# 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
## DECISION SUMMARY
## ASSAY ONLY TEMPLATE

**A. 510(k) Number:**
k053570

**B. Purpose for Submission:**
To demonstrate the safety and effectiveness of TREP-SURE™ EIA test kit

**C. Measurand:**
To detect antibodies to *T. pallidum*

**D. Type of Test:**
Enzyme linked immunoabsorption assay

**E. Applicant:**
PHOENIX BIO-TECH CORP.

**F. Proprietary and Established Names:**
TREP-SURE™ Treponemal Antibody EIA

**G. Regulatory Information:**

1. Regulation section:
   21 CFR 866.3830 Enzyme-linked immunoabsorption assay, *Treponema pallidum*

2. Classification:
   II

3. Product code:
   LIP

4. Panel:
   83 Microbiology

**H. Intended Use:**

1. Intended use(s):
   TREP-SURE™ EIA is a qualitative enzyme immunoassay for the *in vitro* diagnostic detection of *Treponema pallidum* (syphilis) antibodies in human serum or plasma.

2. Indication(s) for use:
   This device is indicated for used as an initial screening test or as a confirmatory diagnostic test.

3. Special conditions for use statement(s):
For prescription use only

The TREP-SURE™ EIA is not intended for use in screening blood or plasma donors.

Warning: A positive result is not useful for establishing a diagnosis of syphilis.  In most situations, such a result may reflect prior treated infection; a negative result can exclude a diagnosis of syphilis except for incubating or early primary disease.

4. Special instrument requirements:
N/A

## I.   Device Description:

TREP-SURE™ EIA is a 96 well microplate format which can be read photometrically. Recombinant treponemal antigens are immobilized on microplate wells coated with treponemal antigens.  Each lot of antigen is pre-qualified using a panel of well-characterized positive and negative sera.

## J.   Substantial Equivalence Information:

1. Predicate device name(s):
Trep-Chek Treponemal IgG Antibody EIA

2. Predicate 510(k) number(s):
k001552

3. Comparison with predicate:

| Similarities | | |
|---|---|---|
| Item | Device | Predicate |
| 1.  Analyte | To detect *T. pallidum* antibodies | To detect *T. pallidum* IgG antibodies |
| 2.  Indication for Use | Same | Aid in the diagnosis of syphilis disease |
| 3.  Specimens | Same | Serum and Plasma |
| 4.  Methodology | Same | Enzyme Immunoassay |
| 5.  Detection | Same | colorimetric |
| 6.  Calculation of results | Same | Quantitative determination with ratiometric values from the Cut-off Calibrator |
| 7.  Quality Control | Same | 2 Controls at different levels |

| Similarities | | |
|---|---|---|
| Item | Device | Predicate |
| | | |

| Differences | | |
|---|---|---|
| Item | Device | Predicate |
| 1. Sample Dilution | Undiluted 100 µL per well | 1:20 100 µL per well |
| 2. Incubation Time | 60 min/ 30 min/ 15 min | 30 min/ 30 min/ 15 min |
| 3. Incubation Temperature | 37°C | Room Temperature (18 - 25°C) |
| 4. Conjugate | Specific *T. pallidum* antigens - HRPO | Specific secondary anti-human IgG - HRPO |
| 5. Test Sensitivity | 0.0019 I.U. (WHO) | 0.10 I.U. (WHO) |

## K. Standard/Guidance Document Referenced (if applicable):

Not Applicable

## L. Test Principle:

Recombinant treponemal antigens are immobilized on the microplate wells. Patient samples and controls are added to the wells. Anti-treponemal antibodies, if present in the patient's serum or plasma, 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 Horseradish Peroxidase (HRPO) conjugated treponemal antigens. The second wash removes the unbound conjugate. A chromogenic reaction takes place on the plate as a result of addition of a substrate for the peroxidase. The resulting color is measured spectrophotometrically after adding the stop solution. Color intensity is proportional to the amount of antibody present in the patient's sample.

## M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   Reproducibility was performed in house and 2 external sites – US and Canada by 3 operators for 3 days.

**Inter-Site** (3 sites X 3 Operators X 3 Runs)

| Sample | Mean Index | SD | CV % |
|---|---|---|---|
| P1 | 16.8 | 4.2 | 24.8 |
| P2 | 13.5 | 2.0 | 14.9 |
| P3 | 1.6 | 0.3 | 15.8 |

| | | | |
|---|---|---|---|
| P4 | 7.5 | 1.4 | 18.5 |
| P5 | 16.4 | 5.0 | 30.4 |
| P6 | 17.2 | 4.6 | 26.4 |
| P7 | 0.1 | 0.2 | 150.2 |
| P8 | 0.2 | 0.3 | 152.7 |
| P9 | 0.6 | 0.1 | 14.2 |
| P10 | 4.9 | 0.7 | 14.5 |

## Intra-Site (3 Operators X 3 Runs)

| Site A | | | Site B | | | Site C | | |
|---|---|---|---|---|---|---|---|---|
| Mean Index | SD | CV % | Mean Index | SD | CV % | Mean Index | SD | CV % |
| 14.2 | 0.9 | 6.1 | 14.1 | 0.8 | 6.0 | 22.1 | 2.8 | 12.9 |
| 12.3 | 0.6 | 4.6 | 13.6 | 0.8 | 5.9 | 14.7 | 3.0 | 20.3 |
| 1.4 | 0.1 | 6.8 | 1.8 | 0.2 | 10.9 | 1.6 | 0.2 | 15.8 |
| 8.1 | 0.8 | 9.4 | 8.3 | 0.9 | 10.8 | 6.2 | 1.4 | 22.8 |
| 12.6 | 1.4 | 11.4 | 13.9 | 0.8 | 5.9 | 22.5 | 3.6 | 15.7 |
| 14.3 | 1.0 | 6.8 | 14.2 | 0.9 | 6.2 | 23.2 | 2.4 | 10.4 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.4 | 0.1 | 25.4 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 | 0.2 | 30.3 |
| 0.5 | 0.1 | 9.7 | 0.6 | 0.1 | 12.8 | 0.6 | 0.1 | 16.9 |
| 4.7 | 0.5 | 9.7 | 5.5 | 0.5 | 8.7 | 4.5 | 0.7 | 16.3 |

### Inter-day Assay Data

Four sera samples (3 positive and 1 negative) were evaluated for inter-day assay reproducibility. These samples were diluted and run on 10 different days as shown below.

| | Sample A | Sample B | Sample C | Sample D |
|---|---|---|---|---|
| Mean | 2.522 | 1.015 | 0.331 | 0.196 |
| S.D. | 0.18 | 0.08 | 0.03 | 0.02 |
| CV(%) | 7.0 | 7.7 | 9.7 | 9.5 |

### Interlot Assay

Eleven random positive and negative sera samples (8 positive and 3 negative) were run in duplicate on 3 different lots to demonstrate lot-to-lot reproducibility. This study was run on one day by the same technician. The following table shows the result of this reproducibility study.

| | Smpl 1 | Smpl 2 | Smpl 3 | Smpl 4 | Smpl 5 | Smpl 6 | Smpl 7 | Smpl 8 | Smpl 9 | Smpl 10 | Sampl 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 0.201 | 1.851 | 3.162 | 2.409 | 1.433 | 0.664 | 0.905 | 0.616 | 0.49 | 0.09 | 0.026 |
| S.D. | 0.008 | 0.039 | 0.016 | 0.08 | 0.205 | 0.056 | 0.057 | 0.049 | 0.028 | 0.015 | 0.0004 |
| CV(%) | 4 | 2.1 | 0.5 | 3.3 | 14. 3 | 8.4 | 6.3 | 7.9 | 5.7 | 16.7 | 1.5 |

### Intra-Assay

Within-run reproducibility was evaluated using five positive and 3 negative samples and run for 12 times in one assay run except for 1 sample which was

only run 7 times since quantity was not sufficient.  Results of this study are shown below.

|  | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| **Mean** | 0.016 | 0.142 | 1.565 | 1.008 | 3.272 | 1.665 | 3.244 | 0.010 |
| **S.D.** | 0.006 | 0.006 | 0.086 | 0.047 | 0.029 | 0.108 | 0.026 | 0.003 |
| **CV(%)** | 35.5 | 4.3 | 5.5 | 4.7 | 0.9 | 6.5 | 0.8 | 29.9 |

Single plate reproducibility was evaluated by using a low positive serum and running it across all 96 wells.  The assay mean was 0.27, SD was 0.02 with a CV of 6.9%.

b. *Linearity/assay reportable range:*

Not applicable (Qualitative assay – Pos, Neg, or Equivocal Results)

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Stability studies were performed in two evaluations to compare performance of different kits over time.  For the first evaluation, a WHO standard was serially diluted and tested with two lots of kits manufactured in 2002 and 2003 and have been in storage for 40 months and 28 months respectively.  These kits were then compared with a 6 month old kit with the following results: the amount of loss of dilutions range from 3% - 45% with the 28 month old kit and 42% – 75% with the 40 month old kit.  Further comparison with the 6 month old kit showed a decline of 1 dilution over a period of 28 months and a decline of 2 dilutions over a period of 40 months.  Results for the first evaluation are shown in the table below.

| Sample IU/mL | Lot # 050510T 6 months | | Lot # 030105T 28 months | | Lot # 020115T 40 months | | 28 mos % loss | 40 mos % loss |
|---|---|---|---|---|---|---|---|---|
| 0.4 | 0.074 | Neg | 0.052 | Neg | 0.034 | Neg | 28 | 54 |
| 0.8 | 0.125 | Neg | 0.074 | Neg | 0.041 | Neg | 41 | 67 |
| 1.6 | 0.223 | **Equi** | 0.133 | Neg | 0.079 | Neg | 40 | 653.1 |
| 3.1 | 0.419 | Pos | 0.229 | **Equi** | 0.104 | Neg | 45 | 75 |
| 6.3 | 0.807 | Pos | 0.443 | Pos | 0.239 | **Equi** | 45 | 71 |
| 12.5 | 1.61 | Pos | 0.954 | Pos | 0.458 | Pos | 41 | 72 |
| 25 | 2.721 | Pos | 1.982 | Pos | 0.955 | Pos | 27 | 65 |
| 50 | 3.24 | Pos | 3.156 | Pos | 1.882 | Pos | 3 | 42 |

For the second evaluation, panels of positive and negative sera were tested against kits that were 2 months and 11 months from date of manufacture.  There appears to be a minimal difference between the calculated indices of the predicate and test device.  A 12 month expiration dating has been assigned to the kits.

Additional stability studies were performed as follows:

1.  Evaluation of kit at elevated temperature (37°C for 7 days) with no change

in end results

2. Demonstration of the effect of temperature on serum samples. Aliquots from 20 negative and 20 positive samples were taken at clot retraction and at specified intervals (1, 3, 5, 7 days) with no observable effect on the final result.

QC studies were performed on each day of testing without any deviations from the expected results.

QC values are not stated in the package insert but certain requirements for validation of the assay were included as follows:

|  | Raw O.D. |
| --- | --- |
| Negative Control | <0.20 |
| Positive Control | >1.0 |
| Cut-Off Control | 0.2 – 0.6 |

Raw O.D. of Negative control < raw O.D. of Cut-Off Calibrator <raw O.D. positive control

d. *Detection limit:*
Not applicable

e. *Analytical specificity:*

**Cross-Reactivity Study**

One hundred nineteen samples from an outside laboratory were evaluated for cross reactivity. The LIA (Line Immuno Assay) had been used as a confirmatory test when there are discordant results. Results are shown in the table below.

Summary of Cross Reactivity Test

| Disease State | # Samples Tested | Trep-Sure™ (#Pos) |
| --- | --- | --- |
| SLE | 6 | 0 |
| Toxoplasma IgM | 5 | 0 |
| Drug Users | 10 | 2 |
| H. pylori | 10 | 1 |
| ANA + | 24 | 0 |
| Arthritis | 64 | 3 |
| Borreliosis | 10 | 0 |
| Pregnancy | 100 | 0 |

**Interference Study**

Eight lipemic samples selected from a collection of frozen sera were evaluated for interference. These were tested against 8 clear randomly selected samples. Additionally,

the WHO standard had been diluted in various concentrations using pooled lipemic and clear sera with no apparent effect on the assay.

Ten samples with varying levels of bilirubin and hemolysis were tested with the Trep-Sure™ assay. Each sample was tested neat and with the addition of a reactive serum at 50% of the volume to assess the effect of each interferent on the reactivity of the sample. No interference was observed.

    *f. Assay cut-off:*

The WHO standard was used to establish the cut-off at a concentration of 0.016 IU/mL. The Cut-off Calibrator provided in the kits is derived from a human positive serum that is adjusted to match the primary WHO standard value of 0.016 IU/ml.

To evaluate the cut-off value, 100 samples were assayed. Negative samples yielded a mean OD of 0.020 and 1 S.D. of 0.026 and positive samples had a mean OD of 3.065 and 1 S.D. of 0.674. When the mean plus 4 S.D. was applied to the negative samples and the mean minus 4 S.D. was applied to the positive samples, the values are 0.125 and 0.366 respectively. Using the WHO standard value of 1.6 mIU/mL yielded an O.D. of 0.21 which this standard is in the approximate center of the 4 S.D.

2. Comparison studies:

    *a. Method comparison with predicate device*

Clinical testing was performed at 1 US site and 2 European sites. All samples were compared to the predicate device, RPR and TP-PA. Discordant samples were further tested with LIA.

Additionally, an inhibition study was performed on 20 IgM positive sera with inconclusive results. It cannot be determined at this time if this assay can detect IgM antibodies.

    *b. Matrix comparison:*

Seventeen positive and 11 negative matched serum and plasma (EDTA) samples were tested to evaluate for potential differences between the two types of matrices. There were no observable differences noted.

Additionally, 25 positive and 15 negative matched serum and citrated plasma were evaluated with no difference in results.

3. Clinical studies:

    *a. Clinical Sensitivity:*

Clinical testing was also performed on 636 samples from patients that are

suspected positive for syphilis and exhibiting symptoms of syphilis. The breakdown of sample distribution are as follows: 73 TP-PA positive samples; 200 RPR positive samples purchased from a commercial source; 113 samples from various stages of disease (treated and not treated) that are RPR pos/TPHA pos; 77 RPR pos (titers <180) /TPHA neg; 5 RPR neg/TPHA <1/80; 17 samples with non-specific agglutinations by TPHA; 75 latent syphilis (RPR neg/TPHA pos); 32 early primary samples (darkfield positive); and 44 from men having sex with men. All samples in this category were tested with the predicate and test device sometimes with the addition of other testing methods not previously done with the other studies. For resolution of discordant results, LIA blots were used.

**Suspected/Known Syphilis**

|  |  | Trep-Chek | | | |
|---|---|---|---|---|---|
|  |  | Positive | Negative | Equivocal | Total |
| **Trep-Sure™** | Positive | 422 | 13* | 15*** | 450 |
|  | Negative |  | 181 | 2**** | 183 |
|  | Equivocal | 0 | 3** | 0 | 3 |
|  | Total | 422 | 197 | 17 | 636 |

*All samples confirmed positive by LIA
**1 sample confirmed negative by LIA, 2 samples unconfirmed
***10 Samples confirmed positive by LIA, 5 samples unconfirmed
****1 sample unconfirmed, and 1 sample Inconclusive by LIA

| Percent Agreement | | | Exact 95% Confidence Interval |
|---|---|---|---|
| Positive | 99.5 % | (437/439) | 98.4 – 99.9% |
| Negative | 91.87% | (181/197) | 87.1 – 95.3% |
| Overall | 97.17% | (618/636) | 95.6 – 98.3% |

Note: Equivocals treated as Positive

An additional 60 samples were performed at US reference laboratory with the following results.

|  |  | Clinical Diagnosis | | |
|---|---|---|---|---|
|  |  | Positive | Negative | Total |
| **Trep-Sure™** | Positive | 27 | 1 | 28 |
|  | Negative | 0 | 32 | 32 |
|  | Total | 27 | 33 | 60 |

| Percent Agreement | | | Exact 95% Confidence Interval |
|---|---|---|---|
| Positive | 100% | (27/27) | 87.2 – 100% |
| Negative | 95% | (32/33) | 84.2 – 99.9% |
| Overall | 98.3% | (59/60) | 91.1 – 100% |

b. *Clinical specificity:*

One thousand six hundred fifty five samples from the normal population were tested with the following results. These samples were randomly collected from both sexes from blood donor and from presumed to be healthy patients. Sixty four of the blood donor samples were from a blood bank in Houston, Texas, and 50 plasma samples were from the New Jersey area.

Normal Population

| | | Trep-Chek | | | |
|---|---|---|---|---|---|
| | | Positive | Negative | Equivocal | Total |
| **Trep-Sure™** | Positive | 9 | 4* | 7** | 20 |
| | Negative | 0 | 1,634 | 1*** | 1,635 |
| | Equivocal | 0 | 0 | 0 | 7 |
| | Total | 9 | 1,638 | 8 | 1,655 |

*3 Samples indeterminate by LIA, 1 sample pos by LIA
**6 samples confirmed positive by LIA, 1 sample indeterminate by LIA
***Negative by LIA

| | Percent Agreement | | Exact 95% Confidence Interval |
|---|---|---|---|
| Positive | 100% | (16/16) | 79.4 – 100% |
| Negative | 99.75% | (1635/1639) | 99.4 – 99.9% |
| Overall | 99.75% | (1651/1655) | 99.4 – 99.9% |

Samples that resulted in Index Values in the Equivocal range (0.8 – 1.2) were considered positive

   c. Other clinical supportive data (when a. and b. are not applicable): Not applicable

4. <u>Clinical cut-off</u>**:**
   Not applicable

5. <u>Expected values/Reference range:</u>

**Calculation of Results**
1) Subtract O.D. of Blank ("Bl" ) from <u>all</u> wells.
   If the resulting O.D. value has a negative value, it is to be taken as 0.001.
2) Determine the mean of the Cut-off Calibrator. Each value should be within 20% of the mean. Sample O.D.'s can be compared to the Cut-Off Calibrator mean O.D. and used to determine a qualitative positive or negative result.
3) Optional: If an Index Value is desired, Divide O.D. of samples (patients) and controls by the mean Cut-off value to obtain "INDEX VALUE"

| Negative | < Cut-Off Cal mean O.D. minus 20% | Index Value <0.8 |
|---|---|---|
| Positive | > Cut-Off Cal mean O.D. plus 20% | Index Value > 1.2 |
| Equivocal | between Cut-off Cal O.D. +/- 20% | Index Value 0.8 – 1.2 |

Samples with values in the Equivocal range (0.8 to 1.2 ratio) or Positive (>1.2 ratio) should be retested.  If the sample remains equivocal on retest, the patient should be considered suspect for disease since a low level of antibody is detected.  A new sample should be obtained and retested.  If the patient remains equivocal, the patient should be monitored for antibody status.

**N. Proposed Labeling:**

The labeling is sufficient and satisfies the requirements of 21 CFR 809.10.

**O. Conclusion:**

The submitted information in this premarket notification is complete and supports substantial equivalence decision.