

New Horizons Diagnostics

TRUST

Toluidine Red Unheated Serum Test

A Qualitative and Quantitative Card Test
for the Serologic Detection of Syphilis

For In Vitro Diagnostic Use

Reorder No.	89-105150 (150 determinations)
	89-105500 (500 determinations)
	89-105004 (5,000 determinations)
	89-105005 (10,000 determinations)

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INTENDED USE

New Horizons Diagnostics Corporation TRUST (Toluidine Red Unheated Serum Test) is a macroscopic, rapid, nontreponemal card testing procedure for the serological detection of syphilis. The TRUST antigen is a modified VDRL antigen containing red pigment particles which visibly agglutinate when mixed with serum or plasma containing antilipoidal antibody formerly called reagin, present in serum or plasma of syphilitic individuals and occasionally in serum or plasma of persons with other acute or chronic conditions.¹

SUMMARY

The host in response to the etiological agent of syphilis, Treponema pallidum, produces two types of antibodies; nonspecific and specific. The nonspecific antibody reacts with the cardiolipin, cholesterol, and lecithin antigen of the TRUST, whereas the specific antibody reacts with antigenic determinants found on T. pallidum and other pathogenic treponemes.

Nonspecific or nontreponemal tests for syphilis are based on the detection of the antilipoidal antibody utilizing a modified Venereal Disease Research Laboratory (VDRL) antigen.²

The original Rapid Plasma Reagin (RPR), a modification of the basic VDRL antigen with choline chloride, allows for the testing of specimens without heating. This same principle was also utilized successfully in the Unheated Serum Reagin (USR) test, RPR (circle) card test, and now TRUST. The RPR card assay utilizes charcoal as the visualizing agent, whereas TRUST utilizes a red pigment as the visualizing agent.

TRUST is recommended when venous blood collection is employed. Use serum or plasma with ethylenediaminetetraacetic acid (EDTA) as the anticoagulant, such as generally prevails in public health and clinical laboratories for testing.

TRUST is a sensitive, nonspecific screening test for syphilis and can be performed in lieu of the RPR Card, USR, or VDRL test.

PRINCIPLES OF PROCEDURE

The TRUST antigen suspension is based on the VDRL antigen but contains a toluidine red pigment. This antigen detects antilipoidal antibody present in serum or plasma from persons with syphilis, and occasionally in the serum or plasma of persons with other acute or chronic conditions. When a specimen contains antibody, the TRUST antigen agglutinates and the reaction appears as red clumps against the white background of the test cards. The test is read macroscopically using a high intensity incandescent lamp. If antibody is not present, the test mixture remains a faint red color and no agglutination occurs.

REAGENT

TRUST antigen consists of VDRL antigen (an alcohol based solution containing 0.03% cardiolipin, 0.9% cholesterol, and sufficient purified lecithin to produce standard reactivity) which is resuspended in a solution resulting in a final concentration of 10% choline chloride, 0.0125 M EDTA, 0.01 M sodium and potassium phosphate buffer, toluidine red pigment particles, and 0.05% sodium azide as a preservative.

**PRECAUTIONS
FOR IN VITRO DIAGNOSTIC USE
TRUST ANTIGEN:**

Storage of the antigen in bright sunlight or temperatures above 30°C (86°F) should be avoided as this may result in a rough appearance of the antigen when used with nonreactive sera. Do not freeze. Antigen should not be used beyond the expiration date.

WARNING: The antigen contains sodium azide which may react with lead and copper plumbing to form highly explosive metal azides. On disposal of antigen, flush with a large volume of water to prevent azide build-up.

SERUM SPECIMEN STORAGE: Keep serum specimens in the original collection tube if the test is to be performed on the day the specimen is collected. Remove serum from the clot and store at refrigerator temperature (2° to 8°C) or frozen (-20°C or lower), if a delay of more than 5 days is anticipated before testing.

PLASMA SPECIMEN STORAGE: Specimens not tested immediately may be stored at 2° to 8°C for up to 48 hours.

TEST CARDS: Designed for use with the TRUST antigen. Care should be taken not to touch the test areas on the card, as this may result in an oily deposit and may affect test results.

When spreading a specimen within the confines of the (18 mm) test circle, avoid scratching the card with the dropper.* If the specimen does not spread to the outer perimeter of the test circle, use another test circle on the card. Do not store opened cards in the refrigerator.

*The dropper is a tubular instrument having an open end for dispensing and a closed end for stirring.

DROPPERS: A dropper is used to transfer the specimen (approximately 0.05 ml) to the test card circle; invert the dropper and spread the specimen within the confines of the test circle. A new dropper must be used for each test specimen.

DISPENSING NEEDLE: Upon completion of the test, to maintain clear passage of the needle for accurate drop delivery, remove the needle from the TRUST antigen bottle and rinse the needle with distilled or deionized water. Do not wipe the needle since this will remove the silicone coating and may affect the accuracy of the drop of antigen being dispensed. Periodically check needle for accuracy per instructions under "User Quality Control".

READING OF TEST RESULTS: Immediately remove the card from the rotator. Tilt the card by hand about 30 degrees from horizontal position. Briefly and gently rock the test card four to five times in a circular motion to better differentiate minimally reactive results from nonreactive results. Read the test reactions horizontally in the "wet" state under a high intensity incandescent light source within 1 minute after removing the card from the rotator.

ROTATION: Recommended speed of rotation is 100 rpm, (98-102 rpm range). Below 98 and above 102 rpm there is a tendency for the clumping of the antigen to be less intense in tests with undiluted specimens. This may result in some minimal reactions being missed. In quantitation, rotation above 102 rpm tends to produce a decrease in titer, approximately one dilution lower.

STORAGE OF KIT COMPONENTS: Store all kit components between 2° to 29°C. After opening kit

and test card package, store the cards in a dry place at room temperature.

SPECIMEN COLLECTION: No special diet is required for the patient prior to specimen collection.

WARNING: 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 the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories (1984) and as outlined in OSHA Standards for Blood Borne Pathogens, 29 CFR 1910.1030.

SERUM COLLECTION: Collect blood by venipuncture into a clean, dry tube without anticoagulant and allow to clot. Centrifuge the specimen at a force sufficient to sediment cellular components. Transfer the serum to a clean, dry test tube or keep the serum in the original collecting tube and test, taking care not to disturb the centrifuged cells. Sera separated from cells may be heated at 56°C for 30 minutes without adversely affecting the test outcome.

PLASMA COLLECTION: Collect blood by venipuncture completely filling a tube containing EDTA as an anticoagulant. Anticoagulants other than EDTA have not been evaluated in TRUST. Gently rock the tube after collection to distribute the EDTA and assure that the sample does not clot. Keep the plasma in the original collecting tube, and if stored, store the specimen at 2° to 8°C. Test the specimen within 48 hours of blood collection. Prior to testing, centrifuge the specimen at a force sufficient to sediment cellular components. When testing, take care not to disturb the centrifuged cells. Plasma should not be used in the quantitative test or in confirmatory test such as the FTA-ABS tests or hemagglutination assays.

PROCEDURES

MATERIALS PROVIDED*:

	150 Determinations	500 Determinations
TRUST antigen	1 Bottle	3 Bottles
50 µl Droppers	150	500
Test Cards (10 Spot, 18 mm)	15	50
Dispensing Needle (20 Gauge)	1	1

*Individual materials and large kit sizes are available.

ADDITIONAL MATERIALS NEEDED BUT NOT PROVIDED:

1. Controls with known patterns of reactivity (Reactive, Reactive Minimal, and Nonreactive) should be run with each day's testing (See User Quality Control).
2. A serological rotator (98-102 rpm), circumscribing a circle 3/4 inch in diameter with a humidifying cover containing a moistened sponge or blotter.
3. Saline (0.9%) for use in quantitative testing. If needed prepare by adding 900 mg dry sodium chloride, QS to 100 ml with distilled water.
4. Serum nonreactive to TRUST diluted 1:50 in 0.9% saline. Used as a diluent for further quantitation of test specimens giving a Reactive quantitative result at the 1:16 dilution. Also required is the necessary equipment and glassware used in a serology testing laboratory for preparation, storage and handling of serologic specimens. See section on "Laboratory Equipment and Supplies", 1990 Manual of Tests for Syphilis.²

USER QUALITY CONTROL

Review "Precautions" prior to the performance of TRUST. When tests are to be performed, the antigen suspension must be checked with controls of known reactivity for Reactive, Reactive Minimal, and Nonreactive, e.g., New Horizons Diagnostics Corporation TRUST Controls order number 89-105002. Antigen not reproducing the established reactivity pattern should not be used.

Check delivery of the needle by placing the needle firmly on a 1 ml serological pipette; fill the pipette with the antigen suspension, and holding the pipette in a vertical position, count the number of drops delivered in 0.5 ml. The correct number of drops is given in the table which follows:

Color of Needle Hub and Size	Number of Drops in 0.5 ml*
Yellow, 20 ga.	30 ± 1 drop

* Replace needle if not meeting these specifications with one that does.

PERFORMANCE OF THE TEST

QUALITATIVE TRUST PROCEDURE (SERUM OR PLASMA)

Antigen controls, specimens, and testing areas must be at 23° to 29°C (73° to 85°F) at time of testing. It is imperative that the techniques as described herein be followed in detail.

1. Attach the needle to the tapered fitting of the plastic bottle containing TRUST antigen. Upon completion of the tests, remove the needle from the dispensing bottle and rinse the needle with distilled or deionized water as per "Precautions" section.
2. Hold a dropper between thumb and forefinger near the sealed end. Squeeze and do not release pressure until open end is below surface of specimen, holding the specimen tube vertically to minimize stirring up of cellular elements when using original blood tube. Release finger pressure to draw up the sample.
3. Holding the dropper in a vertical position approximately one-half inch directly over a clean test card circle (not touching card surface), squeeze the dropper allowing one "free-falling" drop to fall onto the test card circle (approximately 0.05 ml). Sample remaining may be discharged into specimen tube from which it was drawn.
4. Invert dropper and with sealed stirring end, spread the specimen filling entire surface of test circle. Discard dropper, treating as biohazardous material. Repeat procedure for number of specimens to be tested.
5. Gently resuspend the TRUST antigen suspension by inverting bottle several times.
6. Holding the antigen suspension bottle in a vertical position, dispense several drops to clear the needle of air. Preferably when clearing the needle, drop the antigen on Parafilm or into the cap of the dispensing bottle. Place exactly one free-falling drop (1/60 ml) of antigen suspension onto each circle that has a specimen. Do not spread.
7. Place the test card on the rotator under the humidifying cover. Rotate the card for 8 minutes at 100 rpm, ±2 rpm.

8. Immediately remove the test card from the rotator. Tilt the card by hand about 30 degrees from horizontal position. Briefly and gently rock the card four to five times in a circular motion to better differentiate minimally reactive results from nonreactive results. Read the test reactions horizontally in the "wet" state under a high intensity incandescent light source within 1 minute after removing the card from the rotator.

REPORT AS: REACTIVE (R) - Showing characteristic clumping ranging from slight but definite (minimal-to-definite) to marked and intense (i.e., red particle agglutination).

NONREACTIVE (N) - Showing a smooth to slightly rough light red background with no clumping.

NOTE: There are only two possible results with TRUST; Reactive or Nonreactive, regardless of the degree of reactivity.

Reactive minimal-to-definite (showing slight, but definite clumping) is always reported as Reactive.

It is also desirable to quantitate specimens which are questionable (Nonreactive rough), so that an occasional prozone (weaker reaction due to excessive antibody) specimen may be revealed.

Confirm reactive results with a treponemal test.

All Reactive Serum Results Should Be Retested Using the Quantitative Procedure.

QUANTITATIVE TRUST PROCEDURE

1. Quantitate to an end-point titer all serum specimens with Reactive or Nonreactive rough results in the qualitative test. Test specimens undiluted (1:1) and at 1:2, 1:4, 1:8, and 1:16 dilutions.
2. Place 50 µl of 0.9% saline onto circles 2 through 5. Do not spread saline.
3. Using a pipetting device, place 50 µl of specimen onto circle.
4. Using the same pipetting tip draw up an additional 50 µl of specimen into the saline on circle 2. Mix the saline and the specimen in circle 2 by drawing the mixture up and down in the pipetter 5 or 6 times. Avoid forming bubbles.
5. Transfer 50 µl from circle 2 to 3, and mix 5 or 6 times.
6. Transfer 50 µl from circle 3 to 4, and mix 5 or 6 times.
7. Transfer 50 µl from circle 4 to 5, and mix 5 or 6 times. Discard the last 50 µl into an appropriate disposal container (i.e., biohazard container).
8. Using the sealed stirring end of a clean dropper for each specimen to be tested, spread the 1:16

serum dilution within the confines of each test circle. Repeat this action using the same dropper in circles 4(1:8), 3(1:4), 2(1:2), and 1 [undiluted (1:1)]. Discard dropper, treating as biohazardous material.

9. Gently resuspend the TRUST antigen suspension by inverting bottle.
10. Holding the antigen suspension bottle in a vertical position, dispense several drops to clear the needle of air. Preferably when clearing the needle, drop antigen on Parafilm or into cap of dispensing bottle. Place exactly one free-falling drop (1/60 ml, 17µl) of antigen suspension onto each circle. Do not spread.
11. Place the test card on the rotator under the humidifying cover. Rotate the card for 8 minutes at 100 rpm, ±2 rpm.
12. Immediately remove the test card from the rotator. Tilt the card about 30 degrees from horizontal position. Briefly and gently rock the card four to five times in a circular motion to better differentiate minimally reactive results from nonreactive results. Read the test reactions horizontally in the "wet" state under a high intensity incandescent light source within 1 minute after removing the card from the rotator.
13. Report the results in terms of the highest dilution giving any degree of reactivity. See Table 1 for reporting examples.

TABLE 1
REPORTING QUANTITATIVE RESULTS
 Serum Dilutions

Undiluted					
1:1	1:2	1:4	1:8	1:16	Report
Rm	N	N	N	N	Reactive, undiluted (1:1)
R	Rm	N	N	N	Reactive, 1:2 dilution
R	R	Rm	N	N	Reactive, 1:4 dilution*
R	R	Rm	Rm	N	Reactive, 1:8 dilution

R = *Reactive*, Rm = *Reactive minimal*, N = *Nonreactive*

14. If the highest dilution tested (1:16) is Reactive, proceed as follows:
 - a. Prepare a 1:50 (2%) dilution of a human serum Nonreactive to the TRUST antigen by adding 0.1 ml of serum to 4.9 ml of 0.9% saline.
 - b. Prepare a 1:16 dilution of the test specimen by adding 0.1 ml of the specimen to 1.5 ml of 0.9% saline. Mix thoroughly.

- c. Place 50 µl of the 1:50 (2%) Nonreactive serum diluent in circles 2, 3, 4, and 5 of a test card.
- d. With an appropriate pipetting device, place 50 µl of the 1:16 dilution of the test specimen (i.e., prepared in Step 14 b.) on circle 1. Place an additional 1:16 dilution of test specimen into the diluent in circle 2, mixing the specimen 5 to 6 times.
- e. Use the same pipettor to make serial two-fold dilutions and complete the test as described under steps 4 through 13 above.

LIMITATIONS OF PROCEDURE

The diagnosis of syphilis should not be made on a single reactive result without the support of a positive history or clinical evidence. Therefore, as with any serological testing procedure, specimens which are Reactive with TRUST should be subjected to further serologic study. Serum specimens which are Reactive in qualitative testing should be quantitated to establish a baseline from which changes in titer can be determined, particularly for evaluating treatment. Confirm Reactive serum results with a treponemal test. The use of plasma specimens to establish a baseline from which changes in titer can be determined has not been evaluated.

With cardiolipin type antigens, biological false positive reactions have been reported in diseases or conditions such as infectious mononucleosis, pregnancy, leprosy, malaria, narcotic addiction, autoimmune diseases, lupus erythematosus, vaccinia and viral pneumonia.

Pinta, yaws, bejel and other treponemal diseases produce positive reactions with cardiolipin type antigens but should not be considered as false positive reactions.

Lipemia as such will not interfere with TRUST, however, if the degree of lipemia is so severe as to obscure the state of the antigen particles, the specimen should be considered unsatisfactory for testing.

EXPECTED VALUES AND PERFORMANCE CHARACTERISTICS

Each lot of TRUST antigen suspension is tested for established patterns of reactivity against reference antigen suspensions and meets product specifications for performing the New Horizons Diagnostics Corporation TRUST.

The performance of TRUST was determined in a prospective evaluation of 238 clinical specimens from two State Health Departments. Each specimen was screened using the RPR Card Test, FTA-ABS test and the TRUST. The result of this testing is summarized below:

TABLE 2 - QUALITATIVE RESULTS

	RPR CARD		FTA – ABS	
TRUST	Reactive	Nonreactive	Reactive	Nonreactive

Reactive	111	1	105	7
Nonreactive	0	126	0	126

	TRUST vs. RPR Card		TRUST vs. FTA - ABS	
Reactive Agreement	111/111	100%	105/105	100%
Nonreactive Agreement	126/127	99.2%	126/133	94.7%
Overall Agreement	237/238	99.6%	231/238	97.1%

TABLE 3 - QUANTITATIVE RESULTS - TRUST vs. RPR

The results of testing 111 Reactive specimens are noted below:

Titers match exactly	68/103	66.0%
Titers within one dilution	34/103	33.0%
Titers deviate more than one dilution	2/103	1.9%
Others*	8	

- Seven titers were not quantitated beyond 1:16. The corresponding RPR results were at least 1:16. One titer was not performed.

AVAILABILITY*

* Individual materials and larger kit sizes are available:

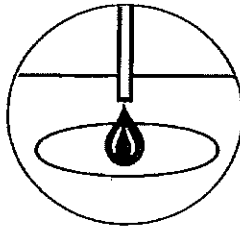
New Horizons Diagnostics Corporation TRUST - 150 Determinations / Order Number 89-105150
 Contains: 1 x 2.8 ml of TRUST antigen, 20 gauge dispensing needle, 15 test cards with ten test circles each, and 150 0.05 ml, 50 µl droppers.

New Horizons Diagnostics Corporation TRUST - 500 Determinations / Order Number 89-105500
 Contains: 3 x 2.8 ml of TRUST antigen, 20 gauge dispensing needle, 50 test cards with ten test circles each, and 500 0.05 ml, 50 µl droppers.

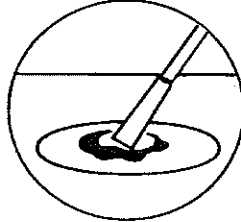
New Horizons Diagnostics Corporation TRUST Controls / Order Number 89-105002
 Contains: One bottle of: Reactive Control, Reactive Minimal Control and Nonreactive Control.

Simple Procedure

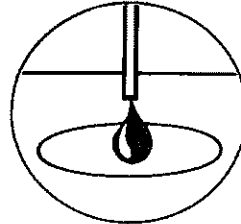
1. Add one drop of patient sample.



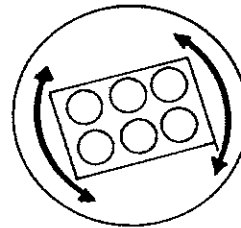
2. Spread to fill circle.



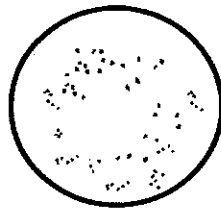
3. Add one drop of TRUST Reagent.



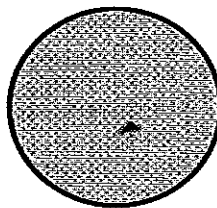
4. Rotate for 8 minutes.



5. Read results visually.



Reactive



Non-Reactive

BIBLIOGRAPHY

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TECHNICAL INFORMATION

New Horizons Diagnostics Corporation: (800) 888-5015, (410) 992-9357

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