Rapid Response™

Syphilis Test Cassette
(Whole Blood/Serum/Plasma)

REF SYP-13C40 Product Insert

For professional *in vitro* diagnostic use only.

Intended Use

The Rapid Response™ Syphilis Test Cassette is an *in vitro* diagnostic rapid immunochromatographic assay for the qualitative detection of antibodies to *Treponema pallidum* in human whole blood, serum, and plasma specimens. The product may be used as an aid in the diagnosis of syphilis. A reactive result should be confirmed by other supplemental assay(s).

Introduction

Syphilis is a sexually transmitted infection caused by the bacterium Treponema pallidum subspecies *pallidum*[1]. The signs and symptoms of syphilis vary depending in which of the four stages it presents (primary, secondary, latent, and tertiary). In latent syphilis, which can last for years, there are few or no symptoms[2].

Syphilis is transmitted primarily by sexual contact or during pregnancy from a mother to her fetus; the spirochete is able to pass through intact mucous membranes or compromised skin[3]. Syphilis is a notifiable disease in many countries, including Canada[4] the European Union[5], and the United States[6]. Approximately 30% to 60% of those exposed to primary or secondary syphilis will get the disease[7]. In 2015, about 45.4 million people were infected with syphilis[8], with 6 million new cases[9]. Untreated, it has a mortality of 8% to 58%, with a greater death rate in males[3]. With early treatment, few complications result[10].

Syphilis is difficult to diagnose clinically early in its presentation. Confirmation is either via blood tests or direct visual inspection using microscopy. Blood tests are more commonly used, as they are easier to perform[3]. Because of the possibility of false positives with nontreponemal tests, confirmation is required with a treponemal test, such as treponemal pallidum particle agglutination (TPHA) or fluorescent treponemal antibody absorption test (FTA-Abs)[3]. Treponemal antibody tests usually become positive 2-5 weeks after the initial infection[10].

Principle

The Rapid Response™ Syphilis Test Cassette detects antibodies to *T. pallidum (TP)* through visual interpretation of color development on the internal strip. Recombinant antigens representing epitopes of TP (15kD, 17kD, 47kD) are immobilized on the test region of the membrane.

During testing, the specimen reacts with recombinant TP-specific antigens conjugated to colored particles and precoated onto the sample pad of the test. The mixture then migrates through the membrane by capillary action and interacts with reagents on the membrane. If there are sufficient antibodies to TP in the specimen, a colored band will form at the test region of the membrane.

The presence of this colored band indicates a positive result, while its absence indicates a negative result. The appearance of a colored band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

Precautions

- For professional *in vitro* diagnostic use only.
- Read these instructions carefully before performing the test.
- Do not use the test beyond the expiration date.
- Do not use the test if the packaging is damaged.
- Do not reuse tests.
- Apply standard biosafety precautions when handling and disposing of potentially infectious materials.
- Handle all specimens as potentially infectious.
- Wear protective clothing such as gloves, laboratory coats, and eye protection when specimens are being assayed.
- The test device and accessory should be disposed in a proper biohazard waste container after testing.
- Do not eat, drink or smoke in the area where the specimens and kits are handled.
- Avoid splashes and clean up spills immediately with appropriate disinfectant.
- The buffer contains 0.02% sodium azide as a preservative which may be toxic if ingested. When disposed of through a sink, flush with large quantities of water.
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Humidity and temperature can adversely affect results.
- Do not use any other specimen than those specified. For venous whole blood and plasma, EDTA, sodium citrate, sodium heparin or potassium oxalate can be used as anticoagulant.
- Used testing materials should be discarded in accordance with local regulations.

Materials

Materials provided

- Test devices
- Buffer
- Droppers
- Product insert

Materials required but not provided

- Specimen collection equipment and container
- Centrifuge
- Timer

Storage and Stability

- The kit should be stored at 35.6-86°F (2-30°C) until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze the kit.**
- Protect the kit from humidity.
- Care should be taken to protect the components of the kit from contamination. Do not use the test if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

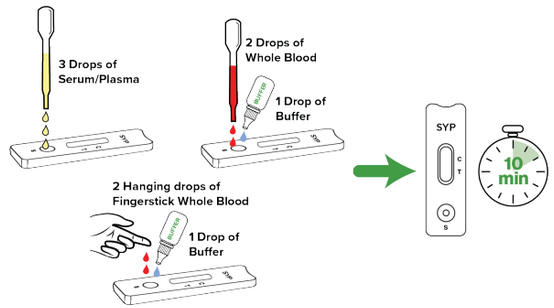
Collection and Storage of Specimens

- The Rapid Response™ Syphilis Test Cassette is intended for use with human whole blood, serum or plasma specimens only.
- Collect specimens according to safe phlebotomy procedure.
- Only clear, non-hemolyzed specimens are recommended for use with this test. Serum or plasma should be separated as soon as possible to avoid hemolysis.
- Perform testing immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 35.6-46.4°F (2-8°C) for up to 7 days. For long term storage, specimens should be kept below -4°F (-20°C). Whole blood collected by venipuncture should be stored at 35.6-46.4°F (2-8°C) if the test is to be run within 3 days of collection. Do not freeze whole blood specimens.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Multiple freeze/ thaw cycles should be avoided.
- If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.
- Icteric, lipemic, hemolysed, heat treated and contaminated sera may cause erroneous results.

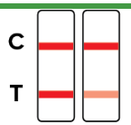
Test Procedure

Bring the test device, buffer and specimen to room temperature (59-86°F; 15-30°C) prior to testing.

1. Remove the test device from its sealed pouch and use it as soon as possible. For best results, the assay should be performed within one hour.
2. Place the test device on a clean, level surface. Label with specimen ID.
   For venous whole blood specimens:
   Hold the dropper vertically and transfer 2 drops of whole blood (approximately 50 µL) to the sample well (S) of the test device, then add 1 drop of buffer (approximately 40 µL) and start the timer.
   For fingerstick whole blood specimens:
   Dispense 2 drops of fingerstick whole blood onto the sample well (S) of the test device, then add 1 drop of buffer (approximately 40 µL) and start the timer.
   For serum or plasma specimens:
   Hold the dropper vertically and transfer 3 drops of serum or plasma (approximately 75 µL) to the sample well (S) of the test device, and start the timer.
   Avoid trapping air bubbles in the specimen well (S), and do not add any solution to the result area.
3. Wait for the colored band to appear. Read results at 10 minutes.

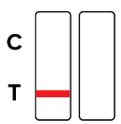


Results Interpretation



POSITIVE: Two colored lines appear. One line should always appear in the control region (C), and another line appears in the test region (T).

NEGATIVE: Only one colored line appears in the control region (C). No colored line appears in the test region (T).



INVALID: No line appears in the control region (C). Results from any test which has not produced a control line at the specified reading time must be discarded. Please review the test procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of color in the test region (T) may vary depending on the concentration of analytes present in the specimen. However, any shade of color in the test region should be considered positive. Note that this is a qualitative test only, and the concentration of analytes in the specimen cannot be determined.
2. Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for control line failure.

Quality Control

- An internal procedural control is included in the test. A colored line appearing in the control region (C) is considered an internal positive procedural control, confirming sufficient specimen volume and correct procedural technique. The control line does not control for the addition of adequate volume of specimen.

- External quality controls are not supplied with this kit. It is recommended that quality controls be tested as a good laboratory practice.

Limitations

1. The Rapid Response™ Syphilis Test Cassette is for professional *in vitro* diagnostic use only, and should be used for the qualitative detection of antibodies to T. pallidum in human whole blood, serum or plasma.
2. The Rapid Response™ Syphilis Test Cassette will only indicate the presence of antibodies to T. pallidum in the specimen and should not be used as the sole criteria for the diagnosis of Syphilis.
3. For confirmation of test results, specimens should undergo further testing using different assays, such as rapid diagnostic tests, EIA, in accordance with a validated syphilis testing algorithm.
4. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
5. False reactive results may arise due to damage to test components by heat or humidity, when other test kit components (e.g. buffer or droppers) are substituted between test kits.
6. False non-reactive results may arise when titers of antibodies to T. pallidum are very low, titers of antibodies to T. pallidum are very high (hook effect), insufficient specimen volume added, excess of buffer added, damage to test components by heat or humidity.

Performance Characteristics

Analytical Sensitivity

The analytical sensitivity of the Rapid Response™ Syphilis Test Cassette has been verified with the WHO International Standard for 1st IS for human syphilitic plasma IgG (NIBSC code 05/122) and IgG+IgM (NIBSC code 05/132). The analytical sensitivity of Rapid Response™ Syphilis Test Cassette is 5 mIU/mL for 05/122, and 10 mIU/mL for 05/132.

Clinical Evaluation

Clinical Evaluation on Serum and Plasma Specimens

The performances of the Rapid Response™ Syphilis Test Cassette have been established in comparison with TPHA. A total of 593 serum and plasma samples, collected from independent subjects, were tested in the study. The testing results were summarized in the table below:

Table 1: Clinical Evaluation on Serum and Plasma Specimens

Relative Sensitivity: 99.6% (97.7%-99.9%)*
Relative Specificity: 99.1% (97.5%-99.7%)*
Overall Agreement: 99.3% (98.3%-99.7%)*
*95% Confidence Interval

		Rapid Response™ Syphilis Test Cassette		
		+	-	Total
TPHA	+	246	1	247
	-	3	343	346
		249	344	593

Clinical Evaluation on Venous Whole Blood Specimens

For venous whole blood specimen validation, 250 clinically diagnosed Syphilis-positive venous whole blood specimens (EDTA as anticoagulant), and 100 negative venous whole blood specimens (EDTA as anticoagulant), collected from independent subjects, were included in the study.

Venous whole blood specimens were collected in anticoagulant tubes with EDTA, and tested by the Rapid Response™ Syphilis Test Cassette within 3 days after specimen collection. The venous whole blood specimens were centrifuged to collect plasma specimens for TPHA test. The testing result was summarized in the table below:

Table 2: Clinical Evaluation on Venous Whole Blood Specimens

Relative Sensitivity: >99% (98.5%-100%)*
Relative Specificity: >99% (96.3 %-100%)*
Overall Agreement: >99% (98.9%-100%)*
(*95% Confidence Interval)

		Rapid Response™ Syphilis Test Cassette		
		+	-	Total
TPHA	+	250	0	250
	-	0	100	100
		250	100	350

Clinical Evaluation on Fingerstick Whole Blood Specimens

For fingerstick whole blood specimen validation, 50 clinically diagnosed Syphilis-positive fingerstick whole blood specimens, and 50 Syphilis-negative fingerstick whole blood specimens, collected from independent subjects, were included in the study. Meanwhile, serum specimens were collected from the 100 donors for TPHA test. Fingerstick whole blood specimens were tested immediately on the Rapid Response™ Syphilis Test Cassette. Serum specimens from these 100 donors were tested by TPHA for comparison.

Table 3: Clinical Evaluation on Fingerstick Whole Blood Specimens

Relative Sensitivity: >99% (92.9%-100%)*
Relative Specificity: >99% (92.9 %-100%)*
Overall Agreement: >99% (96.3%-100%)*
(*95% Confidence Interval

		Rapid Response™ Syphilis Test Cassette		
		+	-	Total
TPHA	+	50	0	50
	-	0	50	50
		50	50	100

Specimen Type Equivalency

Four specimen types (serum, plasma, venous whole blood, fingerstick whole blood) were collected from individuals known to be positive for syphilis (n=50) and from individuals negative for syphilis (n=50). Each specimen was confirmed by TPHA. The result demonstrated 100% agreement between specimen types and with expected results in known positive Syphilis specimens and in negative Syphilis specimens.

Anticoagulant Type Equivalency

To evaluate the equivalency of three anticoagulant types (EDTA, sodium heparin, and sodium citrate) on the assay performance, 25 Syphilis-positive venous whole blood specimens, 25 Syphilis-positive plasma specimens, 25 Syphilis-negative venous whole blood specimens and 25 Syphilis-negative plasma specimens for each anticoagulant type. The results demonstrated that sodium heparin and sodium citrate are equivalent to EDTA for the detection of Syphilis antibody.

Interference Substances

The assay performance of Rapid Response™ Syphilis Test Cassette was not affected by the following analytes at listed amounts:

Analytes	Amount
Acetaminophen	20 mg/dL
γ globulin	60 g/L
Caffeine	20 mg/dL
HAMA	780.73 IU/mL
Aspirin	20 mg/dL
Metronidazole	701 µmol/L
Gentistic acid	20 mg/dL
Quinine	148 µmol/L
Oxalic acid	60 mg/dL
Rifampin	78.1 µmol/L
Creatine	200 mg/dL
Aspirin	4.34 mmol/L
Methanol	10%
Paracetamol	199 µmol/L
Ascorbic acid	2,000 mg/dL
Ibuprofen	2,425 µmol/L
Albumin	2,000 mg/dL
Ethanol	86.8 mmol/L
Hemoglobin	1,000 µg/dL
EDTA	3.4 µmol/L
Bilirubin	1,000 mg/dL
Heparin	3,000 U/L
Rheumatoid	1,035 IU/mL
Citrate	4%
Acetoacetic Acid	2,000 mg/dL

Pregnancy Specimens Testing

To evaluate whether pregnancy would affect the assay performance of the device, specimens from 100 pregnant women including 30 venous whole blood specimen, 30 plasma specimens, and 40 serum specimens were tested. The results showed that the assay performance of the Rapid Response™ Syphilis Test Cassette was not affected by pregnancy specimens.

Cross Reactivity

The assay performance of the Rapid Response™ Syphilis Test Cassette was not affected by the following infections: HIV, HBV, HCV, HAV, Tuberculosis, Toxoplasma gondii, Rubella, CMV, HSV-1, HSV-2, Typhoid, Influenza A, Influenza B, and Dengue.

Bibliography

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Glossary of Symbols

Consult instructions for use
Test per Kit
EC REP Authorized Representative
Store between 35.6°F to 86°F
Use by
Do Not Reuse
LOT Lot Number
IVD For *in vitro* diagnostic use only
REF Catalogue #

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