SUMMARY
Syphilis is a sexually transmitted (venereal) disease caused by the spirochaete Treponema pallidum. After infection the host forms Treponemal antibodies to Treponema pallidum, in addition the host also forms Non Treponemal antilipoidal antibodies in response to the lipoidal material released from the damaged host cell. These antibodies are traditionally referred to as ‘Reagins.’

Toluidine Red Unheated Serum Test (TRUST) is a macroscopic Non Treponemal flocculation test for the detection and quantitation of antilipoidal antibodies. Non-Treponemal tests like REDGEN® are of great value when used for screening and follow up of therapy.

REAGENTS
1. REDGEN® reagent: A particulate suspension containing a red micronised dye coated with lipid complexes.
2. Positive control, reactive with the REDGEN® reagent.
3. Negative control, non reactive with the REDGEN® reagent.
4. REDGEN® detects antilipoidal antibodies in serum or plasma. As against the conventional VDRL reagents, test samples do not require heat inactivation.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, sensitivity and performance.

REAGENT STORAGE AND STABILITY
Store the reagent at 2-8°C. DO NOT FREEZE.
The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. Do not use reagents after the expiry date. Avoid exposure to elevated temperature and air, as the reagent is highly sensitive to denaturation and drying.

PRESENTATION

<table>
<thead>
<tr>
<th>REF</th>
<th>TRUST Ag</th>
<th>Control</th>
<th>Disposable slides with eight reaction circles</th>
<th>Disposable sample / control dispensing pipettes</th>
<th>Rubber teats</th>
<th>Mixing stick ladder</th>
<th>Reagent dropper for dispensing Redgen reagent suspension</th>
<th>Package Insert</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10512075</td>
<td>+ 0.4 ml</td>
<td>10</td>
<td>75</td>
<td>2</td>
<td>3</td>
<td>1</td>
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</table>

PRINCIPLE
During the test procedure, the specimen, serum or plasma is mixed with REDGEN® reagent and allowed to react for eight minutes. If antilipoidal antibodies are present in the specimen, they will react with REDGEN® reagent forming visible red floccules against the white background of the reaction card. If antilipoidal antibodies are not present in the specimen, there will be no flocculation, resulting in an even pink mat on the reaction circle.

NOTE
1. In vitro diagnostic reagent for laboratory or professional use only. Not for medicinal use.
2. The reagent contains 0.1% Sodium azide as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
3. The reagents that are derived from human source have been tested for HBsAg and Anti-HIV antibodies and are found to be non-reactive. However handle the material as if infectious.
4. REDGEN® reagent suspension should be gently but thoroughly mixed before testing to disperse the dye particles
uniformly and improve test readability.

5. Performance of the reagent must be verified with positive and negative controls and it is recommended that controls be run with each test series.

6. Accessories provided with the kit should be used for optimum results.

7. Do not use damaged or leaking reagents.

SAMPLE COLLECTION AND STORAGE
No special preparation of the patient is required prior to sample collection by approved techniques. Haemolysed or lipemic samples are not suitable for testing. In case of oxalated blood samples, it is advisable to avoid excess of oxalate as it may interfere with the test results.

Fresh serum or plasma should be used for testing. Samples not tested immediately may be stored at 2-8°C for upto 48 hrs. Hazy samples should be centrifuged. Use the clear supernatant for testing.

MATERIAL PROVIDED WITH THE KIT
TRUST antigen, Positive control reactive with reagent, Negative control non-reactive with reagent, Disposable slides with eight reaction circles, Disposable sample / control dispensing pipettes. Mixing sticks, Rubber teats, Reagent dropper for dispensing the REDGEN® reagent suspension.

ADDITIONAL MATERIAL REQUIRED
Stop watch, High intensity light source, Isotonic saline, Pipettes, Test tubes, Mechanical rotor at 180 rpm circumscribing a circle 2 cm in diameter on a horizontal plane.

TEST PROCEDURE
Bring all reagents and samples to room temperature before testing.
Thoroughly mix the REDGEN® reagent suspension by gentle agitation before testing.

Qualitative Method
1. Place 50 µl of the test sample, positive and negative controls onto separate reaction circles of the disposable slide using a sample-dispensing pipette provided with the kit.
2. Add one drop of well-mixed REDGEN® reagent next to the test sample or controls by using the reagent dropper provided with the kit. Do not let the dropper tip touch the liquid on slide.
3. Using a mixing stick, mix the test sample and REDGEN® reagent thoroughly, spreading uniformly over the entire reaction circle.
4. Immediately start a stopwatch. Rotate the slide gently and continuously either manually or on a mechanical rotor at 180 rpm.
5. Observe for flocculation macroscopically at 8 minutes.

Quantitative Method
1. Using isotonic saline, prepare serial dilutions of the test sample positive in the qualitative method 1:2,1:4,1:8,1:16,1:32,1:64,1:128 and so on.
2. Perform the qualitative test procedure using each dilution as a specimen.
3. The titre is reported as reciprocal of the highest dilution, which shows a positive test result.

INTERPRETATION OF TEST RESULTS
Qualitative Method
- Large and medium RED coloured floccules against white background : Reactive
- Small RED coloured floccules against white background : Weakly reactive
- No floccules, smooth pink background : Non reactive

Flocculation is a positive test result and indicates presence of antilipoidal antibodies in the test sample.
No flocculation is a negative test result and indicates absence of antilipoidal antibodies in the test sample.

Quantitative Method
The titre of antilipoidal antibodies is the highest dilution of test sample giving a positive test result.

REMARKS
1. Quantitative procedure must be performed to determine response to treatment and detect reinfection.
2. False positive reactions occur not infrequently and have been attributed to a variety of acute and chronic conditions.
3. In absence of supporting clinical, historical or epidemiological evidence, reactive results must be confirmed with more specific Treponemal tests.
4. It is strongly recommended that results of the test should be correlated with clinical findings to arrive at the final diagnosis.
5. Dispose all used and contaminated material as per Standard Biohazard Safety Guidelines.
6. The reagent dropper provided for dispensing the REDGEN® antigen should be thoroughly cleaned with distilled water and air dried after use, to ensure that it does not contaminate the reagent during subsequent use.
7. Very slight roughness should be interpreted as a negative test result.

**PERFORMANCE CHARACTERISTICS**
The results of 100 serum samples obtained with REDGEN® were compared with those obtained using commercial reagent 'A' (a RPR card test using carbon antigen) and another commercial reagent 'B' (modified VDRL reagent).

<table>
<thead>
<tr>
<th>Test Result</th>
<th>REDGEN®</th>
<th>A</th>
<th>B</th>
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<tr>
<td>+ VE</td>
<td>46</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>- VE</td>
<td>54</td>
<td>54</td>
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The results of REDGEN® correlate 100% with both the commercial reagents used. Repeatability and reproducibility (inter-assay and inter-lot) were evaluated on a number of VDRL negative and VDRL positive samples. No variations were found in the outcome of different tests.

**WARRANTY**
This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

**BIBLIOGRAPHY**
4. Data on File: Tulip Diagnostics (P) Ltd.