

Development and Evaluation of an Immunochromatographic Device for the Simultaneous Detection of Non-treponemal and Treponemal Antibodies in Patients with Syphilis

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INTRODUCTION

The diagnosis of syphilis is based on clinical manifestations, direct detection of spirochaetes in early skin lesions and serological assays.

The serological diagnosis of syphilis requires the detection of two distinct antibodies.

Non-treponemal tests are non-specific tests that detect heterophile antibodies to an antigen containing cardiolipin that is released from the treponemes and damaged host cells. These non-treponemal antibodies are the best indicators of active infection and decline with successful treatment. An example is the RPR test.

In contrast, treponemal tests are highly specific and detect antibodies directed against *T. pallidum* or the 15, 17, 47 kD components of the organism. In general, treponemal tests remain positive for life – even after provision of effective treatment. Examples of treponemal tests include the TPPA, FTA-ABS and ELISA tests.

All these assays are performed using a serum sample and must be performed in a clinical setting. Therefore, test results may not be available for several days after the sample is collected. Clearly, a point-of care (POC) test that could provide a diagnosis and therefore treatment at the first clinic visit, or could be used in outreach programs would be a major advance.

The CDC, in collaboration with Chembio Diagnostic Systems, has developed a rapid, disposable, inexpensive, POC test for syphilis, that requires no expertise in interpreting results and serves as a screening and confirmatory test in a lateral flow format (Figures 1-3). The assay can be performed on whole blood (finger stick), on serum or plasma. As little as 5 µl of sample is required to perform the test and the results can be read within 15 minutes.

METHODS

The immunochromatographic test is based on the principle of a dual path platform (DPP). This system has two antigens; one treponemal, one non-treponemal and a control line striped onto the surface of a nitrocellulose membrane within the device. The test is able to screen and confirm the results using a hand held reader within fifteen minutes, which provides a numerical value of test line intensities and requires no expertise in interpreting results. A total of 435 banked serum samples were examined by the rapid test and the results compared to those obtained using a quantitative rapid plasma reagin (RPR) test and the *Treponema pallidum* passive particle agglutination assay (TP-PA).

Figure 1: DPP Syphilis Device Format

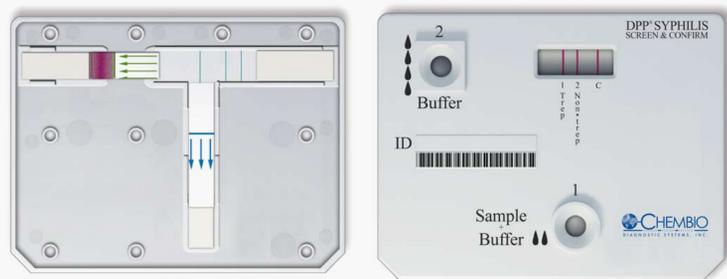


Figure 2: Patterns of Reactivity – DPP Syphilis Assay

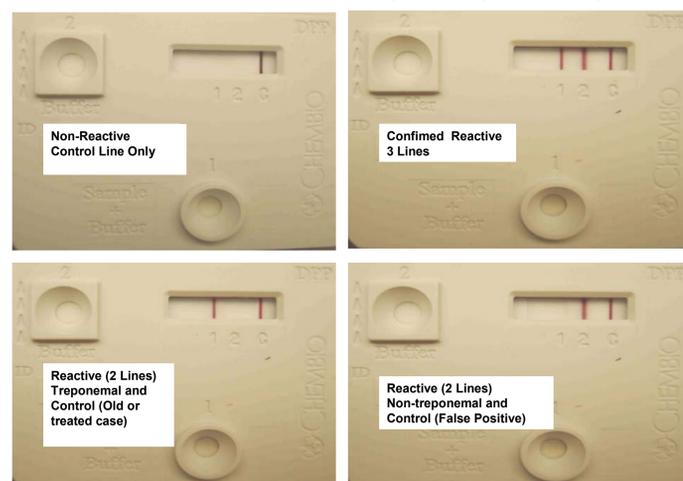
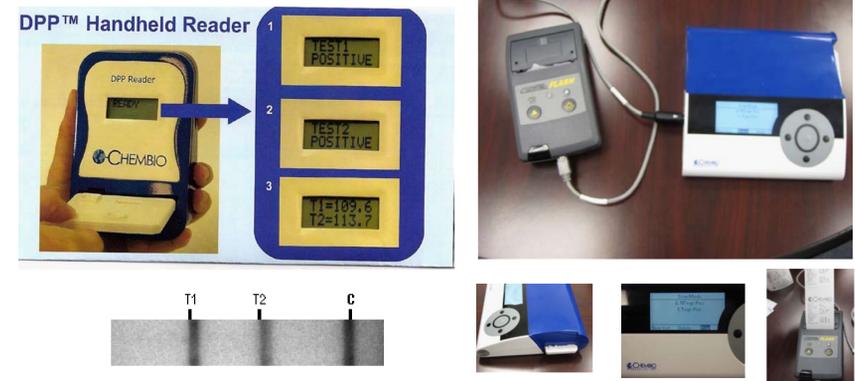


Figure 3. DPP Test Readers



Use of point of care readers, which measure the reflectance of individual test lines, results in less subjectivity in the reading of the test, particularly in settings with poor lighting.

Figure 4. Performance Characteristics of Chembio Dual Syphilis Test (Non-treponemal Line)

		RPR		Total
		+	-	
Chembio Non-treponemal Line	+	201	0	201
	-	14	220	234
		215	220	435

Sensitivity = 93.5% Specificity = 100%

Figure 5. Rates of detection of Anti-cardiolipin Antibody by the POC test by RPR Titer

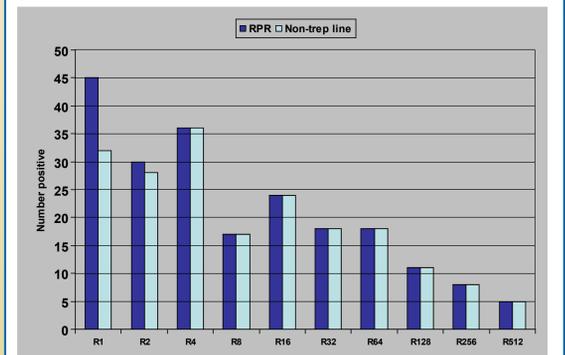


Figure 6. Performance Characteristics of Chembio Dual Syphilis Test (Treponemal Line)

		RPR/TPPA		Total
		Treat	No Treat	
Chembio Dual Test	Treat	195	1	196
	No Treat	20	219	239
		215	220	435

Sensitivity = 90.7% Specificity = 99.5%

Figure 7. Performance Characteristics of the Lateral Flow Dual POC Test for Syphilis when Compared to the RPR and TP-PA tests.

(For a patient to be considered for antibiotic treatment, both non-treponemal and treponemal tests should be positive.)

		TPPA		Total
		+	-	
Chembio Treponemal Line	+	206	13	219
	-	19	197	216
		225	210	435

Sensitivity = 91.6% Specificity = 93.8%

RESULTS

The sensitivity and specificity of the nontreponemal line were 93.5% and 100% respectively when compared to the RPR test (Figure 4). The device detected all positive sera with RPR titers $\geq 1:4$ that were confirmed as true positives by the TPPA test (Figure 5).

The sensitivity and specificity of the treponemal test line were 91.6% and 93.8% respectively when compared to the TP-PA test (Figure 6). Since both non-treponemal and treponemal tests should be positive for a patient to be considered for antibiotic treatment, the overall performance of the lateral flow dual POC test for syphilis when compared to the RPR and TP-PA tests could be considered as, sensitivity 90.7%, specificity 99.5% (Figure 7).

CONCLUSIONS

These results indicate that the DPP dual test could be used as a point-of care test for the serological diagnosis of syphilis in primary health care clinics or in resource poor settings. The use of the hand-held reader permits objective reading of the result, even under suboptimal conditions. When used on whole blood specimens the test can be used in non-conventional settings and permit provision of treatment in situations where patients may not return for test results.