE PROCEDURE

Specific, recombinant treponemal antigens are immobilized on the microplate wells. Patient samples and controls are added to the wells. Anti-treponemal antibodies, if present, will specifically bind to the immobilized antigens; all non-bound proteins are removed during the washing step. The antigen-antibody complex is subsequently reacted with anti-human IgG antibodies conjugated with horseradish peroxidase (HRPO). After a second wash, which removes the unbound conjugate, a chromogenic reaction takes place on the plate as a result of addition of the TMB, a substrate for the peroxidase. The resulting colour is measured spectrophotometrically after adding stop solution. Colour intensity is proportional to the amount of antibody present in the patient's sample.

### IV. COMPONENTS PROVIDED IN THE KIT (96 TESTS)

1. Twelve 8-well strips coated with treponemal antigens. Store unused strips in the resealable plastic bag (up to 30 days at $4^oC$).
2. Cut-off calibrator, 1.0 ml. Stabilized human serum. Use to calculate sample's cut-off ratio.
3. Positive control, 1 vial of 1.0 ml . Stabilized human serum.
4. Negative control, 1 vial of 1.0 ml. Stabilized human serum.
5. Horseradish peroxidase labeled anti-human IgG antibody conjugate 1 bottle of 13 ml (ready to use).
6. Substrate solution TMB (tetramethyl benzidine), 1 bottle of 13 ml (ready to use).
7. Sample diluent buffer (10 x concentrate), 1 bottle of 10 ml.
8. Wash buffer (10 x concentrate), 1 bottle of 100 ml.
9. Stop Solution, 1 bottle of 13 ml (ready to use).

### V. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

1. EIA plate reader able to read OD at 450 nm,
2. Adjustable pipettes to deliver between 10 and 1000 µl,
3. Volumetric laboratory glassware,
4. Freshly distilled water,
5. Disposable microtubes for sample dilution,
6. Wash bottles or automated plate washing system,
7. Timer.

### VI. WARNINGS AND PRECAUTIONS

**This test contains material of human and animal origin and should be handled as a potential carrier or transmittance of disease.**

All reagents supplied are strictly for *in vitro* use only.

1. All controls provided are of human origin. The controls have been tested and found negative for HIV and hepatitis C antibodies, and negative for hepatitis B antigen. However, these sera must be treated as potentially infectious and should be handled according to appropriate procedures.
2. Avoid skin contact with the TMB substrate, stop solution (sulfuric acid), rinsing and dilution buffer. In case of accidental contact, wash thoroughly with tap water.
3. Do not interchange components from different lots. Do not use kit components beyond their expiry date. Wear protective clothing and disposable gloves while handling specimens or kit reagents.
4. Wash hands thoroughly after performing the test.
5. Avoid contact with eyes or skin. In case of contact wash with water immediately and contact a doctor.
6. Never pipette by mouth
7. Do not smoke, drink or eat in areas where specimens or kit reagents are handled

### VII. TECHNICAL DATA

| | |
|---|---|
| Package size: | 96 tests |
| Total test incubation time: | 75 min. |
| Required sample size: | 20 µl |
| Storage: | 2-$8^oC$. |
| Shelf life: | expiry date (or 30 days once opened) |

### VIII. COLLECTION AND STORAGE OF SPECIMENS

Serum is the recommended specimen, although plasma can be used as well. Undiluted samples may be stored at $4^oC$ or up to 5 days. If storage time exceeds 5 days, store at $-20^oC$ to $-70^oC$. Avoid repeated freezing and thawing. Allow serum samples to come to room temperature (21-$23^oC$).

### IX. PREPARATION AND STORAGE OF REAGENTS

**Preparation of Sample Diluent (SD)**:

Dilute the content of the vial containing concentrated sample diluent buffer with distilled water by adding 10 ml (**SD**) to 90 ml $dH_2O$, prior to use.

**Preparation of Wash Buffer (WB):**

Dilute the content of the bottle containing concentrated wash buffer with distilled water by adding 100 ml (**WB**) to 900 ml $dH_2O$.

### X. IMMUNOASSAY PROCEDURE

All materials should be at room temperature prior to starting the assay.

1. Dilute all patient sera and controls 1:20, by adding 20 µl of patient sample to 380µl of sample diluent.
2. Dispense 100 µl of diluted positive and negative control.
3. Dispense100 µl of diluted cut-off calibrator in duplicate in the beginning of run.
4. Dispense 100 µl of diluted patient samples into respective wells. (Transfer within 5 minutes.)
5. Dispense diluted cut-off calibrator in duplicate at the end of run.
5. Incubate plate at room temperature for 30 min.
6. Discard or aspirate the contents of the plate and wash at least 4 times with wash buffer.
7. Add 100µl enzyme conjugate into each well.
8. Incubate at room temperature for 30 minutes.
9. Discard the contents of the wells and wash 4 times with wash buffer.
10. Add 100µl of TMB substrate solution into each well.
11. Incubate at room temperature for 15 minutes, protected from light.
12. Add 100µl of stop solution and let stand for 5 minutes.
13. Read the optical density at 450 nm. and calculate results. Bi-chromatic measurement with a reference filter at 600-690 nm is recommended.

### XI. CALCULATION OF RESULTS

Calculate the ratio of patient OD over OD mean of cut-off calibrator multiplied by lot specific factor $f$:

$$\frac{\text{OD of samples}}{[\text{Mean OD of cut-off calibrator x } (f)]}$$

The value of factor $f$ is lot dependent and is provided in the Quality Control Sheet.

**Interpretation of results:**

The following is intended as a guide to interpretation of the EIA results; each laboratory should establish their own criteria for test interpretation based on sample population.

| Ratio | Interpretation |
|---|---|
| Less than 0.9 | negative |
| 0.9 – 1.1 | equivocal |
| Greater than 1.1 | positive |

**Calibrator:** The optical density of the calibrator should not vary more than 20%.

**Positive control**: The optical density of the positive control should be higher than the mean OD of cut-off calibrator.

**Negative control**: The optical density of the negative control should be lower than 0.25. Values of negative, positive controls and cut-off calibrator should fall within the range specified in the Quality Control Certificate.

### XII. ASSAY CHARACTERISTICS

**Precision:**

Intra-assay variation was determined on 12 determinations of low, medium and high OD samples within single test run.

**Intra-assay variations**

| Sample | Mean OD | CV |
|---|---|---|
| Low | 0.351 | 6.6% |
| Medium | 0.702 | 5.5% |
| High | 2.512 | 2.6% |

**Inter-assay variation**:

Inter-assay precision was calculated from results of 6 independent tests of low, medium and high OD samples.

| Sample | Mean | CV |
|---|---|---|
| Low | 0.258 | 8.5% |
| Medium | 0.691 | 6.2% |
| High | 1.999 | 4.2% |

**Correlation**

A side-by side comparison with a leading assay was performed on 45 difficult patient samples by a reference laboratory. There was a 100% correlation on test samples.

Two hundred patient samples found negative by an STD reference laboratory were tested and also found negative by Trep-Chek assay.

Four hundred patient samples from a clinical laboratory were tested by Trep-Chek. 12 samples were found positive which were confirmed as positive later by western blots.