Assay precision at or near the cut-off

Twenty individual syphilis positive plasma or serum samples with high treponemal antibody values were used to spike syphilis K2EDTA negative venous whole blood from 20 donors. Two samples at 2 different reactivity levels, C5 and C95, were prepared and tested from each of the 20 donor specimens. Additionally, 5 moderate positive samples were prepared and tested by spiking 5 of the syphilis K2EDTA negative venous whole blood specimens. Finally, 5 of the syphilis K2EDTA negative venous whole blood specimens were tested as is.

Sample	Percent Positive	Acceptance Criteria
Non-Reactive (CO)	0%	0%
Weak Reactive (C5)	3%	0-10%
Low Reactive (C95)	100%	90-100%
Moderate Reactive (2-3x LoD)	100%	100%

Prozone (Hook) effect study

High titer RPR positive syphilis samples from ten (10) unique donors representing all syphilis stages were diluted in syphilis negative plasma. The dilutions ranged from neat to 1:1560. Two replicates of each neat and diluted sample were tested with the ADEXUSDx® Syphilis Test. No hook effect was observed. Test results for neat samples and all dilutions less than or equal to 1:160 were positive for all donors. Test results for 1:320 diluted samples were positive for 9/10 donors and negative for 1 primary syphilis donor. Test results for 1:640 dilution samples were negative for both primary syphilis donors and positive for all secondary, early latent, and late latent syphilis donors. Tests results for 1:1280 diluted samples were negative for both primary syphilis donors, positive for 2/3 secondary and early latent syphilis donors, and negative for late latent syphilis donors. Test results for 1:1560 diluted samples were negative for all donors.

Seroconversion Panel

The Syphilis Seroconversion Panel PSS901 is a 9-member panel of undiluted, naturally occurring plasma samples, collected in the United States over 60 days in 2012 from a 41-year-old female. No preservatives were added. This panel converts from negative to positive for both non-treponemal and treponemal-specific tests, demonstrating an early syphilis infection. Each panel sample was performed on three lots of ADEXUSDx® Syphilis Tests. The results were read by three observers. Test results from commercially available syphilis assays are also included as shown below.

Sample ID	01	02	03	04	05	06	07	08	09
Days Since 1st Bleed	0	5	10	13	31	45	48	52	59
ADEXUSDx® Syphilis Rapid Test	NEG	NEG	NEG	NEG	POS	POS	POS	POS	POS
Remel RPR Card Test	NEG	NEG	NEG	NEG	POS	POS	POS	POS	POS
Becton Dickinson Macro-Vue™ RPR Card Test	NEG	NEG	NEG	NEG	POS	POS	POS	POS	POS
DiaSorin Liaison Treponema Syphilis	NEG	NEG	NEG	NEG	NEG	POS	POS	POS	POS
Olympus PK™ TP Syphilis	NEG	NEG	NEG	NEG	NEG	NEG	POS	POS	POS
Fujirebio Serodia® TPPA Syphilis	NEG	NEG	NEG	NEG	NEG	POS	POS	POS	POS
Trinity Biotech MarBlot Syphilis IgG	NEG	NEG	NEG	NEG	NEG	IND	IND	IND	POS
Trinity Biotech MarBlot Syphilis IgM	NEG	NEG	NEG	NEG	NEG	POS	POS	POS	POS
BioRad EIA Syphilis IgM	NEG	NEG	NEG	NEG	NEG	POS	POS	POS	POS
Abbott Architect Syphilis Total (s/co)	NEG (0.0450)	NEG (0.0400)	NEG (0.0300)	NEG (0.0300)	NEG (0.125)	POS (5.04)	POS (9.11)	POS (14.1)	POS (27.4)
Trinity Biotech CAPTIA™ Syphilis IgG (Ab Index)	NEG (0.145)	NEG (0.123)	NEG (0.123)	NEG (0.125)	NEG (0.155)	NEG (0.503)	NEG (0.798)	NEG (0.976)	NEG (1.32)
Trinity Biotech CAPTIA™ Syphilis IgM (Ab Index)	NEG (0.417)	NEG (0.445)	NEG (0.408)	NEG (0.396)	NEG (0.683)	POS (1.17)	POS (1.26)	POS (1.51)	POS (1.87)
Trinity Biotech Trep-Sure™ Syphilis (Index)	NEG (1.07)	NEG (0.475)	NEG (0.419)	NEG (0.576)	POS (3.68)	POS (4.07)	POS (4.98)	POS (4.20)	POS (11.2)

Correlation between whole blood, plasma, and serum

Matched whole blood, plasma and serum were collected from two syphilis negative donors. The samples were spiked with a pool of plasma from 11 syphilis positive donors to attain 4 samples of each matrix (K2EDTA whole blood, K2EDTA plasma, and serum) at different reactivity levels: non-reactive (CO), weak reactive (C5), low reactive (C95), and moderate reactive (2-3x LoD). The reactivity levels were confirmed using a reference assay.

Matrix Information	Sample Type	# Positive	% Positive
K2EDTA Blood	Non-reactive	0/10	0%
	Weak reactive	0/10	0%
	Low reactive	10/10	100%
	Moderate positive	10/10	100%
K2EDTA Plasma	Non-reactive	0/10	0%
	Weak reactive	0/10	0%
	Low reactive	10/10	100%
	Moderate positive	10/10	100%
Serum	Non-reactive	0/10	0%
	Weak reactive	0/10	0%
	Low reactive	10/10	100%
	Moderate positive	10/10	100%

Method Comparison

Patient samples (n=222) were collected for testing with the ADEXUSDx® Syphilis Test and measured quantitatively with a Trep assay, RPR, and TPPA. The final comparator result was determined using a 2 out of 3 rule (Trep assay, RPR, and TPPA).

Trep Assay	RPR	TPPA	Final Comparator Result	ADEXUSDx® Syphilis Test	Number of Subjects
N	R	R	P	P	2
N	R	Inconclusive	P	P	2
P	R	R	P	P	127
N	NR	NR	N	P	1
N	NR	NR	N	N	90
Total					222

P=Positive | N=Negative | NR=Non-Reactive | R=Reactive

The positive percent agreement (PPA) and the negative percent agreement (NPA) of the ADEXUSDx® Syphilis Test when compared to the comparator algorithm, along with the 95% confidence interval, is shown in the table below.

Patient Samples		Final Comparator Algorithm Result		
		Positive	Negative	Total
ADEXUSDx® Syphilis Test (Treponemal Test)	Positive	131	1	132
	Negative	0	90	90
	Total	131	91	222

PPA: 131/131 = 100.0% (95% CI: 97.2-100.0%)
NPA: 90/91 = 98.9% (95% CI: 94.0-99.8%)

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Symbols Used in the Packaging and Package Insert

	Do Not Reuse
	Use by or Expiration Date
REF	Catalog Number or Product Code
	Manufacturer
CE	European Conformity
	Store between 15°C – 30°C
IVD	For in vitro diagnostic use only
LOT	Lot Number
	Tests per kit
EC REP	Authorized Representative

Manufacturer:
NOWDiagnostics, Inc.
1200 Stewart Place
Springdale, AR 72764
1-844-207-3370
techsupport@nowdx.com
www.nowdx.com

Authorized Representative:
NOWDiagnostics Europe Srl
Via Campobello, 1 – 0071 Pomezia
Roma (Italy)
+39 06 87678028

NOWDiagnostics®

PRODUCT INFORMATION

ADEXUSDx®

Syphilis Test

REF 8057

For In-vitro Diagnostic Use

INTENDED USE

The ADEXUSDx® Syphilis Test is a rapid immunochromatographic assay used for the qualitative detection of the presence or absence of antibodies to syphilis in human whole blood, plasma, or serum to aid in the diagnosis of *Treponema pallidum*.

SUMMARY AND EXPLANATION

Syphilis is a sexually transmitted disease caused by the bacterium *Treponema pallidum*. It is transmitted by direct contact with a syphilitic sore, known as a chancre.[1] Syphilis typically follows a progression of stages (primary, secondary, latent, tertiary) with varying symptoms and duration. The primary stage is typically marked by a chancre or chancres at the original site of syphilis infection which subsist for 3-6 weeks before healing. Without treatment, the infection will progress to the secondary stage. Secondary syphilis is marked primarily by skin rashes, and sometimes by swollen lymph nodes, fever, and/or mucous membrane lesions. Like primary syphilis, the symptoms will go away but, without treatment, will progress to the latent stage of disease. During the latent stage, there are no symptoms. Early latent syphilis is specific to infection that occurred within one year. Late latent syphilis is specific to infection that occurred more than one year prior. Latent syphilis can last for years. Tertiary syphilis is rare and develops in a subset of untreated syphilis infections. It can appear decades following primary infection and can affect multiple organ systems, thus symptoms vary. Tertiary syphilis can be fatal.

Syphilis can spread to the brain and nervous system resulting in neurosyphilis and marked by a variety of symptoms including, but not limited to, headache, paralysis, dementia.[2] Syphilis can also spread to the eye resulting in ocular syphilis and marked by changes in vision and even blindness.[1] Women with syphilis can transmit the infection to their babies in utero, resulting in low birth weight babies, and/or early or stillborn deliveries. Untreated babies can become developmentally delayed, have seizures or die.[3]

Diagnosis of syphilis infection relies on both laboratory results and clinical evaluation.[4] *Treponema pallidum* cannot be cultured on artificial media, however this organism produces both treponemal and non-treponemal antibodies. Therefore, diagnosis of syphilis is made primarily by serologic assays.[5] There are two types of tests available for syphilis: (1) non-treponemal tests and (2) treponemal tests. Non-treponemal tests detect antibodies that are non-specific to syphilis. While non-specific antibodies occur in the majority of syphilis infected individuals, many other conditions can give rise to the same non-specific antibodies resulting in false positives. Treponemal tests detect antibodies specific for syphilis. Treponemal antibodies appear earlier than nontreponemal antibodies and usually remain detectable for life, even post treatment.[6] Both non-treponemal and treponemal tests are needed to confirm diagnosis.[7,8,9]

STORAGE

The ADEXUSDx® Syphilis Test is stable unopened at 15°C – 30°C until the expiry date.

WARNINGS

- For *in vitro* diagnostic use only.
- Do not use expired test.
- Do not use if the pouch seal is compromised.
- Do not open the sealed pouch until ready to perform the ADEXUSDx® Syphilis Test.
- Read the Product Insert completely before using this assay. Follow the instructions carefully. Failure to follow instructions may result in inaccurate test results.

MATERIALS

Materials provided:

Each box of fifty (50) tests contains the following items:

- Fifty (50) sealed aluminum pouches each containing one (1) ADEXUSDx® Syphilis Test and one (1) desiccant
- One (1) Product Insert

Each box of twenty-five (25) tests contains the following items:

- Twenty-five (25) sealed aluminum pouches each containing one (1) ADEXUSDx® Syphilis Test and one (1) desiccant
- One (1) Product Insert

Materials reuired but not provided:

- Lancet
- Watch or timer
- Gloves
- Negative and positive control set (available upon request)
- SST or EDTA tube (if relevant)

SAMPLE COLLECTION & HANDLING

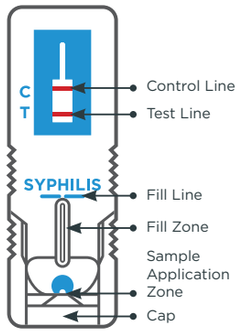
Whole Blood, Plasma and Serum Test

- For whole blood and plasma tests, obtain blood by venipuncture. In a EDTA container, collect blood for the test or process the venous blood to obtain plasma.
- For serum tests, obtain blood by venipuncture. In an appropriate container, collect blood and process the blood to obtain serum.

Samples should be tested as soon as possible. If retained, store plasma or serum samples at 2-8°C for 24 hours or at -20°C or lower for longer periods. Bring stored samples to room temperature for testing.

TEST METHOD

For Whole Blood, Plasma, or Serum Samples



1. Open a sealed pouch containing one (1) ADEXUSDx® Syphilis Test and desiccant packet. Remove the test from the pouch and discard the desiccant packet. DO NOT REMOVE THE CAP. Lay the test on a flat surface.
2. Collect 40 µL of sample using a transfer device.
3. Apply the sample slowly into the Sample Application Zone until the Fill Zone is full and the Fill Line is reached.
4. After filling the Fill Zone, hold the test with the Sample Application Zone facing up and tap the opposite end of the test twice on a hard surface. Then, lay the test on a flat surface.
5. Set a timer for 15 minutes. Read the result after 15 minutes. Do not read the result after 30 minutes.

INTERPRETATION OF RESULTS

"C" & "T" Lines

Lines may appear at two locations, marked "C" and "T".

"C" stands for Control Line; it tells you if the test has worked.

"T" stand for Test Line; it tells you if the test is positive or not.

Positive Result

A positive result has TWO LINES, one at the "C" and one at the "T". The intensity of the lines may vary, so look carefully as **lines might be faint.**

Negative Result

A line at the "C" and no line at the "T" indicate a negative result.

Invalid Result

If NO line appears at the "C", the test has not worked. You must retest using a new test.

EXPECTED RESULTS

A colored line at the Test Line position was designed to be visible when a sample containing syphilis antibody at or above 1 AI is tested. The test was calibrated with the Bio-Rad BioPlex 2200 Syphilis Total Assay.

PERFORMANCE CHARACTERISTICS

Interfering substances

Stock solutions for potential interferents were used to prepare Syphilis low positive and negative whole blood samples at the concentrations indicated below. No interference was observed when the following substances were added to the samples. The negative and positive syphilis venous whole blood gave the expected results regardless of the presence of the interfering substances.

Interfering substances and their final concentrations:

Acetaminophen	0.2 mg/mL
Acetylcysteine	1.66 mg/mL
Acetylsalicylic acid (Aspirin)	0.65 mg/mL
Albumin	20 mg/mL
Ampicillin	0.2 mg/mL
Ascorbic acid	0.2 mg/mL
Bilirubin	2 mg/mL
Cefoxitin	0.66 mg/mL
Cholesterol	2.5 mg/mL
Cyclosporine	1.4 mg/mL
Doxycycline	0.03 mg/mL
Gamma-Globulin	24 mg/mL
Hemoglobin	20 mg/mL
Heparin	3000 U/L
Ibuprofen	0.5 mg/mL
K2EDTA	0.8 mg/mL
Levodopa	6.5 mg/mL
Methyldopa	0.015 mg/mL
Metronidazole	0.12 mg/mL
Phenylbutazone	5.0 mg/mL
Rifampin	0.064 mg/mL
Theophylline	0.04 mg/mL
Triglycerides	2.5 mg/mL

Cross-reactivity Study

Cross reactivity of the ADEXUSDx® Syphilis Test has been evaluated by testing serum and plasma samples positive for the potential cross-reactants. Devices from 1 lot were used in this study. A total of 200 cross-reactant samples were tested. One EBV positive sample and one Rh factor positive sample showed weak positive test bands. The Percent Positive of EBV and Rh factors is 10%. Therefore, patients in these two categories might result false positive with ADEXUSDx® Syphilis test.

Potential cross-reactant	Results (# Negative)
Anti-*Lyme* Positive	10/10
Anti-*Gonorrhea* Positive	10/10
Anti-*Chlamydia Trachomatis* Positive	10/10
Anti-*Leptospirosis* Positive	10/10
Anti-*Trichomonas* Positive	10/10
Anti-*Toxoplasma gondii* Positive	10/10
Anti-CMV Positive	10/10
Anti-EBV Positive	9/10
Anti-HAV Positive	10/10
Anti-HBV Positive	10/10
Anti-HCV Positive	10/10
ANA Positive	10/10
Anti-HIV Positive	10/10
Hemodialysis patient	10/10
Anti-HSV Positive	10/10
Rh factor Positive	9/10
HAMA Positive	10/10
Heterophile antibodies Positive	10/10
Anti-HPV Positive	10/10
Anti-HTLV Positive	10/10

Lot to Lot Proficiency Testing

Four samples were prepared by spiking Syphilis positive plasma or serum to obtain non-reactive (C0), weak reactive (C5), low reactive (C95), and moderate reactive (2-3XLoD) samples. At each of 3 clinical trial sites, 3 site operators performed ADEXUSDx® Syphilis Tests from 3 different lots with each of 4 samples in duplicate on each of 3 days, for a total of 162 tests per sample. Operators were blinded to samples and test lots. Samples and test lots were randomized.

Sample	# of Observations	% Expected Positive	% Actual Positive	95% CI
Non-Reactive (C0)	162	0	0	0.0-2.3%
Weak Reactive (C5)	162	≤5	0	0.0-2.3%
Low Reactive (C95)	162	≥95	99.4	96.6-99.0%
Moderate Reactive (2-3x LoD)	162	100	100	97.7-100.0%

Site to Site Proficiency Testing

Using the same four samples, at each of 3 clinical sites, 3 site operators performed ADEXUSDx® Syphilis Tests from 1 lot with each of the 4 samples in triplicate, on each of 5 days, for a total of 135 tests per sample. Operators were blinded to samples. Samples were randomized.

Sample	# of Observations	% Expected Positive	% Actual Positive	95% CI
Non-Reactive (C0)	135	0	0	0.0-2.8%
Weak Reactive (C5)	135	≤5	0	0.0-2.8%
Low Reactive (C95)	135	≥95	98.5	94.8-99.6%
Moderate Reactive (2-3x LoD)	135	100	100	96.3-100.0%