TREP-SURE™

Anti-Treponema EIA Screen

Catalogue # TS96 / TS960 / TS1920

For *In Vitro* Use Only

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Rev. I July 2010

INTENDED USE

TREP-SURE™ EIA is a qualitative enzyme immunoassay for the *in vitro diagnostic* detection of *Treponema pallidum* (syphilis) antibodies in human serum or EDTA and citrated plasma. This product can be used as an initial screening test or as a confirmatory diagnostic test, but is not cleared (approved) by the U.S. Food and Drug Administration (FDA) 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 a prior treated infection; a negative result can exclude a diagnosis of syphilis except for incubating or early primary disease.

SUMMARY AND EXPLANATION

In response to *Treponema pallidum* subsp. *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, and 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 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 presence of non-specific antigens in preparations of *in vivo* cultivated *T. pallidum*. The use of recombinant treponemal antigens in TREP-SURE™ 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.

The assay methods described above all require visual interpretation and are limited to subjective interpretation by the operator. The use of the more specific Enzyme ImmunoAssay (EIA) has received wide spread acceptance due to the colorimetric results in a 96 well microplate format that can be automated and read photometrically. The TREP-SURE™ Syphilis EIA assay uses recombinant antigens in an enzyme immunoassay format to yield a more specific result with objective reading of absorbencies compared to a Cut-off Calibrator.

PRINCIPLE OF THE TEST

Specific 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. After a second wash, which removes the unbound conjugates, a chromogenic reaction takes place on the plate as a result of addition of TMB, a substrate for the peroxidase. The resulting color is measured spectrophotometrically (450 nm) after adding stop solution. Color intensity is proportional to the amount of antibody present in the patient's sample.

MATERIALS SUPPLIED TS96 / TS960 / TS1920

	TS96	TS960	TS1920
Microwell strips coated with specific recombinant antigens.	12 x 8-well strips	120 x 8-well strips	240 x 8-well strips
Positive Control: stabilized human serum containing <0.1%sodium azide as preservative. ***Ready to use.***	1.8 ml/vial with red cap	1.8 ml/vial x 3 vials with red cap	1.8 ml/vial x 6 vials with red cap
Cut-off Calibrator: stabilized human serum containing <0.1% sodium azide as preservative. ***Ready to use.***	1.8 ml/vial with yellow cap	1.8 ml/vial x 4 vials with yellow cap	1.8 ml/vial x 8 vials with yellow cap
Negative Control: stabilized human serum containing <0.1% sodium azide as preservative. ***Ready to use.***	1.8 ml/vial with green cap	1.8 ml/vial x 3 vials with green cap	1.8 ml/vial x 6 vials with green cap
Conjugate: horseradish peroxides conjugated with specific recombinant antigens. ***Ready to use.***	13 ml	5 x 25 ml	250 ml
Wash Buffer ***(concentrated X 20)***: phosphate buffered saline and Tween 20.	50 ml	500 ml	1,000 ml
Substrate: containing 3,3',5,5' tetramethylbenzidine (TMB). ***Ready to use.***	13 ml	5 x 25 ml	250 ml
Stop Solution: containing 1N sulfuric acid. ***Ready to use.*** **CAUTION:** Solution is toxic. Avoid contact with skin or eyes. If contact is made, flush area with copious amounts of water.	13 ml	5 x 25 ml	8 x 30 ml
Instruction for use and Quality Control certificate.	1	1	1

MATERIALS REQUIRED BUT NOT PROVIDED

1. EIA plate reader able to read OD at 450 nm, reference at 610-630nm.
2. Adjustable pipettes to deliver between 100 and 1000 µl.
3. Volumetric laboratory glassware.
4. Freshly distilled or de-ionized water.
5. Wash bottles or automated plate washing system.
6. Timer.
7. Absorbent toweling.
8. 37°C Incubator.

WARNINGS AND PRECAUTIONS

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

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

1. Warning: The human source components were tested and found negative for anti-HIV (types 1 and 2) and HBsAg by FDA recommended (approved/licensed) tests. Because no test method can offer complete assurance that laboratory specimens do not contain HIV, hepatitis B virus, or other infectious agents, specimens should be handled at BSL 2 as recommended for any potentially infectious human or blood specimen in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, May 1999, and CLSI Approved Guideline M29-A, Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids and Tissue.
2. Avoid skin contact with the TMB substrate, stop solution (1 N sulfuric acid), and washing buffer. In case of accidental contact, wash thoroughly with tap water.
3. Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
4. 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.
5. Wash hands thoroughly after performing the test.
6. Avoid contact with eyes or skin. In case of contact, wash with water immediately and contact a doctor.
7. Never pipette by mouth.
8. Do not smoke, drink or eat in areas where specimens or kit reagents are handled.
9. Negative and Positive Controls, as well as the Cut-off Calibrator must be run with each assay.
10. Use separate pipette tips for each sample, control and reagent to avoid cross contamination.
11. Mix the contents of the microplate wells thoroughly to ensure good test results.

TECHNICAL DATA

Package size:	96 tests; 960 tests; 1920 tests
Total test incubation time:	105 min.
Required sample size:	100µL
Storage:	2-8°C. Do not freeze.
Shelf life:	Unopened kits are stable through the expiration date stated on the label expiry date (or 30 days once opened).

COLLECTION AND STORAGE OF SPECIMENS

- Qualified personnel using approved aseptic venipuncture techniques should collect a whole blood sample.
- The specimen for this kit is human serum and EDTA or citrated plasma. Specimens containing erythrocytes or other visible matter should be centrifuged.
- Do not inactivate plasma.
- Do not use hyperlipemic, hemolytic, or contaminated samples.
- Test the specimen within 48 hours.
  If assays are not completed within 8 hours, separated serum/plasma should be refrigerated (2 - 8°C). If assays are not completed within 48 hours, or the separated serum/plasma is to be stored beyond 48 hours; serum/plasma should be frozen at or below -20°C. Avoid repeated freezing and thawing.
- Serum samples that require transport should be shipped with an ice pack*.

**Special Conditions: Serum samples that are transported under ambient temperatures (up to 40°C) and/or kept "on the clot" must be tested within 5 days of collection. If storage in excess of 5 days is necessary, serum should be removed from the clot as soon as possible and stored at* 2 - 8°C. *However, it is recommended that separated serum be removed from the clot as soon as possible and that separated serum/plasma should remain at room temperature for no longer than 8 hours.*

PREPARATION OF WASH BUFFER WORKING SOLUTION

Dilute 1 volume of the 20X concentrated wash buffer with 19 volumes of distilled or de-ionized water. Mix well before use.
Diluted wash buffer can be stored for 30 days at 2-8°C or up to 7 days at room temperature (15°C – 30°C).

ASSAY PROCEDURE

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

- Bring all reagents to room temperature (15-30°C) before use.
- Remove the amount of strips being used for the day's testing and replace the remainder in a re-sealable plastic bag with the desiccant and store at 2-8°C.
- Allow samples to come to room temperature (15-30°C).

1. Leave first well empty (Blank)
2. Dispense 100µL per well of Negative Control in the second and third wells;
3. Dispense 100µL per well of Cut off Calibrator in wells four, five and six;
4. Dispense 100µL per well of Positive Control in wells seven and eight
5. Dispense 100µL per well of patient samples into respective wells.
6. Incubate plate for **60** (±5) min at **37°C** (cover the plate)
7. Discard or aspirate the contents of the plate and wash 4 times with 300µL per well of Wash Buffer Working Solution.
8. Add 100µL per well enzyme Conjugated Antigens to all wells.
9. Incubate plate for **30** (±3) minutes at **37°C** (cover the plate)
10. Discard or aspirate the contents of the wells and wash 4 times with 300µL per well of Wash Buffer Working Solution.
11. Add 100µL per well of TMB substrate solution to all wells.

12. Incubate plate for **15** (±2) minutes at **37°C**, protected from light.
13. Add 100µL of Stop Solution to each well.
14. Read within **15** minutes with optical density at 450 nm and calculate results. Bi-chromatic measurement with a reference filter at 600-690 nm is recommended if available.

INTERPRETATION OF RESULTS

Validation of the Assay

1. O.D. of Negative control must be < 0.20
2. O.D. of Positive control must be > 1.0
3. O.D. of Cut-off Calibrator must be between 0.15 and 0.50 O.D.
4. O.D. of Negative control < O.D. of Cut-off Calibrator < O.D. positive control.

If any of these conditions are not met, the assay is invalid and needs to be repeated.

Calculation of Results

1. Subtract O.D. of Blank ("Bl") from all 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.
3. To obtain the Index Values, divide the O.D. of samples (patients) or controls by the mean Cut-off Calibrator value (CO).

$$\text{Index} = \frac{OD_{\text{Sample/Control}}}{OD_{\text{Cut-off Calibrator}}}$$

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 its sample population.

Interpretation	O.D.	Ratio
Negative	< Cut-off Calibrator mean O.D. minus 20%	Index Value < 0.8
Positive	> Cut-off Calibrator mean O.D plus 20%.	Index Value > 1.2
Equivocal	Cut-off Calibrator O.D. +/- 20%	Index Value 0.8 – 1.2

Samples with values in the equivocal range (0.8 to 1.2 ratios) should be retested. If the sample remains equivocal on retest, the patient should be considered suspect for infection with *T. pallidum* since a low level of antibody is detected. A second sample should be collected 2 – 4 weeks later and tested. An equivocal result indicates that a low level of antibody is detected, and the patient should be monitored for antibody status.

A negative sample and/or the negative control will have an index value < 0.8. A negative result indicates that no, or very low levels of antibody are present in the sample, but does not rule out a recent or current infection.

A positive sample and/or the positive control will have an index value > 1.2. A positive result indicates that antibody is present in the sample as a result of previous or present infection with *T. pallidum*. The magnitude of the measured result above the cut-off is not indicative of the total amount of antibody present.

Consult "*Quality Control Certificate*" for typical results obtained when run manually.

The following Table provides an algorithm to aid in interpreting and reporting syphilis serology results for diagnosis of *T. Pallidum* infection status.

Non-Treponemal Result (NT)	Treponemal Result	Report/interpretation for all except neonates or infants*
Nonreactive	Negative (Nonreactive)	No serologic evidence of infection with *T. pallidum* (incubating or early primary syphilis cannot be excluded).
Reactive	Negative (Nonreactive)	Current infection unlikely; probability of BFP secondary to other medical conditions (febrile diseases, immunizations, IVDU, autoimmune diseases, etc.). Recommend repeat testing (nontreponemal, and treponemal by other test method.)
Nonreactive	Positive (Reactive)	Probably past infection or potential cross-reactivity with other spirochetes/related antigens; additional testing appropriate to clinical findings/history;** possibility of false negative NT due to prozone and late latent syphilis or neurosyphilis.
Reactive	Positive (Reactive)	Presumptive evidence of current infection (or inadequately treated infection, persistent infection, re-infection, or BFP if prior history); additional testing consistent with clinical assessment.**
Nonreactive	Not done	Current infection unlikely; effectively treated infection if previous diagnosis and treatment; cannot exclude incubating or early primary syphilis; cannot exclude latent or neurosyphilis. Treponemal testing advised if clinical suspicion of latent or neurosyphilis.
Not done	Negative (Nonreactive)	Current or past infection unlikely; cannot exclude incubating or early primary syphilis.

*HIV-infected individuals may have delayed sero-reactivity or negative serology.
**Quantitative non-treponemal testing; clinical history; repeated (sequential) serological testing for changes in titer.

QUALITY CONTROL:

The test results are only valid if the test has been performed following the instructions. The user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable local, state, and federal regulations. Positive and Negative Control sera are supplied with the kit. The Negative and Positive controls validate the assay at the critical absorbance levels to ensure test performance, test integrity and operator reliability. Good laboratory practice dictates running the positive and negative controls each time the kit is used. If the results of the positive and/or negative control are not within the range (as stated on the Certificate of Analysis), the test results are invalid and the assay should be rerun. If the criteria are not met, the run is not valid and should be repeated. Warning: If QC results are “out of range” or invalid, the results must not be reported. Each laboratory should use known samples as further controls. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. It is recommended to participate in appropriate quality assessment trials.

LIMITATIONS:

1. The values obtained from this assay are intended to aid in diagnosis only. As with all serological tests for syphilis, interpretation of results obtained with the TREP-SURE™ Syphilis Antibody test must be used in conjunction with the patient's clinical symptoms, medical history and other clinical and/or laboratory findings to produce an overall clinical diagnosis.
2. The TREP-SURE™ EIA test is specific for detecting *Treponema pallidum* antibodies in serum or plasma samples. It does not detect *T. pallidum* directly.
3. Only if test instructions are rigidly followed will optimum results be achieved.
4. Use fresh serum or plasma or samples frozen only once and thawed. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield spurious results.
5. Specimens giving equivocal results in the assay should be re-tested. A repeated equivocal specimen should be reported as Inconclusive for follow-up and another specimen drawn in two weeks for testing and/or confirmed by other methods, such as FTA-ABS or Western Blot.
6. All treponemal tests tend to remain reactive following treponemal infection; therefore, they should not be used to evaluate response to therapy. Because of the persistence of reactivity, probably for the life of the patient, the treponemal tests are of no value to the clinician in determining relapse or re-infection in a patient who has had a reactive result.
7. Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as rinsing the test wells and thorough mixing of all prepared solutions.
8. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled evenly with Washing Solution, and that there are no residues in the wells.
9. Instructions for using appropriate photometers are to be observed; check adjustment of proper wavelength and reference wavelength respectively.

EXPECTED RESULTS:

To assess the specificity of the TREP-SURE™ test a series of 1,655 serum and plasma samples were obtained from various types of laboratories in different geographical locations. The samples came from a general "presumed" healthy normal population (ages 18 – 72) whose serum or plasma were sent to laboratories for routine testing from clinics, hospital out-patient, and blood donor sites.

A series of studies were performed to confirm the cut-off and determine the specificity of the TREP-SURE™ EIA test using a known normal population. Samples obtained from blood banks were tested by RPR and found negative. All of the samples were tested with the TREP-CHEK™ and TREP-SURE™ EIA tests. Sample results that were discrepant between the two tests were then tested by Line ImmunoAssay (LIA) (Western Blot) to observe the syphilis antibody bands.

To evaluate the use of plasma in the assay, a series of "normal" blood donors from the Northeast and Southern United States and plasma from a hospital blood bank were tested. A total of 206 donor samples were tested. A series of 64 matched donor serum and plasma were collected to demonstrate the use of either serum or plasma in this assay. The results demonstrated that there was no difference between paired serum and plasma (with EDTA as anticoagulant).

Another series of serum samples were collected from various laboratories that were pulled from a presumed normal populations sent in for routine serology testing (not for a disease condition). The samples were tested by TREP-SURE™. Positives were retested with TREP-CHEK™ EIA. Discrepant sample results were tested by LIA to confirm status.

The results of testing the 1,655 presumed normal samples yielded 20 samples that were found to be positive by TREP-SURE™ and confirmed by other reference EIA and LIA methods. Four TREP-SURE™ positive samples were negative by one reference method with 3 of these indeterminate and one positive by LIA. Seven equivocal samples by the reference EIA had six confirmed positive and one indeterminate by LIA. After confirmation of the TREP-SURE™ results by two methods the TREP-SURE™ test correctly identified 16 samples positive with 4 assumed false positive. This resulted in a sensitivity of 100% and specificity of 99.8%. Therefore from this study of 1,655 presumed normal samples 16 were confirmed positive resulting in a 1% positivity rate. These results are consistent with published rates for prevalence of antibody in the adult population. Prevalence may vary depending on a variety of factors such as geography, age, socio-economic status, ethnic background, and type of test employed, specimen collection and handling procedures, clinical and epidemiological history.

PERFORMANCE CHARACTERISTICS:

Comparison

Several studies were conducted which compared the TREP-SURE™ EIA to the Serodia TP-PA, SERA-TEK MHA-TP Kit (Miles Labs.), RPR, Trep-Chek, and Line ImmunoAssay (LIA) , using a total of **712** specimens from patients sent for testing and identified by physicians as suspected positive for syphilis and/or exhibiting symptoms. A total of **1,655** specimens from healthy patients and blood donors considered as normal population, randomly collected from both sexes. These samples were pulled from the laboratory's routine workload of patient testing.
The TREP-SURE™ assay demonstrated 100% agreement to the confirmed positive serum samples tested against at least 2 other methods. The total samples of "presumed" positive and normal donors yielded 99.7% Negative Agreement in the TREP-SURE™ assay compared to the reference methods.

Summary of Comparison Results:

Healthy Adults & Blood Donors	Various Stages of Syphilis Infection

Total # Samples = 1655
Positive = 20
Negative = 1635
 	
% Agreement Positive = 100%
% Agreement Negative = 99.8%
	Total # Samples = 113
	Positive = 112
	Negative = 1*
	
	% Agreement Positive = 99.1%
	
	* confirmed neg. by LIA

Darkfield Positive Primary Samples	Latent Stage

Total # of samples = 32
Positive = 29
Negative = 2
Equivocal = 1
 	
1 patient with "oral lesions"; 2 patients with very low or no antibody detected;
% Agreement Positive = 93.7%
	Total # Samples = 75
	Positive = 75
	Negative = 0
	
	% Agreement Positive = 100%

Men Having Sex with Men	Difficult Samples

Total # Samples = 44
Positive = 5
Negative = 39
% Agreement Negative = 88.6%
	Total # Samples = 60
	Positive = 27
	Negative = 33
	% Agreement Positive = 100%
	% Agreement Negative = 97%

Normal Population:

		Reference EIA			
		Positive	Negative	Equivocal	Total
TREP-SURE™	Positive	9	4*	7**	20
	Negative	0	1,634	1***	1635
	Equivocal	0	0	0	
	Total	9	1638	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

% Agreement Positive: 16/16 = 100% (95% C.I. 79.4 – 100)
% Agreement Negative: 1635/1639 = 99.75% (95% C.I. 99.4 – 99.9)
Overall Agreement: 1651/1655 = 99.75% (95% C.I. 99.4 – 99.9)
Samples that resulted in Index Values in the Equivocal (Inconclusive) range (0.8 – 1.2) were considered positive.

Suspected/Known Positive Syphilis Patients:

		Reference EIA			
		Positive	Negative	Equivocal	Total
TREP-SURE™	Positive	422	13*	15***	450
	Negative	0	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

% Agreement Positive: 437/439 = 99.5% (95% C.I. 98.4 – 99.9)
% Agreement Negative: 181/197 = 91.87% (95% C.I. 87.1 – 95.3)
Overall Agreement: 618/636 = 97.17% (95% C.I. 95.6 – 98.3)
Note: Equivocals treated as Positive

A series of 60 samples yielding unclear syphilis serology results for diagnosis of *T. pallidum* infection status were obtained from state health labs and assayed by a reference lab to aid in determining a syphilis diagnosis. Of these 60 samples, one sample was considered discrepant, but could not be confirmed by repeat of the reference methods. The results of the testing and diagnosis for this series of problematic samples are shown in the following table.

		Clinical Diagnosis		
		Positive	Negative	Total
TREP-SURE™	Positive	27	1	28
	Negative	0	32	32
	Total	27	33	60

% Agreement Positive = 27/27 = 100% (95% C.I. 87.2 to 100)
% Agreement Negative = 32/33 = 95% (95% C.I. 84.2 to 99.9)

Overall Agreement = 59/60 = 98.3% (95% C.I. 91.1 to 100)

Diagnostic Interpretation:

In order to assess the true performance of the TREP-SURE™ test, samples were confirmed positive or negative by two methods and then compared to the TREP-SURE™ results. When a multiple test regime is applied to confirm the status of a patient sample, the following results are obtained. If a sample is found positive by one method it is re-tested by a confirmatory test, i.e. TPHA, EIA, or LIA

Multiple Methods Interpretation

		Positive	Negative	Total
TREP-SURE™	Positive	467*	6**	473
	Negative	0	1,818	1,818
	Total	467	1,824	2,291

* 1 sample Inconclusive (Indeterminate) by LIA
**3 samples indeterminate by LIA, and 3 negative

% Agreement Positive: 467/467 = 100.0% (95% C.I. 99.2 - 100)
% Agreement Negative: 1818/1824 = 99.7% (95% C.I. 99.3 – 99.9)
Overall Agreement: 2285/2291 = 99.7% (95% C.I. 99.4 – 99.9)
Note: Equivocals are considered positive

Potential Cross Reactors:

Panels of **229** samples were obtained to evaluate potential interference from different disease conditions. These samples were confirmed positive for each respective disease condition and were assayed in the TREP-SURE™ EIA. These specimens were medically diagnosed for Systemic Lupus Erythematosus (SLE), Toxoplasma, H. pylori, Arthritis (RF), drug addiction, Borreliosis, and ANA positive patients. One hundred prenatal testing samples from pregnant women were also evaluated for potential assay interference. The categories and number of reactive results obtained are listed in the following table. The TREP-SURE™ test showed no cross reactivity in the samples evaluated. Samples found positive were also positive by other treponemal methods.

Category	Number Tested	TREP-SURE™ Number Reactive
Drug Users	10	2*
Toxoplasma IgM	5	0
SLE	6	0
ANA +	24	0
H. pylori	10	1*
Arthritis	64	3*
Borreliosis	10	0
Pregnant Females	100	0

*These samples were also positive with the reference methods.

REPRODUCIBILITY

Studies were performed to demonstrate the Inter-day, Inter-Lot, and Intra-Assay reproducibility of the TREP-SURE™ Test kit.

Intra-Assay:
Reproducibility of 8 samples in replicates of 12 within one assay run.

Sample	1	2	3	4	5	6	7	8
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
C.V. %	35.5	4.3	5.5	4.7	0.9	6.5	0.8	29.9

Inter-Assay 1
Reproducibility of the test run over 10 days using four samples.

Sample	1	2	3	4
Mean O.D.	2.522	1.015	0.332	0.196
S.D.	0.177	0.078	0.032	0.019
CV (%)	7.0	7.7	9.7	9.5

Inter-Assay 2
Inter- and Intra-Lab Reproducibility was evaluated using 3 sites with 3 operators with 3 assay runs each at each site. The results are shown in the following Tables:

Intra-Site - 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

Inter-Site – 3 sites X 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-Lot

Reproducibility of the TREP-SURE™ EIA Test over 3 lots of kits.

Sample	1	2	3	4	5	6	7	8	9	10	11
Mean	0.201	1.851	3.162	2.409	1.433	0.664	0.905	0.616	0.49	0.090	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 (%)	4.0	2.1	0.5	3.3	14.3	8.4	6.3	7.9	5.7	16.7	1.5

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