

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 despite 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	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.

Condition	Negative			Weak Positive		
	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 (1 st Trimester)	10	0	10	10	10	0
Pregnancy (2 nd Trimester)	10	0	10	10	10	0
Pregnancy (3 rd 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
Ritonavir	0.044 mg/mL
Valganciclovir 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 test 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 1 st 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 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)
3	Reactive	Non-reactive	Non-reactive	- Prior Syphilis (treated or untreated) - False positive EIA - Repeat testing in 2 weeks
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 (chancres), 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 752 plasma and 750 serum 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=502 502/(502+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 table below. 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.

Reference herein to any specific third party by name, trade name, trade-mark, manufacturer or otherwise does not constitute or imply an endorsement or recommendation of this Kit by such third party, or of the products or services of such third party by bioLytical or that such products or services are necessarily best suited for the intended purpose.

GLOSSARY OF SYMBOLS

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



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