ENGLISH

iStatis

Syphilis Antibody Test

Single-use lateral flow immunoassay for the detection of antibodies to *T. pallidum*

REF **90-1150**

2°C – 30°C **Store at 2°C – 30°C. For *in vitro* diagnostic use only.** IVD

It is recommended that the entire Instructions for Use be read prior to beginning the test procedure. Although the assay is designed to be simple to use, conformance with the test procedure is necessary to ensure accurate results.

INTENDED USE - Not for donor screening.

The **iStatis Syphilis Antibody Test** is a single use, rapid, lateral-flow *in vitro* qualitative screening immunoassay for the detection of antibodies to *Treponema pallidum* (*T. pallidum*) in human EDTA-whole blood, fingerstick whole blood, serum or plasma. The test is intended for manual operation by trained personnel in medical facilities, clinical laboratories, emergency care situations, and physicians' offices as an aid to diagnosis for *Treponema pallidum* (*T. pallidum*) infection in adults over 18 years of age by providing results within 15 minutes. Although suitable for near-patient or point-of-care (POC) testing, it is not suitable for home testing.

All required pre and post-test counseling guidelines must be followed in each setting in which the iStatis Syphilis Antibody Test is used. The **iStatis Syphilis Antibody Test** will be referred to as iStatis Syphilis Test in the remainder of this Instructions for Use.

SUMMARY

Treponema pallidum (T. pallidum) is the causative agent of Syphilis. Some of the proteins of this organism are highly immunoreactive and infected persons develop antibodies soon after infection. These antibodies are unaffected by treatment and once induced they remain detectable for years. It is possible for a person to be antibody positive for *T. pallidum* but have been cured of the infection. Following a reactive result for *T. pallidum* antibodies, a venous blood sample must be drawn in an EDTA collection tube (for whole blood or plasma) or red-top tube (for serum) and forwarded to a laboratory for syphilis confirmatory testing. A confirmatory test is required to determine active syphilis or past infection in the patient.

PRINCIPLES OF THE TEST

The **iStatis Syphilis Test** is packaged as a kit containing single-use cartridge units along with a chase buffer bottle. The test cartridge consists of a sample absorption pad, a conjugate reagent pad with specific *T. pallidum* antigens, and a test membrane where a *T. pallidum* antigen, as well as a control antibody, have been striped on two distinct lines to form the entire test strip. Results are visualized in 15 minutes following the addition of the sample and chase buffer to the test cartridge in the form of red control and test lines. Results should not be read after 30 minutes.

Syphilis Antibody Detection: Upon addition of the sample to the iStatis Syphilis Test cartridge, the antibodies to *T. pallidum*, if present, bind to the *T. pallidum* antigens conjugated to colloidal gold. This complex then migrates onto the test strip, aided by chase buffer, and is captured by *T. pallidum* antigen on the test line immobilized on the nitrocellulose membrane. The test strip is designed to filter, absorb, and retain the test specimen and test reagent in such a manner as to limit leakage and exposure of personnel to potentially infectious materials.

Antigen Selection: The syphilis antigens bound to the membrane consist of a recombinant fusion protein derived from the TP15, TP17, and TP47 domains of *T. pallidum*. The conjugate consists of separate TP15, TP17, and TP47 antigens.

Test Complexity: The iStatis Syphilis Test is designed to reduce protocol complexity. The iStatis Syphilis Test does not require sample preparation, a separate reader, or several steps, which include multiple washes and reagents. These requirements increase the complexity of an assay and lead to procedural errors which may adversely affect sensitivity and specificity. Total test time may vary slightly depending on specimen type, but results of valid tests are readable within 15 minutes and up to 30 minutes.

KIT COMPONENTS AND STORAGE

2°C – 30°C iStatis components should be stored at 2-30°C (35.6°F to 86°F).

Components	901150340003-50 (50 tests)	901150340004-100 (100 tests)
Test cartridge	X 50	X 100
Chase Buffer Bottle	1 X 6mL	2 X 6mL
Alcohol Swab	X 50	X 100
Single-use lancet	X 50	X 100
Single-use capillary pipette (50 µL)	X 50	X 100

Each test requires the following materials:

1. **Test cartridge:** individually packaged in a foil pouch with a desiccant.
2. **Chase Buffer bottle (6 mL)**: A bottle that contains chase buffer, a phosphate buffer solution used for aiding the sample across the cartridge.
3. **Alcohol Swab**: For disinfection of the patient's finger prior to pricking.
4. STERILE R **Single-use lancet**: For pricking the patient's finger to allow for fingerstick sample collection.
5. **Single-use capillary pipette (50 µL):** For collection and dispensing of a fingerstick sample into the sample well of the cartridge.

⚠ Chase buffer solution contains 0.08% (w/v) Sodium Azide as a preservative and is harmful if swallowed.

MATERIALS REQUIRED BUT NOT PROVIDED

- Personal protective equipment
- Appropriate biohazard waste containers and disinfectants
- Absorbent cotton balls for fingerstick or venipuncture wound closure
- Timer

FOR VENIPUNCTURE BLOOD COLLECTION AND TESTING:

- Venipuncture apparatus if collecting blood samples
- Appropriate blood collection tubes and shipping containers
- Precision pipette capable of delivering 30 µL or 50 µL of sample

MATERIALS AVAILABLE AS AN ACCESSORY TO THE KIT

iStatis Syphilis Positive and Negative Controls made of human blood components are available from bioLytical Laboratories in user-defined amounts, for use in quality control procedures. Please refer to the section on Quality Control, following the Assay Procedure, and the iStatis Syphilis Test Controls Instructions for Use.

WARNINGS

1. Do not use the test cartridge or the chase buffer if the packaging has been damaged.
2. Do not use if the desiccant in the test cartridge package is exposed or damaged.
3. Do not use the kit beyond the expiry date.
4. This product has been evaluated only for the detection of antibodies to *T. pallidum*, not for any other bacteria, viruses, or pathogens.
5. Do not interchange kit contents from different lots.
6. Avoid microbial contamination and exercise care in handling the kit components.
7. Failure to use the provided chase buffer solution will invalidate the test results.
8. If the kit is refrigerated, ensure it is brought to room temperature (15-30°C) before performing the test. If required, use the iStatis Syphilis Positive/Negative Controls to ensure proper kit performance.
9. ⚠ Sodium Azide is present at 0.08% in the chase buffer solution. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. If products containing sodium azide are discarded into a drain, flush with large amounts of water to prevent azide build-up. Check with local regulatory agencies to determine at what concentration Sodium Azide may cause a product to be regulated as hazardous waste.
10. Individuals with color-impaired vision may not be able to adequately interpret test results.

PRECAUTIONS

1. Wear disposable gloves while handling kit reagents or specimens. Change gloves and wash hands thoroughly after performing each test. Do not pipette by mouth.
2. All specimens should be handled as if capable of transmitting infectious agents.
3. Avoid contact with skin and eyes. If contact occurs, wash affected areas with water.
4. Do not smoke, eat, or drink in areas where specimens or kit reagents are being handled.
5. Avoid forming aerosols.
6. Dispose of used contents as biohazardous wastes in accordance with federal, state, and local requirements.
7. Spills should be cleaned up and decontaminated in accordance with the user facility's established procedures for handling biohazardous spills.
8. Specific training or guidance is recommended if operators are not experienced with specimen collection and handling procedures.
9. Use appropriate precautions in the collection, handling, storage, and disposal of samples and used kit contents.

SPECIMEN STORAGE AND STABILITY

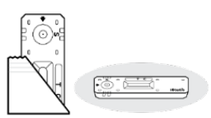
1. For EDTA-whole blood, EDTA-plasma, or serum specimens, follow venipuncture blood collection procedures using lavender-top EDTA anticoagulant tubes (for whole blood and plasma) or red-top (no anticoagulant) tubes for serum.
2. If plasma or serum is to be used, separate from the blood cells by centrifugation.
3. Serum or EDTA-plasma may be stored at 2-8°C for up to 14 days.
4. Whole blood specimens collected venipuncture in EDTA anticoagulant, may be stored at 2-8°C and should be tested within 6 days. **Do not heat or freeze whole blood specimens.**
5. Do not dilute prior to testing.

ASSAY PROCEDURE

NOTE: An iStatis test cartridge must be used immediately once opened from the pouch.

Test Set Up

1. Remove the test cartridge from its packaging and lay flat on the table.



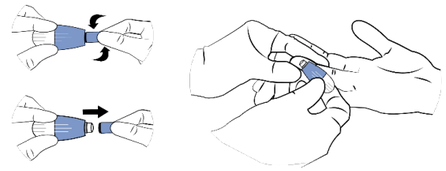
2. Open the Buffer Bottle.

Sampling Fingerstick Blood:

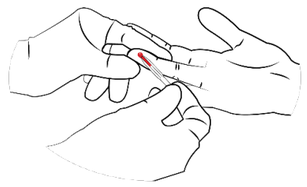
1. Gather support materials (alcohol swab, lancet, capillary pipette), one sealed test cartridge pouch, and Chase Buffer for each test to be performed.

⚠ **CAUTION! The amount of sample (fingerstick blood) is critical**. To ensure that the proper amount of blood is achieved, follow these instructions carefully:

2. Massage the finger to allow the blood to move to the surface (the fingertip will become pink). Use a heating pad if available to warm the hand. The hand must be positioned at waist level or lower.
3. Wipe the fingertip with the alcohol swab.
4. As soon as the finger is dry, twist and remove the protective insert from the lancet. Press the finger firmly at the point just below where the lancet will be applied. With the other hand, place the lancet on the side of the fingertip and press hard until it clicks. Immediately dispose of the used lancet into a proper sharps container.



5. As the blood droplet forms, hold the pipette horizontally and touch the tip of the pipette to the blood sample. Capillary action automatically draws the sample to the fill line and stops. If very little blood trickles out of the puncture, gently apply intermittent pressure below the puncture site to obtain the required blood volume. If blood is inadequate, open a new test kit and perform a second skin puncture using a new lancet.



⚠ **CAUTION!** Filling is automatic: Never squeeze the pipette bulb while sampling.

6. Follow General Procedure after Sampling below. Test the sample immediately.

Sampling Venous Whole Blood, Serum, Plasma:

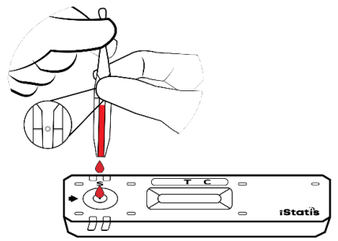
1. Bring specimens to room temperature and mix each specimen thoroughly prior to use.
2. A volume of 50 µL must be used for venous whole blood (see note)
3. A volume of 30 µL must be used for serum, or plasma (see note).

NOTE: In POC settings, it is important to use a 30 µL pipette to add the plasma or serum to the cartridge. A 50 µL pipette must be used for venous whole blood. Do not use the disposable single-use pipette provided for finger stick blood collection with the test kit.

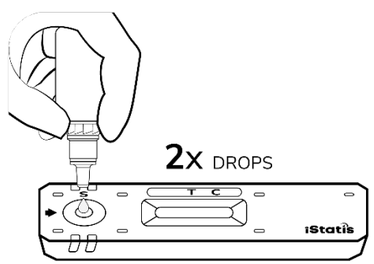
4. Follow General Procedure after Sampling below.

General Procedure after Sampling:

1. Transfer the fingerstick whole blood sample held in the pipette to the sample well (marked "S") of the test cartridge. For serum/plasma samples, transfer 30 µL; for venous whole blood samples, transfer 50 µL using a precision pipette. For fingerstick whole blood collected using the pipette provided in the kit, align the tip of the pipette with the sample well and squeeze the bulb to dispense the sample. **NOTE:** if the sample does not expel, hold the pipette vertically and slide a finger over (without pressing) the vent hold, then squeeze the bulb.



2. Immediately add 2 drops of Chase Buffer to the sample well of the test cartridge, holding the bottle vertically.



IMPORTANT: Do not move or lift the cartridge during this step.

3. Start a timer for 15 minutes. The results of the test can be read in 15 minutes. The test result should NOT be read after 30 minutes.
NOTE: iStatis tests should be read and interpreted under adequate lighting.

QUALITY CONTROL

The control line is a procedural control and indicates that the chase buffer has been applied successfully and that the active ingredients of the main components on the strip are functional.

Kit Controls:
iStatis Syphilis Positive and Negative Controls are available from bioLytical Laboratories for use with the iStatis Syphilis Test. The controls are used to verify test performance and interpretation of results. Kit controls should be run under the following circumstances:

- Once for every new lot of kits and every new user operator verification prior to performing testing on patient specimens.
- When temperature during storage of the kit falls outside of 2° to 30°C (35.6°F to 86°F).
- When the temperature of the test area falls outside of 15° to 30°C (59°F to 86°F).
- At regular intervals as determined by the user facility.

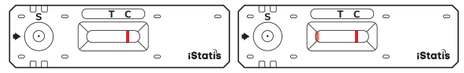
Refer to the iStatis Syphilis Test Controls Package Insert for additional information on the use of these samples. It is the responsibility of laboratory or POC site using the iStatis Syphilis Test to establish an adequate quality assurance program to ensure the performance under their specific locations and conditions of use.

⚠ **CAUTION! It is not recommended to use external controls that have not been validated for the iStatis Syphilis Antibody Test as these may not produce the expected results.**

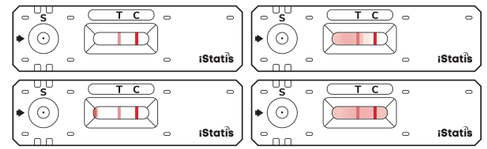
INTERPRETATION OF RESULTS

- Do not read the results if more than 30 minutes have elapsed following the addition of the sample and chase buffer to the test cartridge.
- If using the iStatis syphilis control samples provided by bioLytical Laboratories:
  - A syphilis Positive Control must be positive with iStatis Syphilis Test.
  - A Negative Control must be negative with iStatis Syphilis Test.
  - Controls that produce incorrect or invalid results must be re-tested with a new iStatis Syphilis Test. If results are still incorrect or invalid, contact bioLytical Laboratories immediately.
- Control and test lines appear red in color.

NEGATIVE► Control line is clearly visible at the "C" marking following development. Additionally, absence of any test line at the "T" marking indicates the specimen does not contain any syphilis antibodies and indicates a negative test result.

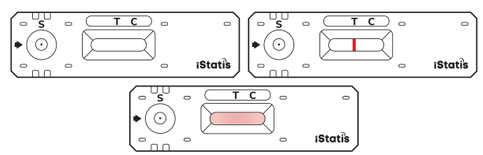


POSITIVE ► Control line is visible at the "C" marking following development. Additionally, any test line development at the "T" marking, be it faint or distinct is present. This indicates the presence of syphilis antibodies and indicates a positive test result.



NOTE: The Test and Control Lines can be very faint. Any red line visible here indicates a positive test result. Following a positive result, a venous blood sample must be drawn in an EDTA collection tube (for whole blood or plasma) or red-top tube (for serum) and forwarded to a laboratory for syphilis confirmatory testing. Depending on the antibody titer, a reactive specimen may be less intense in color than the procedural control.

INVALID ► Absence of control line on the cartridge indicates an invalid result. If the smearing background makes it impossible to read the result, the test is considered invalid. An invalid test result means that the test may have been run incorrectly or insufficient specimen was added. Invalid test results cannot be interpreted. Repeat the test with a new iStatis Syphilis Test.



NOTE: Invalid tests with fingerstick blood should be repeated with a fresh sample using a new test kit. Invalid tests with whole blood, plasma, or serum samples should be repeated using a new test cartridge and kit components.

LIMITATIONS OF THE TEST

- The performance of the device has not been established in individuals under the age of 18.
- In some instances, samples may exhibit longer than normal flow times (from the time the specimen is added into the sample well, to the time the Chase Buffer has fully flown through the membrane). This is due to various factors such as cellular components, especially with whole blood.
  In these instances, a venous blood sample should be drawn in an appropriate collection tube and forwarded to a laboratory for syphilis confirmatory testing.
- Insufficient data are available to interpret tests performed on other body fluids, pooled blood or pooled serum or products made from such pools; therefore, testing of these specimens is not recommended.
- The presence of antibodies to *T. pallidum* may indicate current or past syphilis infection, and a blood sample should be collected and sent to a laboratory for confirmation of infection status. Antibodies in patients may persist for decades, even in spite of successful therapy. A positive syphilis test may not be an indication of an ongoing infection.

PERFORMANCE CHARACTERISTICS

Analytical Specificity (Cross Reactivity):

Cross reactivity with the potentially interfering medical conditions/diseases was evaluated with the iStatis Syphilis Antibody Test. A total of 101 clinical specimens were evaluated in singlicate (n=1) on one lot of iStatis Syphilis Test materials. The results are summarized in the following table. All tested samples showed no cross-reactivity with the iStatis Syphilis test.

Medical Conditions/diseases (n=101)	Number of Specimens	iStatis Positive	iStatis Negative
HIV	8	0	8
Hepatitis B infection	5	0	5
Hepatitis C infection	8	0	8
acute Hepatitis A infection	5	0	5
Cytomegalovirus (specimens representing acute infection – IgM)	5	0	5
Epstein-Barr virus (specimens representing acute infection – IgM)	5	0	5
Herpes Simplex Virus (HSV) 2	5	0	5
Chlamydia trachomatis (CT)	5	0	5
Human papillomavirus (HPV)	5	0	5
Trichomoniasis Infection	5	0	5
Malaria	5	0	5
Visceral Leishmaniasis	5	0	5
Tuberculosis	5	0	5
Brucellosis	5	0	5
Leptospirosis	5	0	5
Leprosy	5	0	5
Influenza vaccine recipient	5	0	5
Vaccine-induced HIV seropositivity	5	0	5
Lyme Disease – genus Borrelia	5	0	5

Analytical Specificity (Endogenous Interferences):

To evaluate the effect of elevated levels of various endogenous substances on the iStatis Syphilis Antibody Test, both plasma and whole blood samples were spiked with the following levels of endogenous interferences and assayed at two syphilis antibody levels: negative and weak positive. No interference was observed at the following tested concentrations:

Interferent Tested	No Interference up to
Hemoglobin	10 mg/mL
Bilirubin, Conjugated	0.40 mg/mL
Bilirubin, Unconjugated	0.40 mg/mL
Cholesterol	4.0 mg/mL
Albumin	60 mg/mL
Intralipid	20 mg/mL
Hyperglobulinaemia (IgG)	20 mg/mL

To evaluate the effect of various autoimmune conditions, pregnancy, heterophile antibodies and vaccination on iStatis Syphilis Antibody Test performance, both plasma/serum and whole blood samples were tested as is (negative) and spiked with syphilis antibodies to a weak positive level. The results are presented in the following table and no interference was observed for each condition.

	Negative			Weak Positive		
Condition	**N**	**iStatis Positive**	**iStatis Negative**	**N**	**iStatis Positive**	**iStatis Negative**
Heterophilic Antibodies	10	0	10	10	10	0
Human Anti-Mouse Antibodies	10	0	10	10	10	0
Systemic Lupus Erythematous	10	0	10	10	10	0
Anti-Nuclear Antibodies	10	0	10	10	10	0
Rheumatoid Factor	10	0	10	10	10	0
Flu Vaccine Specimen	10	0	10	10	10	0
Pregnancy (1st Trimester)	10	0	10	10	10	0
Pregnancy (2nd Trimester)	10	0	10	10	10	0
Pregnancy (3rd Trimester)	10	0	10	10	10	0
Multiparous Pregnancy	10	0	10	10	10	0
Sickle Cell Disease	10	0	10	10	10	0
Thyroiditis	10	0	10	10	10	0
Hemolysis	10	0	10	10	10	0

Drug Interferences (Exogenous Interference):

A drug interference study was performed with fifteen common therapeutic drugs representing common over-the-counter, anti-inflammatory drugs and anti-bacterial and anti-viral drugs. Each drug was evaluated at the highest concentration recommended by CLSI EP07-A3 and listed in CLSI EP37 and spiked into both plasma and whole blood samples at two syphilis antibody levels: negative and weak positive. No interference was observed at the following tested concentrations:

Compound	Concentration
Acetaminophen	0.156 mg/mL
Acetylsalicylic acid	0.03 mg/mL
Ampicillin	0.075 mg/mL
Cefoxitine	6.6 mg/mL
Chloroquine	789 ng/mL
Doxycycline	0.018 mg/mL
Entecavir	0.024 µg/mL
Isoniazid	0.06 mg/mL
Mefloquine	9.84 µg/mL
Metronidazole	0.123 mg/mL
Rifampicin	0.048 mg/mL
Quinine	0.054 mg/mL
Ribavirin	0.011 mg/mL
Ritronavir	0.044 mg/mL
Valgancidovir Hydrochloride	0.021 mg/mL

Analytical Sensitivity:

The WHO 1st International Standard for human syphilitic plasma IgG and IgM (NIBSC 05/132) was tested with the iStatis Syphilis Antibody Test. The LoD was determined to be 0.0120 IU/mL for plasma samples, and 0.0151 IU/mL for whole blood samples.

Seroconversion Sensitivity:

Seroconversion sensitivity of the iStatis Syphilis Antibody Test was evaluated by testing one commercially available seroconversion panel which demonstrated a range of antibody levels. Of a total of 9 specimens, 4 were detected by the iStatis assay versus 4 specimens detected by the benchmark assay. Overall, the iStatis Syphilis Antibody Test has a similar performance in comparison with a benchmark assay.

Panel Member	Days since 1st bleed	DiaSorin Liaison Treponema Syphilis	iStatis Syphilis
01	0	NR	NR
02	5	NR	NR
03	10	NR	NR
04	13	NR	NR
05	31	NR	NR
06	45	R	R
07	48	R	R
08	52	R	R
09	59	R	R

Performance Panel Sensitivity:

Analytical sensitivity of the iStatis Syphilis Antibody Test was evaluated by testing one commercially available performance panel which demonstrated a range of antibody levels. Of a total of 20 specimens, 20 were correctly classified by the iStatis assay. Overall, the iStatis Syphilis Antibody Test has a similar performance in comparison with a benchmark assay.

Test	# of Reactive Samples	# of Non-Reactive Samples	Total # of Samples Analyzed	Reactivity Rate (%)
iStatis Syphilis Test	19	1	20	100.0%
DiaSorin Liaison Test	19	1	20	100.0%
INNO-LIA Syphilis Score (TpN15; TpN17; TpN47)	19 **TpN15+; TpN17+; TpN47+** for all 19 samples	1	20	100.0%

Hook Effect:

iStatis Syphilis Antibody Test could detect 20 Syphilis high titer positive samples (defined by Abbott Architect Syphilis TP S/CO of ≥20) as positive.

Reproducibility:

Reproducibility testing was conducted using three unique lots of iStatis Syphilis Antibody Test kits over five days and three testing sites with one run per day on a blinded, seven-member panel of specimens consisting of plasma and whole blood matrices at Syphilis negative, weak reactive, and medium reactive levels. Two operators per site participated in the study. The iStatis Syphilis Test demonstrated a reproducibility rate of 100% for between-site, between-day, between-operator, and between-lot variables.

CLINICAL PERFORMANCE

The prospective clinical evaluation conducted in South Africa compared the performance of the test from fingerstick, venous whole blood, and plasma specimens with standard laboratory tests. Of the 1500 participants with eligible syphilis POCT results, 83.5% were female, 42.5% were between 26-35 years, and 877 were pregnant. In summary, the test performance on completed fingerstick, venous whole blood, and plasma POCT are as follows (with any RPR value): 96.4% PPA and 100.0% NPA for fingerstick, 98.8% PPA and 100.0% NPA for venous whole blood, and 99.0% PPA and 100.0% NPA for plasma. Out of 500 EIA positive plasma samples, iStatis Syphilis detected 495 positives. Of those 495 detected positives, the iStatis Syphilis has equal PPA for both less than and greater than or equal to 8 dilutions. The PPA, NPA, and RPR sub-analysis of the tests are summarized in Tables 1a and 1b below for primary analysis.

Table 1a Primary iStatis Syphilis Test Performance Calculations

iStatis Syphilis Test	PPA (%) (95% CI)	NPA (%) (95% CI)
Fingerstick Capillary blood	96.4% (94.4%-97.9%) N=500 482/(482+18)	100.0% (99.6%-100.0%) N=1000 1000/(1000+0)
Venous whole blood	98.8% (97.4%-99.6%) N=500 494/(494+6)	100.0% (99.6%-100.0%) N=1000 1000/(1000+0)
Plasma	99.0% (97.7%-99.7%) N=500 495/(495+5)	100.0% (99.6%-100.0%) N=1000 1000/(1000+0)

Table 1b Primary iStatis Syphilis Test RPR Sub-Analysis Performance Calculation – Plasma

	EIA positive sample size (N)	iStatis Syphilis positive sample size (N)	PPA (%) (95% CI)
	500	495	99.0% (97.7%-99.7%) N=500 495/(495+5)
Syphilis sub-analysis for different RPR dilution values:			
Syphilis (RPR Non-reactive)	61	56	91.8% (81.9%-97.3%) N=61 56/(56+5)
Syphilis (RPR <8 dilutions)	204	204	100.0% (98.2%-100.0%) N=204 204/(204+0)
Syphilis (RPR ≥8 dilutions)	235	235	100.0% (98.4%-100.0%) N=235 235/(235+0)

Out of 1500 included in the analysis, 500 participants had reactive EIA results. Table 2 shows 97.9% of the newly diagnosed cases and 99.7% of the previously diagnosed cases were correctly identified with the iStatis Syphilis test using plasma samples.

Table 2 iStatis Syphilis test performance on positive plasma samples compared to reference test

Syphilis for EIA positive Results by iStatis Results (N=500)			
	iStatis Syphilis Test Results		Total
	Positive n (%)	Negative n (%)	
Newly Diagnosed	185 (97.88%)	4 (2.12%)	189
Previously Diagnosed	310 (99.68%)	1 (0.32%)	311
Grand Total	495 (99.00%)	5 (1.00%)	500

Table 3 outlines different serologic patterns and their possible interpretations. Out of 1500 participants, 419 exhibited symptoms, with majority (36.5%) being skin rash. Among the 500 positive samples, 436 showed reactivity in EIA, RPR, and TPPA tests, 3 samples showed reactivity in EIA and RPR but non-reactivity in RPPA, 3 samples showed reactivity in EIA, but non-reactivity in RPR and TPPA tests, and 58 samples showed reactivity in EIA and TPPA, but non-reactivity in RPR.

Table 3 Syphilis Serology Patterns and Interpretation

Sample Size (n)	Serologic pattern			Possible interpretations
	EIA	RPR	TPPA	
436	Reactive	Reactive	Reactive	- Syphilis infection - If patient has been treated for Syphilis and RPR titre is declining, this may be consistent with treated Syphilis
3	Reactive	Reactive	Non-reactive	- Treponemal tests do not agree, which may indicate: - Early infection (TP-PA not yet positive) - Prior Syphilis (treated or untreated) - False positive EIA - *Repeat testing in 2 weeks*
3	Reactive	Non-reactive	Non-reactive	
58	Reactive	Non-reactive	Reactive	- Previously treated Syphilis - Early Syphilis (RPR not yet positive)
0	Nonreactive	-	-	- Not consistent with Syphilis; if concern for primary Syphilis (chancre), should treat and repeat testing in 2 weeks

The retrospective clinical evaluation that was also conducted in South Africa compared the performance of the test from plasma and serum specimens with standard laboratory tests. Of the 750 samples with eligible syphilis POCT results, majority were female with 73.7% for serum samples and 71.8% for plasma samples. The largest age group was between 20-24 years. In summary the test performance on completed retrospective plasma and serum POCT reveals a PPA of 100.0%, and NPA of 100.0% for both sample types (with any RPR value). The PPA and NPA of the tests are summarized in below table 4 for primary analysis.

Table 4 Primary iStatis Syphilis Test Performance Calculations

iStatis Syphilis Test	PPA (%) (95% CI)	NPA (%) (95% CI)
Plasma	100.0% (98.5%-100.0%) N=250 250/(250+0)	100.0% (99.3%-100.0%) N=500 500/(500+0)
Serum	100.0% (98.5%-100.0%) N=250 250/(250+0)	100.0% (99.3%-100.0%) N=500 500/(500+0)

A total of 752 samples were included in the below table. Out of 250 positive plasma samples, 199 revealed RPR reactivity at dilutions > 8. Meanwhile, 45 samples displayed RPR reactivity at dilutions < 8. The iStatis Syphilis demonstrated 100% concordance results with EIA testing at RPR dilutions < 8 (Table 5).

Table 5 iStatis Syphilis Test RPR Sub-Analysis Performance – Plasma

iStatis Syphilis Test Results (N=752)	EIA reactive			EIA Non-reactive
	RPR Reactive (≥8 dils)	RPR Reactive (<8dils)	RPR Non-Reactive (Null)	RPR not done (Null)
Positive	199	45	6	0
Negative	0	0	0	502
Total:	199	45	6	502

PREGNANT SAMPLES

In the prospective study, a total of 877 pregnant samples were evaluated, of those 301 participants had first time pregnancies and 576 participants experienced multipara pregnancy. For first pregnancy samples, 111/111 of the syphilis positive samples were reactive and 190/190 of the syphilis negative samples were non-reactive with EIA testing. Additionally for multipara pregnant samples, 228/230 samples were reactive and 346/346 of the syphilis negative samples were non-reactive with EIA testing. Additionally, in the retrospective study a total of 319 serum and 296 plasma pregnant samples were evaluated. Out of 149 first pregnancy serum samples, there were 17 syphilis positives and 132 syphilis negatives. Out of 170 multipara pregnancy plasma samples, there were 20 syphilis positives and 150 syphilis negatives. Furthermore, out of 143 first pregnancy plasma samples, there were 15 syphilis positives and 128 syphilis negatives. Lastly, out of 153 multipara pregnancy plasma samples, 18 were syphilis positives and 135 were syphilis negatives.

TECHNICAL INFORMATION

For further information, assistance, or problem reporting, contact Customer Service at +1-604-644-4677.

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GLOSSARY OF SYMBOLS

2°C 30°C	Store at 2°C to 30°C		Manufacturer
IVD	*In Vitro* Diagnostic Medical Device		Caution Harmful if swallowed
	Consult Instructions for Use	STERILE R	Sterilization using irradiation
	Do not reuse	N	Contains sufficient for "N" tests
	Do not use if damaged		Keep dry
REF	Catalogue Number		Keep away from direct sunlight
	Use By Date		This side up
LOT	Lot Number		

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