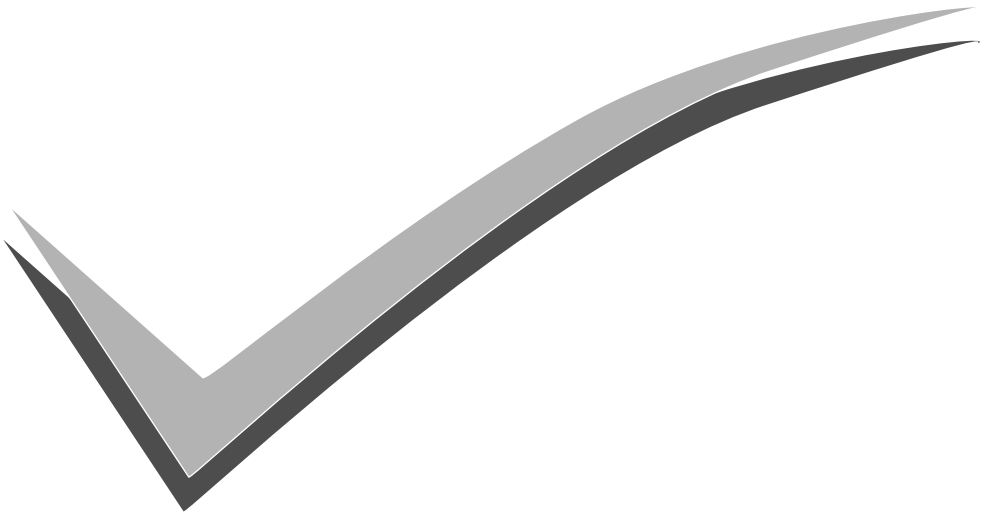
Performance Evaluations



SYPHICHECK®-WB

Rapid test for Syphilis (Modified TPHA)

Tulip Group

Performance Evaluations

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RAPID DIAGNOSTICS

A multi-centre evaluation of nine rapid, point-of-care syphilis tests using archived sera

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Objectives: To evaluate nine rapid syphilis tests at eight geographically diverse laboratory sites for their performance and operational characteristics.
Methods: Tests were compared ''head to head'' using locally assembled panels of 100 archived (50 positive and 50 negative) sera at each site using as reference standards the *Treponema pallidum* haemagglutination or the *T pallidum* particle agglutination test. In addition inter-site variation, result stability, test reproducibility and test operational characteristics were assessed.
Results: All nine tests gave good performance relative to the reference standard with sensitivities ranging from 84.5–97.7% and specificities from 84.5–98%. Result stability was variable if result reading was delayed past the recommended period. All the tests were found to be easy to use, especially the lateral flow tests.
Conclusions: All the tests evaluated have acceptable performance characteristics and could make an impact on the control of syphilis. Tests that can use whole blood and do not require refrigeration were selected for further evaluation in field settings.

The World Health Organization (WHO) estimates that approximately 12 million new cases of venereal syphilis occur worldwide each year, most of which are in developing countries where access to sexually transmitted diseases (STD) laboratory services are limited.[1] Nonetheless, the disease remains a global health priority. The recent re-emergence of syphilis in the developed world, as seen in Russia and eastern Europe, has been associated with social upheaval and is potentially a contributor to burgeoning HIV epidemics.[2] In North America and Western Europe, resurgent syphilis has been associated mainly with men who have sex with men or illicit drug users.[3]

In most countries where prenatal screening for syphilis is available, the rapid plasma reagin (RPR) test is used. To prevent stillbirth and other adverse outcomes of pregnancy, women who test positive at peripheral clinics are treated without recourse to a confirmatory treponemal test. Given the importance of early treatment, and the efficacy and safety of intramuscular benzathine penicillin, this has proved to be a sound strategy, even though it may lead to unnecessary treatment in some cases. Screening and treatment of pregnant women for syphilis remains cost-effective even when the prevalence is low.[4] However, it is estimated that less than 30% of pregnant women are screened for the disease in sub-Saharan Africa,[5] while a study in Bolivia showed that, although 76% of the study population received antenatal care, only 17% were screened for syphilis during pregnancy.[6] Among the many reasons for low rates of screening, one major barrier is that current screening using a non-treponemal test requires a laboratory with trained personnel and a source of electricity to run a refrigerator to store the RPR reagent, a centrifuge to separate serum from whole blood, and a shaker to perform the serology. Since such facilities are generally not available in primary health care settings, blood or serum samples have to be transported to regional or central facilities for testing. Often results are only available days or weeks after testing. Studies have shown that only a small proportion of infected women receive treatment when RPR testing is performed off-site, because women do not return for their results or specimens or results are lost in transit.[7] Even when testing is available at clinical sites, there are technical difficulties associated with maintaining trained personnel and assuring quality standards and supplies of tests and treatment.[8]

A number of simple, rapid treponemal tests have recently become commercially available. Most are ''lateral flow'' tests in which antibodies are transported by capillary flow over antigen immobilised on a nitrocellulose membrane strip (also termed immunochromatographic strips). Antibodies in the specimen become bound at the antigen site on the strip and are revealed with dye bound to an anti-immunoglobulin. These tests are simple, robust, affordable and can be stored and transported without need for refrigeration. Initial evaluations suggested that their performance was comparable with the best laboratory-based diagnostics.[9–17] Used alone, they would be unable to distinguish active from cured disease but they can facilitate a crucial intervention—the screening of pregnant women to reduce the occurrence of stillbirth and congenital syphilis where access to laboratory services is a problem[18 19] The WHO/Sexually Transmitted Diseases Diagnostics Initiative (SDI) is conducting an ongoing comprehensive evaluation of these rapid tests with panels of well-characterised archived serum specimens from geographically diverse settings. The results of these evaluations are used to select a number of the most promising tests for further evaluation in field settings. This paper reports the first results.

METHODS

The initial phase of the work involved the recruitment of laboratories to undertake the evaluation and two reference laboratories to provide a measure of quality assurance. A request for applications was posted on the WHO/SDI website and laboratories on the SDI mailing list were contacted.

Abbreviations: SDI, Sexually Transmitted Diseases Diagnostics Initiative; STD, sexually transmitted diseases; RPR, rapid plasma reagin; SOP, standard operating procedures; TPHA, *Treponema pallidum* haemagglutination assay; TPPA, *Treponema pallidum* particle agglutination assay; WHO, World Health Organization

Responding laboratories were sent a questionnaire to establish their access to patients, sera and a suitably constituted ethics committee, their general experience of evaluations and, importantly, their access to field sites for subsequent testing. In addition, the principal investigators were asked to submit 20 sera to the reference laboratories together with details of their results of both treponemal (*Treponema pallidum* particle agglutination assay (TPPA) or *T pallidum* haemagglutination assay (TPHA)) and non-treponemal (RPR) antibody tests. This was to establish that they were proficient in performing the reference tests used in the evaluation. The eight laboratories selected by this process are shown in table 1.

Tests for evaluation

An ad hoc SDI expert working group for laboratory-based evaluations decided that the tests to be included should have the following characteristics:

- rapid-test result is available in less than 30 minutes
- simple test can be performed in a few steps, requiring minimal training and minimal extra equipment
- easy-to-interpret card or strip format with visual readout.

In the initial round of evaluation, 13 manufacturers with tests that conform to the above characteristics were invited to participate, of whom six manufacturers submitted tests for evaluation at eight SDI sites on four continents. In the second round of evaluations, three more tests were evaluated in six of the SDI sites. One of the tests evaluated in the second round was an improved version of the test submitted in the first round (SyphiCheck made by the Tulip Group in India). Details of the tests are given in table 2 and their major features are listed in table 3. The serum panels used in round 2 were not identical to those used in round 1.

Several parameters of the tests were evaluated including sensitivity and specificity relative to a ''gold'' or reference standard laboratory test together with inter-reader variability, result stability, reproducibility, ease of use and between-site variability. These laboratory comparisons represent the first part of a full evaluation of these tests, the final and definitive phase being the field evaluation.

Development of the standard protocol and performance of the evaluation

All participating laboratories collaborated in the development of a standard protocol for the evaluation which was then reviewed and approved by the WHO and the local site ethical committees. Before beginning the evaluation, the study protocol was piloted with one positive and one negative serum.

Each laboratory assembled an evaluation panel from archived specimens containing 50 TPHA/TPPA positive sera (40 RPR+, 10 RPR−) and 50 TPHA/TPPA negative sera (40 RPR−, 10 RPR+). Haemolysed sera were avoided and, if a precipitate was visible, the serum was clarified at 12 000 *g* for five minutes. All patient identifiers were unlinked from specimens before the evaluations.

The reference test was either the TPPA (Serodia, Fujirebio Inc, Tokyo) or the TPHA.

The standard operating procedures (SOP) for the assays were the manufacturer's product inserts. In addition, SOPs were produced to ensure that the testers were blinded to reference standard results and that, in the inter-observer variability trial, both testers were truly independent.

Each kit was tested with all 100 sera in batches of 25 sera before evaluating another test kit to avoid comparison of results between kits. Indeterminate results were recorded as such and any repeat testing was only performed after all 100 sera had been tested. To allow result stability to be assessed, each result was read at the recommended time and after one hour. At each site, each test was read by two project technicians to allow inter-operator variability to be estimated.

Each test was assessed for its operational characteristics by the same technicians. Tests were scored for clarity of kit instructions, technical complexity or ease of use and ease of interpretation of results. Each of these characteristics was allotted marks out of 3 and an additional score of 1 was given to tests not requiring additional equipment, giving a maximum of 10. This was not done in the second round as it was felt that this would be better evaluated by field staff than highly trained laboratory technicians.

Test reproducibility was investigated in the reference laboratories. Lot-to-lot reproducibility was tested using 25 sera and two lots of each rapid test. Operator reproducibility was compared by two technicians who ran each test with the same 20 sera. Run-to-run reproducibility was investigated using nine sera that were tested on five successive days for each test.

Quality control measures were included in the data management instructions in the protocol. Results were recorded in the laboratory notebooks of each technician which was signed off by the supervisor at the end of each day. Data were then entered into a laboratory data collection spreadsheet provided by SDI. The spreadsheet was then double-checked against the notebooks of both technicians.

Table 1 Sexually Transmitted Diseases Diagnostics Initiative (SDI) sites for laboratory-based evaluations of rapid syphilis diagnostics

Site location	Institution	Principal investigators
Africa		
South Africa, Durban	University of Natal	AW Sturm
The Gambia, Fajara*	MRC Laboratories	B West, RA Adegbola
Tanzania, Mwanza*	National Institute for Medical Research	J Changalucha
Asia		
China, Nanjing*	National Center for STD and Leprosy Control	YP Yin
Sri Lanka, Colombo	National STD/AIDS Control Programme	S Mananwatte
Americas		
Haiti, Port au Prince*	Les Centres GHESKIO (Groupe Haitien d'Etudedu Sarcome de Kaposi et des Infections Opportunistes)	JW Pape, DW Fitzgerald
USA, Birmingham Alabama*	University of Alabama	E Hook III
Europe		
Russian Federation, Moscow*	Central Institute for Skin and Venereal Diseases	A Kubanova, E Filatova

*These laboratories undertook both rounds of the evaluation.

Table 2 Syphilis tests evaluated and their manufacturers

Test	Company
Round 1	
Determine Syphilis	Abbott Laboratories, Chicago, USA; www.abbottdiagnostics.com
Syphilis Fast	DIESSE Diagnostica, Senese SpA, Milan, Italy; www.diesse.it
Espline TP	Fujirebio Inc, Tokyo, Japan; www.fujirebio.co.jp
Syphicheck-WB	Qualpro Diagnostics, Goa, India; www.tulipgroup.com
SD Bioline Syphilis 3.0	Standard Diagnostics, Inc, Kyunggi-do, Korea; www.standardia.com
Visitect Syphilis	Omega Diagnostics Ltd, Scotland, UK; www.omegadiagnostics.co.uk
Round 2	
Syphicheck-WB (new version)	Qualpro Diagnostics, Goa, India; www.tulipgroup.co
Bioline Syphilis 3.0 (new manufacturer)	Pacific Biotech Co, Ltd, Petchaboon, Thailand
Syphilis^{onsite} Rapid Screening Test	CTK Biotech, Inc, San Diego, USA

Data analysis

Sensitivities and specificities were calculated relative to the reference standard TPPA or TPHA results obtained for each serum specimen at each site and validated by the reference centres. Sample size calculations showed that the use of 600–800 sera, of which 50% are positive, would allow estimation of the sensitivity and specificity of the test with a 95% confidence interval of $\pm$5%. No discrepant analyses were undertaken. The Breslow-Day test for homogeneity was used for determining site to site variation and κ values were calculated for each test as a summation of the overall performance (combined correlation of test sensitivity and specificity) of each test against the reference standard for all sites. A κ value of $\geqslant$0.75 is considered excellent.

Interobserver variability was calculated as the number of tests for which different results are obtained by two independent different readers, divided by the number of specimens tested.

RESULTS

Round 1

Owing to insufficient quantities of characterised sera at some sites, the final results were available for 789 sera, 399 of which were TPHA or TPPA positive. (The requirement for biological false positive sera (TPPA−, RPR +) was a particular problem for some sites.) The sensitivity and specificity of each test for each site is shown in table 4. The overall sensitivity and specificity for the combined results with 95% confidence intervals are also shown.

The Fujirebio Espline, Abbott Determine, and Standard Bioline tests showed the highest sensitivity (97.7%, 97.2% and 95%, respectively; table 4). The sensitivities of these three tests were not significantly different from each other but were significantly different from those of the Diesse, Omega and Qualpro tests ($p<0.03$).

The Omega Visitect and the Qualpro Syphicheck tests showed the highest specificity (98% and 97.7%, respectively; table 4). These are not significantly different from each other but were significantly higher than the other four tests.

For estimation of overall test performance, the κ value was used. This determined the combined correlation of test sensitivity and specificity for all the sites against the reference standard. A κ value of 0.75 is considered excellent. Thus all the rapid tests had excellent correlation with the reference standard tests at each site, with κ values for the initial six tests ranging from 0.84–0.95.

Site-to-site variation for each test was measured using the Breslow-Day test for homogeneity of odds ratios. The three tests that gave the most variation were the Omega Visitect, the Abbott Determine, and the Diesse Syphilis Fast tests, with p values of 0.03, 0.0086 and 0.0002, respectively. There was no significant difference between malaria endemic and malaria-free sites with respect to test specificity.

Test results were stable after one hour for the Abbott Determine, Fujirebio, Qualpro Syphicheck and Omega Visitect tests with five or less results different from the original results. The Standard Bioline had 12 results different from the original with most of these becoming false-positive after one hour. The Diesse Syphilis Fast was affected by drying, making reading difficult after an hour. By the second reading, 22 results were different from the original test result, turning from negative to false-positive.

The scores for operational characteristics are summarised in table 5. The Abbott test obtained the best score (7.5 out of 10) with the Omega Visitect, Qualpro Syphicheck, Fujirebio Espline and Standard Bioline all less than 10% different from each other (6.5–7.1 out of 10). The Diesse Syphilis Fast test scored lowest (4.3 out of 10) on technical complexity and ease of interpretation.

The results for test reproducibility are summarised in table 6. Overall, the variability was low. The maximum observed was 10% for the Omega test for operator-to-operator variation in the

Table 3 The major features of the evaluated tests

Test	Design	Cassette mounted	Specimen utilised			Time to result (min)	Extra supplies*	Shelf life/storage temp
			Blood	Plasma	Serum			
Determine Syphilis	Lateral flow	No	Yes	Yes	Yes	5–20	Yes	24 months/2–30˚C
Syphilis Fast	Latex agglutination	Not applicable	No	No	Yes	8	Yes	18 months/reagents; 6 months after reconstitution
Espline TP	Lateral flow	Yes	No	Yes	Yes	15	Yes	9 months/2–10˚C
Syphicheck-WB	Lateral flow	Yes	Yes	Yes	Yes	15	No	18 months/4–30˚C
SD Bioline Syphilis 3.0	Lateral flow	Yes	Yes	Yes	Yes	5–20	Yes	18 months/room temp
Visitect Syphilis	Lateral flow	Yes	Yes	Yes	Yes	15	No	24 months/4–30˚C
Syphicheck-WB (new version)	Lateral flow	Yes	Yes	Yes	Yes	15	No	18 months/4–30˚C
Bioline Syphilis 3.0 (new manufacturer)	Lateral flow	Yes	Yes	Yes	Yes	5–20	No	Unknown/4–30˚C
Syphilis^{onsite} Rapid Screening Test	Lateral flow	No	No	Yes	Yes	5–10	No	Unknown/4–30˚C

*In all cases, where additional equipment was required it consisted of handheld micropipettes and micropipette tips.

Table 4 Performance of rapid diagnostic tests for syphilis in round 1

Sites	Determine Syphilis TP Abbott Labs		Syphilis Fast Diesse Diagnostica		Espline TP Fujirebio Inc		Syphicheck-WB Qualpro Diagnostics		SD Bioline Syphilis 3.0 Standard Diagnostics		Visitect Syphilis Omega Diagnostics	
	Sens (%)	Spec (%)	Sens (%)	Spec (%)	Sens (%)	Spec (%)	Sens (%)	Spec (%)	Sens (%)	Spec (%)	Sens (%)	Spec (%)
Moscow, Russian Fed.	100	100	72	98	100	83	80	100	92	98	76	100
Birmingham, USA	88	92	57	92	98	88	82	94	94	90	80	94
Port au Prince, Haiti	100	98	100	92	98	100	90	98	100	100	90	100
Nanjing, China	98	93	79	89	94	93	77	95	89	96	81	93
Mwanza, Tanzania	96	94	94	90	98	100	80	100	94	94	82	100
Colombo, Sri Lanka	100	98	96	100	98	96	88	100	96	100	92	100
Durban, South Africa	96	90	94	96	96	94	82	100	94	98	86	100
Fajara, The Gambia	100	88	94	86	100	92	96	94	100	84	92	96
Overall results	97.2	94.1	86	92.8	97.7	93.4	84.5	97.7	95	94.9	85	98
95% CIs	95.6 to 98.8	91.8 to 96.4	82.5 to 89.4	90.3 to 95.4	96.3 to 99.2	90.9 to 95.8	80.9 to 88.0	96.2 to 99.2	92.8 to 97.1	92.7 to 97.1	81.4 to 88.5	96.5 to 99.4
Homogeneity* p values	0.0086		0.0002		0.2529		0.1427		0.1132		0.03	
κ† (95% CI)	0.95 (0.93 to 0.97)		0.87 (0.84 to 0.90)		0.95 (0.93 to 0.97)		0.84 (0.80 to 0.87)		0.9 (0.87 to 0.94)		0.85 (0.82 to 0.89)	

*The Breslow-Day test for homogeneity of odds ratios was calculated to determine the extent of site-to-site variation of test performance.
†The κ value for each test is a summation of the overall performance of each test against the reference standard for all sites. A value of 0.75 or greater is considered excellent.
CI, confidence interval; Sens, sensitivity; Spec, specificity.

reference laboratories. However, this test performed well for this parameter at the evaluation sites. Results of the first round of evaluations have been posted on the SDI website.

Round 2

A total of 600 sera from the six laboratory sites were used to evaluate a further three rapid syphilis tests; 299 were reference standard positives and 301 were reference standard negatives. The serum panels were not identical to those used in round 1. The performance data for these tests is summarised in table 7.

The CTK Syphilis On Site Rapid Q and the Qualpro Syphicheck WB tests showed the highest sensitivities (96.3% and 95.3%, respectively). These values were not significantly different from each other but there were significant differences between the sensitivities of the Bioline Syphilis anti-TP and CTK Syphilis On Site Rapid. The Bioline Syphilis Anti-TP test showed the highest specificity (97%). The only marginally significant difference was between the Qualpro Syphicheck WB and the Bioline Syphilis anti-TP. All other performance comparisons were not significantly different. The κ values for the three tests ranged from 0.89–0.92. Similarly, all three tests gave excellent values for the Breslow-Day test for site to site variation.

In round 2, the reproducibility testing was restricted to measuring lot-to-lot variations for two different lots using 20 serum samples. There was one discrepant result with the Qualpro Syphicheck-WB, two with the Bioline Syphilis anti-TP test and five with CTK's Syphilis Rapid Screening Test.

In result stability testing after one hour the Qualpro test showed seven changes in result; five negative tests became false positive. The Bioline and the CTK tests were less stable with 12 and 14 changes, respectively. (In the Bioline test, nine became false positives and in the CTK test 10 became positive.)

Since all the lateral flow tests in round 1 were found to be simple in operation and the three tests in round 2 were essentially identical, the site technicians were not asked to score the operational characteristics of the tests in round 2. Given that these rapid tests are intended to be used in field settings, it was decided that the ease of operations would be better assessed in field settings.

DISCUSSION

Most of these rapid tests utilise one or more similar recombinant treponemal antigens, and it is likely that small differences in antigen concentration, detection system and the volume of serum used account for the small variations observed in performance. The nine rapid tests evaluated all showed good performance in terms of sensitivity and specificity relative to the reference standard TPHA or TPPA tests using archived serum specimens. As has often been noted in such trials of diagnostic tests, there was a trend towards an inverse relationship between sensitivity and specificity. Thus, for a diagnosis such as syphilis that can carry a risk of stigma for the patient, there may be an advantage in using a high specificity test to confirm diagnosis with a high sensitivity assay. There would also be an advantage for interpretation of disease status if the tests could be combined with a non-treponemal antibody assay. Although the RPR is simple enough to be performed under field conditions, it requires some laboratory equipment and refrigeration. In addition, there are problems with reading in differentiating between weak positive and negative reactions. Therefore, an anti-cardiolipin antibody test in a lateral flow format would be a substantial advance.

The multicentre design of this trial also allowed inter-site variation to be assessed. This also showed that the tests performed well in geographically distinct areas with differing

Table 5 Operational characteristics of rapid diagnostic tests for syphilis

Operational characteristic	Determine Syphilis TP Abbott Labs Mean score	Syphilis Fast Diesse Diagnostica Mean score	Espline TP Fujirebio Inc Mean score	Syphicheck-WB Qualpro Diagnostics Mean score	SD Bioline Syphilis 3.0 Standard Diagnostics Mean score	Visitect Syphilis Omega Diagnostics Mean score
Clarity of kit instructions	2.625	1.875	1.875	2.125	2.125	2.5
Technical complexity	2.875	1.125	2.625	1.875	2.375	2
Ease of interpretation of results	2	1.25	2.125	1.875	2	1.625
Equipment required but not provided	0	0	0	1	0	1
Total score	7.5/10	4.3/10	6.6/10	6.9/10	6.5/10	7.1/10

The technicians were asked to give, for each test, a score out of 3 for each of the first three characteristics, with 3 being the best, and then a score of 1 if the test does not require any additional equipment.

Table 6 Test reproducibility

	Determine Syphilis TP Abbott Labs	Syphilis Fast Diesse Diagnostica	Espline TP Fujirebio Inc	Syphicheck-WB Qualpro Diagnostics	SD Bioline Syphilis 3.0 Standard Diagnostics	Visitect Syphilis Omega Diagnostics
Lot-to-lot variation*	1/50	4/50	3/50	0/50	3/50	1/50
Day-to-day variation†	0/45	3/45	1/45	1/45	0/45	3/45
Operator-to-operator at reference labs‡	1/40	0/40	0/40	3/40	1/40	4/40
Operator-to-operator at sites	4/789	20/789	0/789	6/789	0/789	5/789

Values given as number of discordant results/total number of tests performed.
*Two lots of rapid tests performed using the same 25 sera.
†Nine sera tested on 5 days.
‡Ten sera run by 2 operators at each of 2 reference laboratories.

patient populations, despite inevitable variations in the sera selected for each panel, test performance and reading, and the subjective nature of result interpretation.

These studies were able to detect significant variations in the stability of the results after one hour. This measurement was made to anticipate the use of these tests in a busy clinic setting where staff may not be able to read the tests at the manufacturers designated time. The Syphilis Fast latex agglutination assay particularly should be read after the recommended 8 minutes but, given its speed, this was not perceived as a problem. Similarly, all the lateral flow tests were perceived as very easy to use. The Syphilis Fast latex agglutination test was found to be marginally more difficult to do as sometimes the stick for stirring the reaction broke and it is also more difficult to interpret, especially when the reaction dried before the designated reading time. None of the tests were technically complex to perform and all tests were considered suitable for field use. The true evaluation of the operational characteristics of the tests will emerge from the subsequent field evaluations.

The SDI ad hoc expert working group considered which tests should be further evaluated in field settings after round 1 and felt that it was difficult to select one or two tests based on test performance characteristics alone. The final consensus was that the four rapid tests in round 1 that can use whole blood and do not require refrigeration should be taken forward to SDI field trials (see Mabey *et al* in this supplement). The three tests evaluated in round 2 also warrant field testing.

Given the simplicity and low cost of these rapid tests, it is hoped that they may prove to be effective tools in the control of syphilis and for screening pregnant women to prevent congenital syphilis in primary health care settings.

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Table 7 Performance of rapid diagnostic tests for syphilis in round 2

Site	Syphicheck WB Qualpro Diagnostics Sens (%)	 Spec (%)	Bioline Syphilis Anti-TP Pacific Biotech Co Ltd Sens (%)	 Spec (%)	Syphilis On Site Rapid CTK BioTech Inc Sens (%)	 Spec (%)
Moscow, Russia	92	98	92	100	96	98
Birmingham, USA	90	100	92	100	92	100
Port au Prince, Haiti	96	90	94	94	92	94
Nanjing, China	100	92	86	98	98	94
Mwanza, Tanzania	94	94	100	96	90	100
Fajara, Gambia	100	88	100	90	100	86
Overall values	95.3	93.7	92.2	97	96.3	94.6
95% CIs	92.5 to 99.9	91.3 to 99.9	88.9 to 95.1	95.1 to 98.9	93.8 to 99.9	92.5 to 99.9
Homogeneity (p value)	0.4451		0.3552		0.2911	
κ (95% CI)	0.89 (0.86 to 0.93)		0.9 (0.86 to 0.93)		0.92 (0.89 to 0.95)	

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Immunochromatographic strip syphilis tests had high sensitivity and specificity for detection of T. pallidum antibodies in samples from patients seen at antenatal and STI clinics.

Accelerating worldwide syphilis screening through rapid testing: a systematic review.
Tucker JD, Bu J, Brown LB, Yin Y-P, Chen X-S, Cohen MS.
Lancet 2010;10:381-86.

Summary:

Question
What are the test characteristics of immunochromatographic strip (ICS) syphilis tests that detect T. pallidum antibodies? Do syphilis clinical stage of infection, non-treponemal syphilis titer, HIV co-infection, clinic type, specimen type, or manufacturer affect test characteristics?

Design
Research papers that retrospectively analyzed ICS syphilis test characteristics at STI clinics and antenatal clinics were included. Study criteria included use of finger stick blood or serum for testing, use of at least one treponemal reference comparator test performed on all samples, inclusion of total number of patients tested, provision of the number of positive and negative test results, and use of tests that met the ASSURED criteria. Studies that used archived blood samples instead of samples collected and immediately tested were excluded. If several studies used the same population, only the most recent study or the study with the largest sample was included.

Participants

Description of Tests and Diagnostic Standard
Two-by-two tables were constructed for each of the studies. Investigators were contacted if further data were needed. Sensitivity and specificity were calculated. The type of reference test was recorded. The diagnostic odds ratio (DOR) was used to obtain an overall measure of accuracy. The DOR is the odds of a positive test result in patients with the disease (true positive rate) divided by the odds of a positive test result in patients without disease (false positive rate).

Main Outcome Measures

Main Results
Fifteen studies representing 23,055 individual test results were selected. All were retrospective cross-sectional surveys. Thirteen of 15 studies were conducted in low or middle income countries; 12 were conducted in urban health centers. Eight studies were at antenatal clinics and 7 were at STI clinics. Four, 3, 2, and 3 studies used finger-prick, whole blood other than finger-prick, serum, and several specimen types, respectively. Three studies included HIV positive patients; one included test characteristics in the basis of HIV status. Three studies calculated test characteristics by syphilis titer, 3 by clinical syphilis stage.

The ICS tests used were Determine Syphilis TP (Abbott Laboratories), Visitect Syphilis (Omega Diagnostics), Syphicheck-WB (Qualpro Diagnostics), SD Bioline 3.0 (Standard Diagnostics), Rapid Syphilis Test (Quorum Diagnostics), Syphilis Ultra Rapid Test Strip (Acon), Phoenix Biotech Trep-Strip IV (Phoenix Bio-Tech Corp.), and Guardian Biosciences One Step (Testmedica Diagnostics, Guardian Biosciences). Five studies used both treponemal and non-treponemal tests as the reference standard. There were no significant differences in ICS sensitivity and specificity between studies that used only treponemal reference tests and those that used a combined reference. The prevalence of syphilis ranged from 0.3 to 53.0%.

The sensitivity and specificity of each ICS syphilis test are shown in the table by test name, clinic type, and study location. The median sensitivity and specificity were 86% and 99%, respectively, at both clinic types. Two studies reported higher sensitivity when serum samples were used compared to finger-stick blood. There was limited data that the sensitivity of rapid testing is the same in HIV positive compared to HIV negative individuals and in patients with high titer syphilis infections. The median DOR was 737 and ranged from 59 to 23,733; 11 studies had DORs greater than 1000. The ICS syphilis tests had greater than 80% positive predictive value of syphilis when the prevalence was greater than 0.3%.

Sensitivity and specificity of ICS syphilis tests by test name, clinic type, and study location

Test name	Clinic type	Study location	Sensitivity	Specificity
Determine	Antenatal	South Africa	85.7	90.9
		Mexico	100	100
		Vietnam	100	98.6
		Tanzania	59.6	99.4
		Bolivia	91.8	98.5
		Bolivia	98.0	99.8
	STI	Italy	95.0	97.7
		Brazil	88.5	97.9
		China	81.9	99.4
		Haiti	72.5	98.5
		USA	88.0	100
Visitect	Antenatal	Tanzania	75.0	99.8
	STI	Brazil	57.0	99.0
		Brazil	96.1	98.5
		China	73.5	99.7
		Haiti	72.7	99.1
Syphicheck-WB	Antenatal	Tanzania	78.6	99.1
	STI	Brazil	84.3	99.6
		China	64.0	99.7
		Haiti	80.5	97.8
SD Bioline 3.0	Antenatal	Tanzania	85.7	98.1
		Mozambique	86.0	96.8
	STI	Brazil	88.2	99.4
		China	87.6	99.4
		Haiti	100	98.3
Rapid Syphilis Test	Antenatal	Gambia	75.0	95.2
Syphilis Ultra Rapid	STI	Bangladesh	94.4	92.6
Guardian Biosciences One Step	STI	USA	72.0	100
Phoenix Biotech Trep-Strip IV	STI	USA	70.0	100

Authors' Conclusions

This review concludes that ICS syphilis tests have a high sensitivity and specificity when testing specimens from patients seen at STI and antenatal clinics. The sensitivity and specificity of the ICS syphilis tests were similar to those of conventional non-treponemal tests. ICS syphilis tests are simple, rapid, and inexpensive. However, disadvantages include false negative results due to suboptimum sensitivity, persistent positive tests, and lack of titers to follow, which are important for clinical management algorithms. Because unnecessary treatment may occur in areas with many patients treated for syphilis, subsequent clinical management will require a non-treponemal test.

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Rapid Immunochromatographic Test for Syphilis

Dear Editor,

Confirmatory test for syphilis such as fluorescent treponemal antibody-absorption test (FTA-ABS), *Treponema pallidum* immobilization test (TPI), *Treponema pallidum* haemagglutination (TPHA) test are technically demanding and not available in most developing country settings outside of reference laboratories. By far, TPHA test has the sensitivity and specificity almost similar to that of FTA-ABS test and is considered as an attractive alternative to the expensive and technically demanding FTA-ABS and TPI test for serodiagnosis of syphilis,[1] however, simple rapid treponema specific tests are urgently required for the use in primary health care, private settings, and for high risk patients who are often untraceable. Therefore, one step dipstick test for syphilis namely Syphicheck kit was used in our hospital for rapid confirmation.

Our study group consisted of 300 pregnant females randomly selected from antenatal clinic (ANC) and 300 high risk patients attending dermatology department of Post Graduate Institute of Medical Sciences, Rohtak, with genital ulcers. Previous history of syphilis was excluded. All samples were initially screened qualitatively and quantitatively by Venereal Disease Research Laboratory (VDRL) test using antigen obtained from Serology Laboratory, Kolkata by standard protocols. All VDRL positive samples were followed by one step dipstick test for syphilis (Syphicheck) obtained from Qualpro diagnostics, Goa, India (Rs. 1375/- for 25 tests). Test was done and interpreted according to manufacturer's instructions.

Out of 300 ANC cases 210 (70%) were both VDRL and Syphicheck negative. Among ANC only two cases were Syphicheck positive out of which one was VDRL positive in titre R4 and other was VDRL negative. Also Syphicheck detected 88 (29.3%) biological false positive cases (Table).

Out of 300 genital ulcer cases from dermatology department, 81 (27%) were both VDRL and Syphicheck negative. A total of 216 cases were diagnosed as syphilis on clinical and serological grounds; out of which 207 (122 titre >R8, 77 titre R1-R8 and 8 among non-reactive VDRL) were Syphicheck positive while rest 9 (6.8%) cases were VDRL reactive in titre more than R8 but Syphicheck negative and compatible with clinical illness. These nine cases were Syphicheck positive after 15 days. Only three (1.7%) gave biological false positive results and these were still Syphicheck negative after 15 days (Table).

Table: Results of VDRL and Syphicheck

VDRL titre	Syphicheck	Antenatal	Genital ulcers	**Total (%)**
>R8 (n=131)	Positive	0	122	**122 (93.1)**
	Negative	0	9	**9 (6.8)**
R1-R8 (n=169)	Positive	1	77	**78 (46.1)**
	Negative	88	3	**91 (53.8)**
Non-reactive (n=300)	Positive	1	8	**9 (3)**
	Negative	210	81	**291 (97)**
Total		**300**	**300**	**600**

Correlation between negative VDRL test and Syphicheck was 97%. With VDRL titre >R8, 93.1% samples were Syphicheck positive, however, with VDRL titre R1-R8, 46.1% were Syphicheck positive. Also Syphicheck detected 9 (3%) cases which were VDRL negative (Table).

VDRL or Rapid Plasma Reagin (RPR) test is most widely used screening test for syphilis in India as they are rapid and economical. Because neither of these tests assay for syphilis - specific antibodies, there are problems associated with both their specificity and sensitivity. In early primary disease antilipoidal antibodies may not have developed and in late syphilis (late latent and tertiary) upto 30% of individuals may lack antilipoidal antibodies. In addition, because a variety of

conditions (e.g., lupus and increased age) lead to antilipoidal antibodies and false positive results,[2] hence a rapid, effective, practical confirmatory test is often required for diagnosis and treatment. Syphicheck is a one step rapid self performing test which can qualitatively detect presence of IgG and IgM class of treponema specific antibodies in serum or plasma within 15 minutes. It uses the principle of immunochromatography, a unique two site immunoassay on membrane. Positive results indicate a past or present infection, however a positive result should always be evaluated in correlation with clinical condition before arriving at final diagnosis. Manufacturers have reported 100% correlation between syphicheck and standard TPHA.[3] Low levels of antibodies to *Treponema pallidum* at a very early primary stage of infection can give a negative result, nine such cases were detected in our study. However, Syphicheck in our study proved to be very helpful to exclude biological false positive results as well as to institute therapy in low titre (R1-R8) VDRL positive cases. As the results are available in 15 minutes and reproducible, it is better than standard TPHA which takes at least 3-4 hours.

Therefore, we conclude that syphicheck is a simple, rapid, point of care type treponema specific test suitable for use in primary health care settings for the diagnosis of syphilis. Evaluating the performance of rapid tests, their utility in a disease control programme and acceptability to patients and health care providers will improve the diagnosis of syphilis in primary health care settings in developing countries and reduce over treatment.

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Rapid tests for diagnosing syphilis: validation in an STD clinic in the Amazon Region, Brazil

Testes rápidos para diagnóstico de sífilis: validação em clínica de DST na Região Amazônica, Brasil

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Abstract

Correct, early diagnosis and treatment of syphilis are essential for its control. Traditional diagnostic tests depend on specialized equipment, installations, and human resources. In the search for quick, simple tests, a project was conducted on the validation and reproducibility of four different tests, previously assessed by WHO reference laboratories. The study also verified the operational characteristics and acceptance by patients and health professionals. Samples obtained at an STD clinic were from 541 and 248 patients with 51 and 52 positive results according to FTA-Abs (gold standard) in studies 1 and 2, respectively. The sensitivity varied from 84 to 96%, specificity was greater than 98%, and PPV was > 90%. Reproducibility was > 97% and kappa index 0.94, comparing the results obtained by different health workers. The tests took less than 20 minutes to perform, and more than 90% of patients agreed to wait up to two hours for the results. The tests presented the necessary requirements for use in diagnosis of syphilis, thus providing an additional option for controlling this disease.

Syphilis; Sexually Transmitted Diseases; Reproducibility of Results; Diagnosis

Introduction

Syphilis is one of the primary causes of adverse events during pregnancy, aside from being one of the most prevalent sexually transmitted diseases (STD) [1]. It has a global distribution, but it affects developing countries in Africa, Asia, Latin America, and the Caribbean with greater intensity. The World Health Organization (WHO) estimated that in 1999 there were 12 million new cases of syphilis of which more than 75% of them reported in poor countries, with a tendency of continuous increasing in the last years [2,3,4,5,6,7].

Fetal deaths and morbidity through congenital syphilis can be prevented if identified and treated adequately in the mothers infected before the beginning of the third trimester. Nevertheless, unfortunately, the majority of pregnant women infected by syphilis are asymptomatic and it is only possible to identify them if they are included in programs of prevention and control and perform serological tests.

In the case of adults, the algorithms designed to manage genital ulcer syndrome include syphilis as one of the most probable causes, making possible a proper treatment of this pathology. Meanwhile, most of the time, patients are totally asymptomatic and, not being diagnosed correctly, are not treated, and have important complications related to gestation and childbirth, facilitating the sexual transmission of HIV [8,9].

Many developed countries have established tracking activities in their syphilis control programs. They have been using tests called nontreponemal tests, like the Venereal Disease Research Laboratory (VDRL) and the Rapid Plasma Reagent test (RPR). Aside from being easy to carry out, their low cost, and their relatively rapid results, they cannot be applied in all the country's primary health care units in virtue of the fact they require refrigeration or other laboratory facilities like electricity, or a centrifuge, for example. Together with these factors, when these tests are employed, principally on pregnant women, up to 28% percent of positive results are biologically false reactions and. even in ideal conditions require additional tests of greater specificity (treponemal tests, such as the fluorescent treponemal antibody absorption test-FTA-Abs, the microhemagglutination test and hemoagglutination test for *Treponema pallidum* antibodies-TPHA, etc.) [1,10].

In Brazil, there has been an investment in the scale-up of the primary health care network by means of the implementation of the Family Health Program (FHP) and the work of community health agents, and an ample network of diagnostic laboratories has been installed. Even so, the distribution of these services is unequal, and reflects the different realities of regions and subregions.

For these reason, the Special Programme for Research and Training in Tropical Disease (TDR)/Sexually Transmitted Diseases Diagnostics Initiative (SDI) of the WHO stimulates the search for new tests for diagnosing syphilis that comply with the necessary requisites: rapid results (less than 15 minutes), ease of use by professionals who work directly with the patients, not requiring the resources of traditional laboratories, being stable at room temperature, possessing good sensitivity and specificity, and low cost. Thus they can be used on large scale in primary health care facilities in developing countries to adequately identify and treat the greatest number of infected people.

More than twenty commercially available rapid tests exist [11,12,13,14]. The SDI program first selected six of the most promising ones for evaluation of their performance, utilizing serum banks in eight countries (the United States, Russia, China, Sri Lanka, Tanzania, Gambia, South Africa, and Haiti). In this research 789 samples were used, and sensitivity values of 85-98%, and a specificity of 93-98% were obtained, when they were compared with test of hemogglutination and agglutination in particles against *T. pallidum* (TPHA and TPPA) as a gold standard [15].

Afterwards, in the year 2003, four different sites in Asia (China), Africa (South Africa), and America (Haiti and Brazil) were selected to realize and validate their operational characteristics and acceptance by the patients and health professionals that work with the care of patients living with STD. For this stage, four rapid tests were selected, fundamentally based on their common characteristics, of using whole blood samples, serum or plasma, and not requiring refrigeration. The validation of the four rapid tests at a specialized clinic in the city of Manaus, Amazonas State, Brazil, is discussed in this article.

The objective of this study was to evaluate the operational characteristics (validation and reproducibility) of four diagnostic tests proposed, under the denomination of "rapid tests for syphilis". Also evaluated were the feasibility and acceptability of their use by health professionals who work directly in caring for cases of suspected syphilis or other STD, as well as for the possibility of this clientele to get the results of the test before terminating the consultation.

Material and methods

Comparative validation research of four rapid treponemic tests for the diagnosis of syphilis

Whole blood samples from patients who presented themselves in a consecutive manner at a clinic specialized in STD in Manaus were used, and serum samples from the same patients in the laboratory. The FTA-Abs test was used as a "gold standard".

The tests validated were: (1) Syphicheck-WB (Qualpro Diagnostics, India); (2) SD Bioline Syphilis 3.0 (Standard Diagnostics, South Korea); (3) Determine Syphilis TP (Abbott Laboratories, U.S.A.); (4) VisiTect Syphilis (Omega Diagnostics, Scotland). All these producing companies donated the quantities necessary for the validation tests to the WHO.

During the research all the patients were treated based on the results of routine clinical exams (VDRL and FTA-Abs).

The validation stage activities were carried out at four different sites: Asia (China), Africa (South Africa), and America (Haiti and Brazil). The following phases were carried out at a specialized clinic in Manaus:

- The investigation was divided into two stages, because the tests were delivered at different times. In the first part the Syphicheck-WB and the SD Biolina Syphilis tests were validated (study period 1). Afterwards the tests of VisiTect Syphilis

and Determine Syphilis TP were completed (study period 2).

- The participating patients were recruited from among those who presented themselves at the specialized STD clinic in a voluntary and consecutive manner, starting on March 1, 2003, being randomly selected for study 1, and starting in on January 15, 2004 for study 2.
- All patients signed an Informed Consent Form stating that their participation in the research was voluntary.
- Criteria for inclusion were men and women over 18 years old with no previous history of syphilis, while criteria for exclusion were those under 18 years old and/or with previous history of syphilis or positive serology for syphilis.
- Two teams were prepared for the execution of the exams: (a) professionals who work directly providing health care in clinics (nurses and paramedics), and (b) biochemists and regular field laboratory technicians.
- From each participant in the studies 10ml of venous blood was drawn, in vacutainer tubes. From this total 1ml was immediately used for evaluating the rapid tests in the clinic, while the rest was sent to the laboratory for centrifuging and the execution of the same rapid tests by the laboratory team, in the same way that is done when carrying out routine tests (VDRL, serum anti-HIV etc.) and the gold standard exam (FTA-Abs). An aliquot of 2ml was stored in a freezer at -70ºC for future tests and quality control to be carried out by the reference laboratories of SDI/WHO in all samples that tested positive, and 10% of those that tested negative.
- Identification, epidemiological data, as well as test results from each patient were recorded on a form and double entered into a database using the Epi Info 6.4 software (CDC; Centers for Disease Control and Prevention, Atlanta, U.S.A.).
- The sample size was determined through multicentric protocols, according to each site characteristics, and had to incorporate a number of patients large enough to reach fifty positive patients using the gold standard test (FTA-Abs). In this study project, the sample was 541 people for study 1, and 248 for study 2. A possible explanation for the difference in sample size was the fact that the period when study 2 was being carried out coincided with an increment of sex workers seeking treatment.
- The samples of studies 1 and 2 were analyzed to verify if they were able to minimize the random error. It is known that the appropriate sample size for evaluating a specific test is determined by the formula as follows [16]: $N = Z^2 [p (1-P)]/D^2$ and this applied to the syphilis prevalence obtained in the patient group (9.4% and 21.1%) has enough power to arrive at a maximum acceptable error of 3% and 5% in each of the samples, respectively.
- For validation of the tests, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated with their respective confidence interval of 95% (95%CI). To evaluate the reproducibility of each test, global agreement rates and kappa indices were employed. To investigate the existence of statistically significant differences between the validation results for each test, chi-square tests for proportional differences were carried out (with Yates correction).
- To get opinions about the operational qualities of each test an opinion questionnaire was created and filled out by the 13 professionals responsible for the execution of rapid test evaluations.

Results

The validation of the tests was carried out in two different time stages in distinct samples of patients, each of them used to evaluate a pair of tests. The comparison of the validation results, therefore, cannot be extended to the four tests together and only can be referred to by separate pairs, denominated studies 1 and 2. The same professionals worked on all four tests,minimizing statistical bias.

In the first study to validate the tests Syphicheck-WB and SD Bioline Syphilis, a total of 541 consecutive patients were studied during the period from March 1 through June 14, 2003 until 51 cases had tested positive for FTA-Abs. In the second study to validate the tests VisiTect Syphilis and Determine Syphilis TP a total of 248 consecutive patients were studied from January 15 through June 25, 2004 until 52 cases had tested positive for FTA-Abs.

The principal characteristics of the patients in the two samples studied are presented in Tables 1 and 2.

The epidemiological variables of the patients included in the samples under analysis and the laboratory results of the two studies were recorded on registration forms and in the laboratory ledgers.

The reproducibility of the tests, when done by the project teams (patient care team and laboratory team) were evaluated through the percentage of agreement and the kappa coefficient. For the purpose of this study when these indicators had values of more than 0.80 the reproducibility was considered good and when they were greater than 90% and 0.90, as excellent.

Table 1

Characteristics of the sample employed for the validation of rapid tests at a specialized STD clinic in. Manaus, Amazonas State, Brazil

Characteristics	Study 1 Syphicheck-WB and SD Bioline Syphilis	Study 2 VisiTect Syphilis and Determine Syphilis TP
Period the study was carried out	May to June/2003	January to May/2004
Sample size	541	248
Positive FTA-Abs	51	52
Prevalence of syphilis (%)	9.4	21.0
Average age (years)	24	25
Percentage of men	72	43.7
Percentage of women	28	56.3

Table 2 shows the prevalences of syphilis according to the principal reasons for patient consultation. In the two groups studied, suspicion of latent syphilis and patients directed to the service under suspicion of secondary syphilis presented the highest prevalence values: 66.6% and 66.6%, 76.9% and 62.5%, respectively. Altogether, all the groups included had high or moderately syphilis prevalence rates, 9.4% in the first period and 21% in the second, as was to be expected at a STD specialized clinic.

The performance of the four tests, relative to their principal validation indicators are shown in Table 3. However, as was explained earlier, these results can only be analyzed independently in relation to the two pairs that were analyzed each time (study 1 and study 2).

In both the studies, the four rapid tests were compared with the FTA-Abs and the VDRL (routinely employed in health service). The tests carried out by the professionals working in the clinic were compared to those carried out in the laboratory by professionals more experienced in this type of work (Table 3).

The validation of sensitivity compared to FTA-Abs showed that in study 1 SD Bioline Syphilis (88.2% and 90%) had slightly higher values than Syphicheck-WB (84.3% and 90%) whether they were performed by the clinical professionals or by the laboratory professionals. However the 95CI% intervals of both tests overlap and, therefore, it cannot be stated that the sensitivity of one test is higher than the sensitivity of the second one.

Table 2

Cases studied and number and percentage of positives for FTA-Abs according to the main reason for visiting a specialized STD clinic in Manaus, Amazonas State, Brazil.

Reason for visiting the STD clinic	Study 1 Syphicheck-WB and SD Bioline Syphilis			Study 2 VisiTect Syphilis and Determine Syphilis TP		
	n	FTA-Abs positives	%	n	FTA-Abs positives	%
Vesicular genital ulcers	32	1	3.1	6	1	16.7
Non-vesicular genital ulcers	37	6	16.2	9	4	44.4
Suspicion of secondary syphilis	3	2	66.6	8	5	62.5
Suspicion of latent syphilis	21	14	66.6	13	10	76.9
Contact with cases of syphilis	17	4	23.5	8	2	25.0
Other STD	367	20	5.4	97	4	4.1
Spontaneously seeking anti-HIV test	20	2	10.0	51	18	35.3
Other	44	2	4.5	56	8	14.3
Total	541	51	9.4	248	52	21.0

Table 3

Performance of each one of the rapid tests for syphilis in comparison with FTA-Abs at a specialized STD clinic in Manaus, Amazonas State, Brazil.

Test	Sensitivity	Specificity	PPV	NPV
VDRL				
Study 1	80.4	97.4	75.9	98.0
Study 2	65.4	97.9	89.5	91.4
SD Bioline Syphilis				
Ambulatory	88.2	99.4	93.8	98.8
Laboratory	90.2	99.4	93.9	99.0
Syphicheck-WB				
Ambulatory	84.3	99.6	95.6	98.4
Laboratory	88.2	99.6	95.7	98.8
VisiTect Syphilis				
Ambulatory	96.2	98.5	94.3	99.0
Laboratory	96.2	98.5	94.3	99.0
Determine Syphilis TP				
Ambulatory	88.5	97.9	92.0	97.0
Laboratory	88.5	97.9	92.0	97.0

PPV: positive predictive value; NPV: negative predictive value.

Table 4

Reproducibility of rapid tests for syphilis carried out in a specialized STD clinic in Manaus, Amazonas State, Brazil.

Tests evaluated/Work teams	Concordance (%)	Kappa index
SD Bioline Syphilis (A) vs. SD Bioline Syphilis (L)	99.0	0.99
Syphicheck-WB (A) vs. Syphicheck-WB (L)	99.0	0.97
VisiTect Syphilis (A) vs. VisiTect Syphilis (L)	100.0	1.00
Determine Syphilis TP (A) vs. Determine Syphilis TP (L)	100.0	1.00
SD Bioline Syphilis (A) vs. Syphicheck-WB (A)	99.4	0.96
SD Bioline Syphilis (L) vs. Syphicheck-WB (L)	99.6	0.98
VisiTect Syphilis (A) vs. Determine Syphilis TP (A)	97.9	0.94
VisiTect Syphilis (L) vs. Determine Syphilis TP (L)	97.9	0.94

A: ambulatory team; L: laboratory team.

The specificity of the two rapid tests was similar and, in general, with very high values, higher than 98% and the VPP showed a slightly better performance of Syphicheck-WB, but both with a very similar 95%CI.

The analysis of reproducibility or reliability was carried out through a comparison of the results obtained for each test when it was executed by clinic professionals and when it was executed by the laboratory team, employing for this the overall agreement indicators, or the percentage of agreement or the kappa index.

The reproducibility achieved by the SD Bioline Syphilis and Syphicheck-WB proved excellent when executed by both teams, with agreement values of 99% and kappa index > 0.95% for both tests (Table 4).

In study 2, the VisiTect Syphilis test showed sensitivity values of 96.2% and 96.2%, specificities of 98.5% and 98.5%, and VPP of 94.3% and 94.3%, respectively, when carried out by the health care or the laboratory team. In both cases results were higher to those obtained with Determine Syphilis TP that had sensitivities of 88.5% and 88.5%, specificities of 97.9% and

97.9%, and VPPs of 92% and 92% when carried out by both teams.

The 95%CI for the sensitivity value of VisiTect Syphilis when carried out by the laboratory team was found to be between 85.7 and 99.3, surpassing that of Determine Syphilis TP with between 75.9 and 95.2, respectively. A chi-square test for proportion differences p = 0.002, confirmed that the VisiTect Syphilis was more sensitive than the Determine Syphilis TP.

The reproducibility in the second study was excellent (1.0) for both tests, with similar results when carried out by both the health care and laboratory teams.

The possibility of patients being able to wait for exam results at the clinic was high: 92.1% in the first study, 97% in the second, and, in total, for both studies, of 93.7% (739/789). When asked how long they were willing to wait, 100% of the participant in both studies agreed to wait up to 30 minutes, 59.1% in up to an hour, and 33% up to two hours, however as the average wait for test results was 15 minutes (between 10 and 20 minutes), it was confirmed that more than 90% of the participants in both studies were willing to wait for their results (Table 5).

The results of the opinion questionnaire applied to those professionals performing the tests showed that instruction comprehension, manageability, and results interpretation were rated 100% easy, or very easy, for all four tests. The speed of obtaining results with different executors was always less than 15 minutes, for SD Bioline Syphilis (100%), Syphicheck-WB (75%), VisiTect Syphilis (89%), and Determine Syphilis TP (78%).

Discussion

When compared with FTA-Abs, the four rapid tests showed sensibility, specificity and VPP performance superior to that obtained using VDRL, which is the technique most used in the routine tracking of syphilis in Brazil, nevertheless the specificity of the four tests was very similar to that of VDRL.

In general it can be said that the sensitivity, specificity, and VPP of the four tests, when dealing with patients who had high syphilis prevalence rates, was satisfactory, as well as its reproducibility when comparing the results obtained by the health care team with those of the laboratory team, giving evidence that all four tests are easily executed.

In study 1, the SD Bioline Syphilis presented a sensitivity (90.2%) higher to that of Syphicheck-WB (88.2%). This difference, however, was not statistically significant, suggesting that the performance of both of them, when dealing with high prevalence syphilis cases, was similar.

The reproducibility, measured by employing indicators of overall agreement and with the kappa index, was also very similar for both teams, demonstrating that it is possible to use them, independent of the team of professionals carrying them out.

In study 2, the VisiTect Syphilis test (96.2%) had greater sensitivity, statistically significant (p = 0.002), when compared with that of Determine Syphilis TP (88.5%). The specificity values (98.5% and 97.5%, respectively) were high, and similar, in both of the tests. The reproducibility was excellent (of 100%) possibly in virtue of the

Table 5

Acceptability and willingness of patients to wait for results of rapid tests at a specialized STD clinic in Manaus, Amazonas State, Brazil.

	Study 1 Syphicheck-WB and SD Bioline Syphilis n (%)	Study 2 VisiTect Syphilis and Determine Syphilis TP n (%)	Total n (%)
Willingness to wait			
Yes	497 (91.7)	239 (96.4)	736 (93.2)
No	45 (8.3)	9 (3.6)	54 (6.8)
Total	541 (100.0)	248(100.0)	790 (100.0)
Waiting time (minutes)			
Up to 30	497 (100.0)	239 (100.0)	736 (100.0)
Up to 60	324 (65.2)	111 (46.4)	435 (59.1)
Up to 120	206 (41.4)	43 (18.0)	249 (33.8)

fact that the professionals had become more experienced and trained with the new technology after study 1.

Despite the fact that VisiTect Syphilis, of the four tests studied, showed the greatest sensitivity, it is not possible to be certain that it is better than SD Bioline Syphilis and Syphicheck-WB as the samples employed for validation were not the same.

When comparing the results of these tests found by the laboratory professionals with those of the first SDI [11] study, the following performances for each of the four tests researched were observed.

SD Bioline Syphilis

In Manaus, the sensitivity of (90.2%) was below the weighted mean for the eight SDI reference laboratories (95%). Only one of them, from Nanjin (China), was slightly lower (89%). Its specificity in the Brazilian site was found to be (99.4%), higher than the mean of the SDI studies (94.9%), and only the studies carried out in Port-au-Prince (Haiti) and Colombo (Sri Lanka) obtained 100%.

Syphicheck-WB

The point sensitivity found in Manaus (88.2%) was higher than the weighted results of the eight studies (84.5%), and only one of them, the Gambian study, with 96%, was higher. The specificity (99.6%) was also slightly superior to that pondered in the eight studies (97.7%), although four of them had higher values than those achieved in the Brazilian city.

VisiTect Syphilis

The mean point value obtained in Manaus (96.2%) was much higher than the mean of the SDI studies (85%), and was also higher than those obtained in the eight studies; on the other hand, the specificities were very similar (98.5% and 98%).

Determine Syphilis TP

In Manaus, the value obtained (88.5%) was lower than the mean of the SDI study (97.2%), only being similar to that obtained in Birmingham (U.K). The specificity was higher in the Brazilian city (97.9%), compared to the SDI mean (94.19%).

The Determine Syphilis TP was also recently evaluated in a joint study carried out by the CDC, the Pan American Health Organization/WHO, and the Instituto Evandro Chagas e Instituto Oswaldo Cruz [Evandro Chagas Institute and Oswaldo Cruz Institute] in Brazil [17], being interpreted by three different observers. The sensitivity in relation to the gold standard employed (TPHA) varied between 95.6% and 98%, which were higher than those encountered in Manaus. The specificity, on the other hand, varied between 95.7% and 97.3%, a similar gradient to the one made evident by the present research.

Another study of Determine Syphilis TP, carried out in São Paulo, Brazil (Instituto Adolfo Lutz/Adolfo Lutz Institute), found a sensitivity of 93.6%, a specificity of 92.5%, and a VPP of 95.2% when compared to FTA-Abs and the TPHA [18].

A more pronounced difference was observed between the results of different validations carried out in reference to the VisiTect Syphilis test which showed a higher sensitivity in Manaus when compared to the results obtained in the eight SDI laboratories.

The prevalence of positive cases in the sample submitted in the first SDI study was higher (around 50%) than that of the one studied in Manaus (20.1%), which may explain the different findings. It will be necessary, however, to collect additional information about its performance in populations with low prevalence (3% or less), to confirm that this explanation is satisfactory.

In the peer-reviewed international bibliography, only studies about Determine Syphilis TP were found, and it seems to be, of the four tests studied, the best known and studied. The studies carried out by more than dozen different observers demonstrate a sensitivity that oscillates between 88% (Birmingham and Manaus) and 100% (Moscow [Russia], Colombo, and Gambia) and that, combined with a high specificity, varying from 88% (Gambia) to 100% (Moscow), and excellent reproducibility (Manaus and Rio de Janeiro), defines it as an excellent test. Meanwhile, in the Manaus study, when compared with the VisiTect Syphilis test, this last one had a stistically significant higher sensivity, possibly due to variations of temperature and humidity which are very high in the Brazilian city, possibly causing alterations in the strip of Determine Syphilis TP (the only one of the four tests which uses such strips). This hypothesis should be verified in the future.

Another element that should always be remembered is that a positive result of any one of these rapid tests (as also occurs with other treponemic tests) does not necessarily mean a recent or active infection. This is a negative factor for their use as a tracking test in areas of high prevalence where there are many people that have already had syphilis and have been treated

and cured of it and will still test positive with rapid tests. This implies the additional use of the tests currently used, VDRL or RPR, with titration, to avoid unnecessary treatment.

Finally, it was shown that more than 90% of the participants in both studies were willing to wait up to 30 minutes, a large enough time to get results in all four of the tests. The health professionals responsible for their execution considered that all of the four tests were relatively easy to execute and interpret.

Conclusion

To sum up, the tests validated presented quite high sensitivity, specificity and PPV. Easily manipulated by health professionals, they had high acceptability among both the patients and the health professionals that participated in the studies. The study demonstrated that it is possible to guarantee treatment to people on their first contact with the health system. These results lead the authors to believe in the necessity of the incorporation of these rapid tests as one more tool in the fight against syphilis, emphasizing their utilization in hard-to-reach populations.

Resumo

O diagnóstico e o tratamento corretos e precoces da sífilis são essenciais para o seu controle. Os testes diagnósticos tradicionais dependem de equipamentos, instalações e recursos humanos especializados. Na busca de testes de execução simplificada e rápida, realizou-se projeto de validação e da reprodutibilidade de quatro diferentes testes anteriormente avaliados pelos laboratórios de referência da Organização Mundial da Saúde. Verificaram-se também as características operacionais e aceitabilidade dos pacientes e dos profissionais de saúde. As amostras obtidas numa clínica de DST constaram de 541 e 248 pacientes com 51 e 52 positivos no FTA-Abs (padrão ouro) nos estudos 1 e 2, respectivamente. A sensibilidade variou entre 84 e 96%, especificidade superior a 98% e valor preditivo positivo > 90%. A reprodutibilidade foi superior a 97% e 0,94 no índice de kappa, comparando-se os resultados obtidos pelos diferentes profissionais. A execução dos testes foi de menos de vinte minutos, e mais de 90% dos pacientes concordaram em esperar o seu resultado até duas horas. Os testes apresentaram requisitos necessários para serem empregados no diagnóstico da sífilis, dando assim mais uma opção para o controle desta infecção.

Sífilis; Doenças Sexualmente Transmissíveis; Reprodutibilidade dos Testes; Diagnóstico

Contributors

A. S. Benzaken participated in the elaboration of the project, the execution coordination of it, and the writing and editing of the article. E. G. Garcia contributed to the epidemiological data analysis. J. C. G. Sardinha and J. C. Dutra Junior participated in the execution of the project and the writing and editing of the article. R. Peeling collaborated on the final discussion of the article.

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Introduction of Rapid Tests for Large-Scale Syphilis Screening Among Female, Male, and Transgender Sex Workers in Mumbai, India

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Background: Despite widespread availability of rapid plasma reagin (RPR) for syphilis screening at sex worker (SW)-dedicated project clinics, uptake of syphilis testing remains low and prevalence of syphilis remains high among SWs in Maharashtra, India. The primary reasons given for refusal of RPR were fear of venipuncture and long waiting times for results.

Methods: Between December 2007 and February 2008, rapid point of contact diagnostic tests (Syphicheck-WB, Qualpro Diagnostics, India) using finger-prick samples were introduced for syphilis screening, with RPR confirmation test of positives.

Results: Uptake of syphilis screening among clinic attenders increased to 63.1% compared with an average of 14.3% before the intervention. Among the 19,809 SWs who were screened, 598 tested positive (3% prevalence of lifetime infection). Of these, 395 (66.1%) accepted RPR confirmation test; 337 (88.3%) were seroreactive, 160 (40.5%) had titers ≥1:8 (active syphilis). The projected overall prevalence of active syphilis among all SWs screened was 1.2% but varied by site and typology of sex work (brothel-based, 2.4%; bar-based, 0.5%; street-based, 2.3%; male SWs, 0.2%; transgender, 11.3%; home-based, 0.6%).

Conclusions: The introduction of rapid tests dramatically increased the uptake of syphilis screening in this large-scale intervention among a high-risk population in India. However, only two-thirds of SWs with a positive rapid test accepted a confirmatory RPR test. The high proportion (40.5%) of active syphilis among those testing positive on the rapid screening test justifies treatment even if confirmatory testing is declined. A commercially available, simple, rapid nontreponemal test is needed to further strengthen syphilis screening.

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The World Health Organization (WHO) estimates that 12 million new cases of syphilis occur every year globally. South and South East Asia, the region with the highest number of cases, accounts for one-third of the global disease burden.[1] Reported syphilis prevalence varies widely among female sex workers (FSWs) in India, ranging from 3.1% to 51.0%, depending on geographic location, sex worker (SW) typology, and laboratory definition of syphilis.[2–6] A recent study demonstrated that incidence rates among FSWs are nearly double the rate among female STI patients who are not SWs (9.9 per 100 person-years vs. 4.5 per 100 person-years).[7] The prevalence of syphilis among men who have sex with men and transgenders (TGs) in India is also high (3.5%–28.0%).[4,8,9]

Because of the painless nature of the primary chancre and the symptom-free latency period, syphilis often remains undiagnosed and this result in continued transmission, as well as adverse pregnancy outcomes and serious sequelae in the later stages of infection. In addition to the morbidity and mortality associated with adult and congenital *Treponema pallidum* infection, syphilis increases HIV risk.[7,10] With an estimated 1.04 million FSWs, 235,000 male and TG SWs,[11] and 30 million men who regularly buy sex services in India,[12] diagnosing and treating syphilis infection among SWs is a key intervention for prevention of HIV transmission in the country.

The Aastha Project, under the India AIDS Initiative (Avahan) of the Bill and Melinda Gates Foundation, provides focused HIV prevention services to more than 30,000 female, male, and TG (hijra) SWs in the cities of Mumbai and Thane, Maharashtra.[13] The project includes behavior change communications, condom provision, and an essential service package for STI management, including 6 monthly syphilis screening.[14] The following services are provided free of charge in SW-dedicated, accessible settings: fixed clinics, satellite clinics (scheduled times at private practitioner clinics), and mobile clinics (conducted at houses, bars, and in brothel and lodge rooms).

Despite the widespread availability of rapid plasma reagin (RPR) tests through project-supported clinics and recommendations for semiannual screening, project-monitoring data indicate that only 10% to 15% of SWs in the project catchment areas accept routine syphilis screening each quarter. Results from a qualitative assessment indicated that the main factors contributing to the low screening coverage among SWs were fear of venipuncture (both the needle stick and the amount of blood taken) and reluctance to wait 1 hour for results in fixed clinic settings. In addition, health care providers reported operational difficulties of RPR testing at mobile clinics, citing inadequate illumination for blood drawing, and the need to transport samples to distant laboratories after late night sessions.

Rapid point of care (POC) treponemal tests have been evaluated for performance in multiple geographic areas[15–18]

and have shown to be a cost-effective tool for syphilis screening in antenatal clinic settings,[19,20] in STI clinics,[21] and in field conditions when carried out by low-skilled paramedics.[18,22,23] They are particularly valuable for SW populations in developing countries because they can be performed by trained nonlaboratory personnel using whole blood specimens obtained by finger prick, with results available within minutes. In addition, the storage of test kits at room temperature (up to 30°C) and lack of need for electricity or laboratory equipment make them ideally suited to mobile clinic conditions.

Because rapid treponemal test kits could address all of the key barriers to syphilis screening identified in the assessment, they were introduced along with a package of general health screening in a campaign to increase attendance at the clinics and increase uptake of syphilis screening.

MATERIALS AND METHODS

A health campaign was implemented during a 3-month period from December 2007 through February 2008 in Mumbai and the adjacent city of Thane in the Indian state of Maharashtra. Expanded health check-ups were offered at all project-supported clinic sites which included static clinics, satellite clinics (scheduled times at private practitioner clinic space), and mobile clinics (conducted at houses, bars, and rooms in brothels and lodges).

In addition to routinely offered STI check-up services (genital examination and syndromic management of symptomatic STIs, presumptive treatment for gonorrhea and chlamydia, at first visit or if no clinic visit within past 6 months) and limited general health care, SWs were offered a blood pressure check, hemoglobin level, blood type determination, and syphilis screening (POC with RPR confirmation) during the campaign period. All services were provided free of charge. Results were recorded on a health card that was given to the patient.

Stakeholders, such as brothel owners, bar managers, pimps, and members of the SW community, were engaged in the planning process for the campaign. Before the launch, clinic-based staff, outreach staff, and peer educators distributed promotional materials and interpersonal communication messages that included information on the importance of syphilis screening and the availability of a new test using finger-prick blood with results available within 15 minutes. Additional mobile clinics were planned (over and above the routine schedule) to ensure maximum geographic coverage of services in areas of active sex trade as determined by prior mapping exercises.

Syphilis screening was carried out on whole blood obtained by finger prick, using an immunochromatographic rapid test to detect antibodies to *T. pallidum* (Syphicheck-WB) at a cost of 18.50 Indian rupees (USD 0.39) per patient. Positive rapid test results were confirmed by RPR testing (Agappe Diagnostics Ltd, Kerala, India) in accordance with the recommended WHO algorithm.[24] RPR testing was performed on-site at fixed clinics. In satellite and mobile clinics, samples were transported to the fixed clinic laboratory in cold boxes and stored between 2°C to 8°C. RPR testing was conducted within 24 hours after the collection of the sample. Internal quality controls were conducted for the Syphicheck-WB and RPR kits and an External Quality Assurance System was established for RPR testing, with the Topiwala National Medical College in Mumbai as the reference laboratory.

SWs who tested positive on both Syphicheck-WB and RPR were treated for syphilis with oral doxycycline (100 mg) t.d.s. for 14 days and azithromycin 1 g OD STAT as per the national guidelines.[25] Penicillin injection, as per the State Government guidelines, is permitted only in secondary level health facilities because of the reported incidence of adverse reactions. In addition, those who tested positive on rapid test but refused RPR confirmatory testing were offered treatment if they did not have a history of syphilis treatment. A total of 598 individuals were provided the treatment regimen, and RPR was conducted with 395 randomly selected individuals to confirm effectiveness of treatment, of which 160 individuals had significant titres of $\geq$1:8 (active syphilis). Sixty-nine individuals were randomly selected to confirm effectiveness of treatment, of which 61 (88%) of the cases showed a decrease in RPR titres. All cases were followed up through PEs to ensure compliance of treatment.

SW typologies were defined by gender and site of client solicitation. FSWs were divided into the following 4 categories: brothel-based, bar-based, street-based, and home-based. Male and TG SWs (hijras) in Maharashtra are predominantly street-based.

The data presented in this article are from a retrospective analysis of routine monitoring data, with unique personal identifiers collected by the Aastha Project's computerized monitoring information system using Microsoft Excel.

RESULTS

Uptake of Syphilis Screening

In total, 31,395 SWs attended clinic services during the 3-month intervention period; 91.6% female (16,294 bar-based FSWs, 6489 home-based, 4352 brothel-based, and 1627 street-based), 5.6% (1742) male, and 2.8% (891) TG SWs. Services were delivered at 15 fixed clinic sites, 36 satellite clinic sites, and 1211 mobile clinics. The number of mobile clinic sessions increased during the intervention period to an average of 403 per month, up to 19% from an average of 340 per month in the previous 9 months. More than two-thirds (69.4%, 21,777) of SWs reached by the intervention attended the mobile clinics. The availability of fixed and satellite clinic services did not change compared with the preintervention period. The average monthly overall clinic attendance increased by 15.8% from 9032 SWs in the 9 months before the intervention to 10,465 during the intervention period.

The uptake of POC rapid syphilis screening among SWs attending clinic services during the 3-month intervention period was 63.1%, more than 4-fold higher than the monthly average uptake of 14.3% at clinic sites during the preceding 9-month period (Fig. 1). Acceptance of screening was highest among male SWs (76.0%) and lowest among bar-based FSWs (57.1%) (Table 1).

Testing Outcomes

Among the 19,809 SWs who accepted rapid syphilis testing, 598 tested positive (3.0% prevalence of lifetime syphilis infection). The proportion testing positive was lowest among male SWs (0.3%) and highest among TGs (15.3%). Among those screened with a positive rapid test, two-thirds accepted a confirmatory RPR test, with a reactivity rate of 85.3% (any titer) and 40.5% for active syphilis (RPR titer $\geq$1:8). The proportion with positive confirmatory RPR tests for active syphilis varied widely by SW typology, ranging from 27.5% for home-based FSWs to more than 70% for male and TG SWs.

Applying the RPR reactivity rate to all SWs with positive rapid test screening results gives an estimated overall active syphilis prevalence among all SWs screened of 1.2%

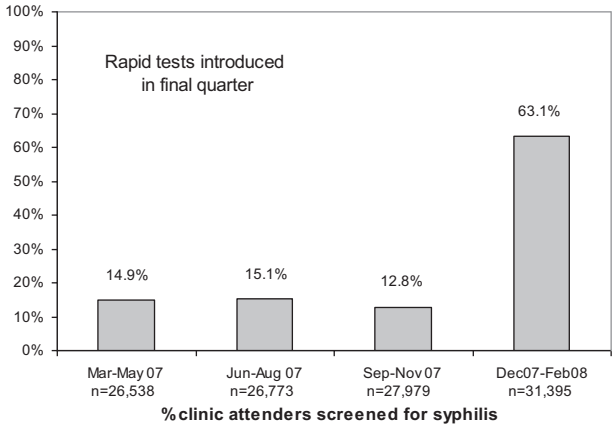

Figure 1. Uptake of syphilis screening among sex workers attending clinic services (fixed clinic sites, mobile clinics and health camps). Screening was carried out by rapid plasma reagin tests until the final quarter which was further replaced by rapid tests. n = number of sex workers attending clinic services during the period.

(titer ≥1:8) and 2.6% for syphilis with any RPR titer. The prevalence varied by site and typology of sex work and was substantially higher among TGs (11.3% and 13.1%, respectively) (Table 1).

DISCUSSION

To our knowledge, this is the first reported large-scale application of rapid diagnostic tests for syphilis screening in mobile settings among high-risk populations outside of a research environment. The introduction of rapid tests dramatically increased the uptake of syphilis screening among female, male, and TG SWs attending STI service sites during the intervention period.

In the Avahan Project, it has been difficult to screen a high proportion of the target population. Introduction of rapid tests increased the voluntary uptake of screening more than 4-fold. Several aspects of this intervention contributed to the improved uptake. The introduction of rapid POC screening tests directly addressed the main barriers voiced by both the SWs and the health care providers in the findings of the preintervention assessment. In addition, the intervention was planned with a broad range of stakeholders in a participatory manner, increasing the subsequent support and buy-in of the community.

Syphilis screening using RPR has been difficult to implement at mobile clinic sites where blood samples are sent to a central laboratory for testing and SWs are often lost to follow-up before results and treatment can be delivered days later. SWs in this rapid test screening intervention (with results generally available within 15–30 minutes) were more likely to agree to the test.

Nevertheless, only two-thirds of SWs testing positive on initial rapid test agreed to RPR confirmatory testing. At the time of writing, commercially available rapid syphilis tests are limited to treponemal tests which cannot distinguish between active syphilis and previously treated syphilis. WHO recommends a confirmatory RPR test for individuals with positive rapid tests to avoid overtreatment, an exchange of the usual process of using a treponemal test to confirm positive nontreponemal test result. Because the possible consequences of not treating an active infection outweigh the potential harm of overtreatment, WHO also suggests treating all those with positive rapid tests in situations where RPR is not available.[24] The high proportion (40%) of SWs with positive rapid tests who had confirmed active syphilis (even in this relatively low prevalence series) justifies the practice adopted during the health camp campaign of providing treatment when confirmatory testing is refused.

The estimated prevalence of active syphilis in this population was lower than expected, based on results from recent surveillance among FSWs in Mumbai and Thane.[4] The prevalence of syphilis (defined as RPR positive at any titer with TPHA confirmation) among brothel-based FSWs was reported as 13% (95% confidence interval [CI], 9.3%–17.9%) in Mumbai and 9.1% (95% CI, 5.9%–13.8%) in Thane, compared with 6.6% in this intervention. The prevalence among street-based FSWs was also higher: 14.6% (95% CI, 10.6%–19.7%) in Mumbai and 4.7% (95% CI, 2.4%–9.0%) in Thane, compared with 2.9% in this intervention.

Although the Syphicheck-WB test compared favorably to other rapid tests on multicenter performance evaluation with a sensitivity of 85% to 98% compared with gold standards using serum samples,[15–17] the sensitivity of the test was much lower (64%–73%) when performed on whole blood specimens as in this series.[16,23] However, the increased case-finding due to the 4-fold increase in acceptance of syphilis testing on introduction of POC tests more than offsets the missed cases due to

TABLE 1. Results of Syphilis Screening Among Sex Workers, by Typology

Typology	No. Attending Services	No. (%) Screened With Rapid Test	No. (%) Positive Rapid Test (Lifetime Syphilis)	No. (%) Accepted RPR	No. (%) Positive RPR	No. (%) RPR Titer ≥1:8	Estimated Prevalence* (RPR [+], Any Titer)	Estimated Prevalence* (RPR titer ≥1:8)
FSW brothel-based	4352	2858 (65.7)	221 (7.7)	150 (67.9)	123 (82.0)	46 (30.7)	6.6%	2.4%
FSW bar-based	16,294	9296 (57.1)	144 (1.6)	106 (73.6)	81 (76.4)	37 (34.9)	1.3%	0.5%
FSW street-based	1627	1199 (73.7)	41 (3.4)	38 (92.7)	38 (100.0)	26 (68.4)	2.9%	2.3%
FSW home-based	6489	4512 (69.5)	93 (2.1)	51 (54.8)	45 (88.2)	14 (27.5)	1.8%	0.6%
Male SW	1742	1324 (76.0)	4 (0.3)	4 (100.0)	4 (100.0)	3 (75.0)	0.3%	0.2%
Transgender SW	891	620 (69.6)	95 (15.3)	46 (48.4)	46 (100.0)	34 (73.9)	13.1%	11.3%
Overall	31,395	19,809 (63.1)	598 (3.0)	395 (66.1)	337 (85.3)	160 (40.5)	2.6%	1.2%

*Assuming all SW with positive rapid test accepted confirmatory RPR with reactivity rate matching the typology-specific subgroup of SW accepting RPR during the intervention.
RPR indicates rapid plasma regain; FSW, female sex worker; SW, sex worker.

their lower sensitivity compared with RPR screening in this population.

Another factor that may have contributed to the lower than expected syphilis prevalence in this series is that all SWs in the intervention were registered in the Aastha Parivaar (the Aastha Project family) and were regularly contacted by outreach workers, attended monthly clinic services, and enrolled in Aastha ghats (community self-help groups). In contrast, the surveillance protocol randomly sampled SWs, and only 34% of the SWs in Mumbai reported of being visited by a peer educator or outreach worker in the previous year.[26] It is also likely that presumptive treatment with cefixime 400 mg and azithromycin 1 g provided at the initial clinic visit in this population treated many cases of early latent syphilis.[27]

In conclusion, the data presented in this study suggest that rapid diagnostic tests are a valuable addition to syphilis control efforts among SWs, a key population for HIV prevention efforts in India and Asia. The introduction of the rapid test increased the acceptability and feasibility of syphilis screening in all clinical settings, including mobile venues. A commercially available rapid, simple, nontreponemal test would further improve screening efforts by increasing the acceptability of confirmatory testing in this high-risk population.

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REVISIÓN DE TEMA

Las pruebas rápidas en la promoción, prevención y diagnóstico de la sífilis

Rapid tests in the promotion, prevention and diagnosis of syphilis

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Resumen

Se presenta una corta revisión sobre el impacto que pueden tener las pruebas rápidas en el diagnóstico, promoción y prevención de la sífilis y se mencionan algunos aspectos clínicos y de diagnóstico de la sífilis y de la sífilis congénita.

Palabras clave: pruebas rápidas, sífilis, sífilis congénita, diagnóstico

Summary

A short review about the potential impact of rapid tests in the diagnosis, promotion and prevention of syphilis is presented. Some clinical and laboratory aspects of postnatal and congenital syphilis are mentioned as well.

Key words: rapid test, syphilis, congenital syphilis, diagnostic

Introducción

La sífilis es una enfermedad infectocontagiosa curable, producida por *Treponema pallidum,* de transmisión predominantemente sexual, aunque puede también transmitirse de madre a hijo durante el embarazo. Su forma de presentación clínica es variada y se acompaña de compromiso multisistémico.

La Organización Mundial de la Salud (OMS) estima que, aproximadamente, 12 millones de casos nuevos de sífilis sexualmente transmitida ocurren anualmente en el mundo, distribuidos así: 100.000 casos en Norteamérica, 3 millones en Latinoamérica y el Caribe, 140.000 en Europa del este, 370.00 en África del norte y del medio este, 4 millones en el África subsahariana, 100.000 en el este de Europa y Asia central, 240.000 en el este de Asia y el Pacífico, 4 mi-

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llones en el sur y sureste asiáticos y 10.000 en Australia y Nueva Zelanda [1,2]**.** Aun en regiones como la Europa del este, donde se consideraba que la sífilis había sido controlada, se encontró que su incidencia había aumentado, especialmente en el grupo de hombres que tiene sexo con hombres [3]. En muchos países en desarrollo la sífilis permanece como la principal causa de complicaciones durante el embarazo [4-7].

En casi todos los países existe una política de ofrecer tamización para sífilis a toda mujer embarazada, pero la realidad es muy diferente. Se calcula que menos del 30% de las mujeres embrazadas en el Africa subsahariana son tamizadas para sífilis [4,8]. Un estudio en Bolivia demostró que, aunque el 76% de la población recibía cuidado prenatal, sólo el 17% había sido tamizada para sífilis durante el embarazo [7]. Todas estas cifras demuestran claramente el impacto de la sífilis como una enfermedad vigente y su gran dimensión en todo el mundo.

Definiciones clínicas de la sífilis

La sífilis pasa por varios estadios clínicos conocidos y definidos como [9, 10]:

Sífilis primaria: es un estadio de la infección por *T. pallidum*, caracterizado por uno o más chancros. Los chancros pueden diferir considerablemente en apariencia clínica. Sin embargo, el chancro clásico de la sífilis se define como una lesión ulcerosa, indolora, de bordes levantados y fondo limpio, acompañada generalmente de adenopatía satelital.

Sífilis secundaria**:** es otro estadio clínico de la sífilis con manifestaciones generalizadas que comprometen la piel y las mucosas, a menudo asociada a linfadenopatía generalizada. El chancro primario puede estar aún presente.

Sífilis latente: se define como un paciente con serorreactividad, sin evidencia clínica de la enfermedad. La sífilis latente se subdivide en temprana, tardía y de duración desconocida.

Sífilis latente temprana: es una subcategoría de la sífilis latente; cuando la infección inicial ha ocurrido en los 12 meses previos, esta sífilis se clasifica como latente temprana.

Sífilis latente tardía: es una subcategoría de la sífilis latente; cuando la infección inicial ha ocurrido y lleva más de un año.

Sífilis latente de duración desconocida: es una subcategoría de la sífilis latente; cuando no es posible conocer los datos de la infección inicial, la edad del paciente oscila entre 13 y 35 años, y tiene títulos no treponémicos iguales o mayores de 1:32.

Neurosífilis**:** evidencia del compromiso del sistema nervioso central atribuibles a *T. pallidum.*

Sífilis tardía con manifestaciones clínicas diferentes de la neurosífilis: las manifestaciones de esta sífilis tardía incluyen lesiones inflamatorias del sistema cardiovascular, piel y hueso. Pocas veces compromete otras estructuras como el sistema respiratorio superior o inferior, la boca, los ojos, los órganos abdominales, los ganglios linfáticos o el sistema músculo-esquelético. La sífilis tardía usualmente se manifiesta después de un periodo de 15 a 30 años de una sífilis no tratada.

Sífilis congénita: es la que ocurre durante el embarazo.

Mortinato sifilítico: una muerte fetal que ocurre después de la semana 20 de gestación o en la cual el feto pesa más de 500 g y la madre tuvo una sífilis no tratada o inadecuadamente tratada.

Diagnóstico de la sífilis

Para el diagnóstico de la sífilis existen varios tipos de pruebas que se utilizan según el estadio clínico de la enfermedad y para las cuales se deben tener en cuenta las características de sensibilidad y especificidad. Dentro de este grupo de pruebas existen varias técnicas [11-14], como las siguientes.

***Examen microscópico directo*:** es una prueba que permite la observación directa del treponema, la cual se recomienda cuando hay lesiones presentes y se asume que es posible observar la bacteria, como en el chancro, los condilomas y las lesiones tempranas de la sífilis congénita. Esta prueba se considera confirmatoria de sífilis.

De esta prueba existen dos técnicas:

Campo oscuro: con esta técnica se puede observar el treponema con forma y movimientos característicos, razón por la cual el informe se presenta de la siguiente manera: "Se observó o no se observó *T. pallidum* con forma y movimientos característicos". Es importante que cuando se vaya a practicar la técnica del campo oscuro se utilice un raspado gingival como control positivo, el cual permite observar treponemas no patógenos llamados *T. denticola*; por esta razón, el campo oscuro no está indicado en lesiones de la cavidad oral, las cuales se deben estudiar por inmonofluorescencia directa. Se considera que el campo oscuro tiene una sensibilidad del 80%. Para poder observar treponemas en el campo oscuro se requiere un microscopio con un condensador de campo oscuro.

Inmunofluorescencia directa contra T pallidum (IFD-TP): la técnica de IFD-TP detecta y diferencia los treponemas patógenos de los no patógenos, mediante una reacción antígeno-anticuerpo. Debido a que el conjugado que se usa en esta técnica es específico para cepas patógenas, la prueba se puede realizar en muestras tomadas de lesiones orales, rectales e intestinales. Las muestras se colorean con inmunoglobulina anti-*T. pallidum* preparada en seres humanos o en conejos con sífilis, y marcada con isotiocianato de fluoresceína. Cuando se observan treponemas, el laboratorio debe informar: "Se observaron por IFD treponemas inmunológicamente específicos para *T. pallidum*". Esta técnica alcanza una sensibilidad cercana al 100% y es útil en lugares donde no es posible practicar el campo oscuro. Para la realización de esta prueba se requiere un microscopio de fluorescencia.

***Pruebas no treponémicas*:** estas pruebas detectan anticuerpos no treponémicos de tipo inmunoglobulina M (IgM) y anticuerpos IgG contra el material lipídico liberado de la célula huésped. Su principal uso es como pruebas diagnósticas de tamización y seguimiento del tratamiento. Las más usadas son la *Venereal Disease Research Laboratory* (VDRL) y la *Rapid Plasma Reagin* (RPR).

Características propias de estas pruebas: aunque fáciles de usar y con sensibilidad y especificidad variables y aceptables de acuerdo con el estadio clínico de la enfermedad [11], para su uso se requiere de un laboratorio con personal entrenado, los reactivos deben conservarse refrigerados, se debe contar con electricidad para el refrigerador y la centrífuga para separar el suero de la sangre de los pacientes y un agitador para llevar a cabo la prueba; la de VDRL, además, requiere de microscopio [14].

En la tabla 1 se observan las diferentes características de la VDRL y RPR.

Tabla 1. Algunas características diferenciales entre la RPR y la VDRL

Característica	RPR	VDRL
Sensibilidad	86-100%	78-100%
Especificidad	93-95%	98-100%
Facilidad de uso	Fácil	Fácil
Equipos	Rotador y centrífuga	Microscopio de luz y centrífuga
Entrenamiento	Mínimo	Mínimo
Comentarios	La mayoría de los reactivos requieren refrigeración	Los reactivos requieren refrigeración

Pruebas treponémicas: usan como antígeno *T. pallidum* y detectan anticuerpos contra los componentes celulares del treponema; su mayor utilidad es distinguir los falsos positivos y negativos de las pruebas no treponémicas y establecer el diagnóstico. Se usan como pruebas confirmatorias.

Características propias de estas pruebas: su sensibilidad y especificidad también varían según el estadio clínico de la enfermedad. Sus limitaciones son la complejidad de la técnica, los equipos requeridos, especialmente para el *Fluorescent Treponemal Antibody-Absorption* (FTA-ABS), el cual requiere microscopio de fluorescencia. Las técnicas *Treponema pallidum particle agglutination* (TPPA) y *Treponema pallidum hemagglutination assay* (TPHA), aunque no requieren microcopio de fluorescencia, sí requieren de otros equipos adicionales que necesitan electricidad [11,14]. En la tabla 2 se pueden ver las características de las pruebas treponémicas.

Tabla 2. Algunas características diferenciales entre las pruebas treponémicas

Característica	TPHA/TPPA	FTA-ABS
Sensibilidad	85-100%	70-100%
Especificidad	98-100%	94-100%
Facilidad de usar	Compleja	Compleja
Equipo	Incubador, microplatos, lavador y lector	Microscopio de fluorescencia
Entrenamiento	Extenso	Extenso
Comentarios	Prueba confirmatoria: no diferencia entre infección pasada y activa	Prueba confirmatoria: no diferencia entre infección pasada y activa

Pruebas rápidas

Puesto que el acceso a los laboratorios generalmente no es posible en muchas áreas remotas de los países en desarrollo debido a la distancia que se debe recorrer para llegar a ellos y, además, el transporte de la sangre o el suero se hace también difícil, no sólo por la distancia que se debe recorrer, sino por el embalaje a una temperatura que garantice su conservación [15] (tabla 3), se realizaron varios estudios que demostraron que las pruebas rápidas para el diagnóstico de sífilis se pueden recomendar teniendo en cuenta sus características [1,2,14-17]:

- rapidez: resultados en menos de 30 minutos,
- fácil montaje: se realizan en 3 a 4 pasos,
- mínimo entrenamiento y equipo,
- de fácil interpretación: formato de tarjeta o tirilla con reacción visual (figura 1).
- conservación: se pueden mantener a menos de 30 °C y no requieren refrigeración.

Tabla 3. Distancia que se debe recorrer en algunos países para acceder a un centro de salud

País	Distancia (km)
Bolivia	11,8
Haití	8,0
Madagascar	15,5
Nigeria	26,9
Tanzania	4,7
Uganda	4,7
Zimbawe	8,6

Tomado y adaptado de: referencia 1

Teniendo en cuenta estas características, se evaluaron algunas marcas comerciales y se compararon sus sensibilidades y especificidades con TPHA/TPPA [14,15]; los resultados se pueden ver en la tabla 4.

Las pruebas rápidas para sífilis pertenecen al grupo de las pruebas treponémicas y, al compararlas con las pruebas treponémicas estándar, FTA-ABS y TPHA/TPPA, se puede observar que su sensibilidad y especificidad, aunque están casi en los mismos rangos, muestran una leve diferencia frente a la especificidad que no alcanza el 100%, lo que las colocan en leve desventaja como pruebas confirmatorias; no obstante, por su facilidad de uso, el no requerir equipo especial, el poco entrenamiento que se requiere, la facilidad para conservar los reactivos, su bajo costo y su alta sensibilidad, las presentan como una muy buena alternativa para su uso como prueba diagnóstica inicial (tabla 5). Sin embargo, en algunos lugares donde se cuenta con una prueba como la RPR, se puede utilizar la prueba rápida como prueba inicial para definir el seguimiento del paciente sin necesidad de realizar primero la RPR. En caso de que no se cuente con la RPR, se puede utilizar una de estas pruebas rápidas y, de esta forma, causar un impacto desde el punto de vista epidemiológico en la diseminación de esta enfermedad. Uno de los grandes impactos es su utilidad en lugares donde el acceso a los servicios de salud es imposible y el costo de pruebas no treponémicas no permite su uso [1,2,14,18].

La OMS recomienda claramente las pruebas rápidas para la sífilis para mujeres en embarazo (prevención de la sífilis congénita), realizando la tamización al comienzo y al final del embarazo, en personas con riesgo de padecer una infección de transmisión sexual (ITS), en trabajadores sexuales, clientes de trabajadores sexuales, hombres que tiene sexo con hombres y usuarios de drogas inyectables [1].

Tabla 4. Resultados de la sensibilidad y especificidad de las diferentes pruebas rápidas para el diagnóstico de sífilis, ordenadas en orden alfabético

Prueba	*Sensibilidad (%)	*Especificidad (%)
Abbott (Determine)	97,2	94,1
Diesse (Syphilis fast)	86,0	92,8
Fujirebio (Espline)	97,7	93,4
Omega (VISITECT)	85,0	98,0
Qualpro (Syphicheck)	84,5	97,9
Standard (BIOLINE)	95,0	94,9

*Comparado con la prueba de referencia: TPHA/ TPPA

Tabla 5. Características de las pruebas treponémicas estándar comparadas con la pruebas rápidas

Característica	TPHA/TPPA	FTA-ABS	Pruebas rápidas
Sensibilidad	85-100%	70-100%	84-98%
Especificidad	98-100%	94-100%	94-98%
Facilidad de usar	Compleja	Compleja	Fácil
Equipo	Incubador, micropla-tos, lavador y lector	Microscopio de fluorescencia	Ninguno
Entrenamiento	Extenso	Extenso	Mínimo
Comentarios	Prueba confirmatoria: no diferencia entre infección pasada y activa	Prueba confirmatoria: no diferencia entre infección pasada y activa	La mayoría se pueden conservar a temperatura ambiente por 9-18 meses

En este mismo documento se dan algunas recomendaciones que se deben tener en consideración cuando se van a implementar estas pruebas, las cuales se basan en varios puntos, a saber:

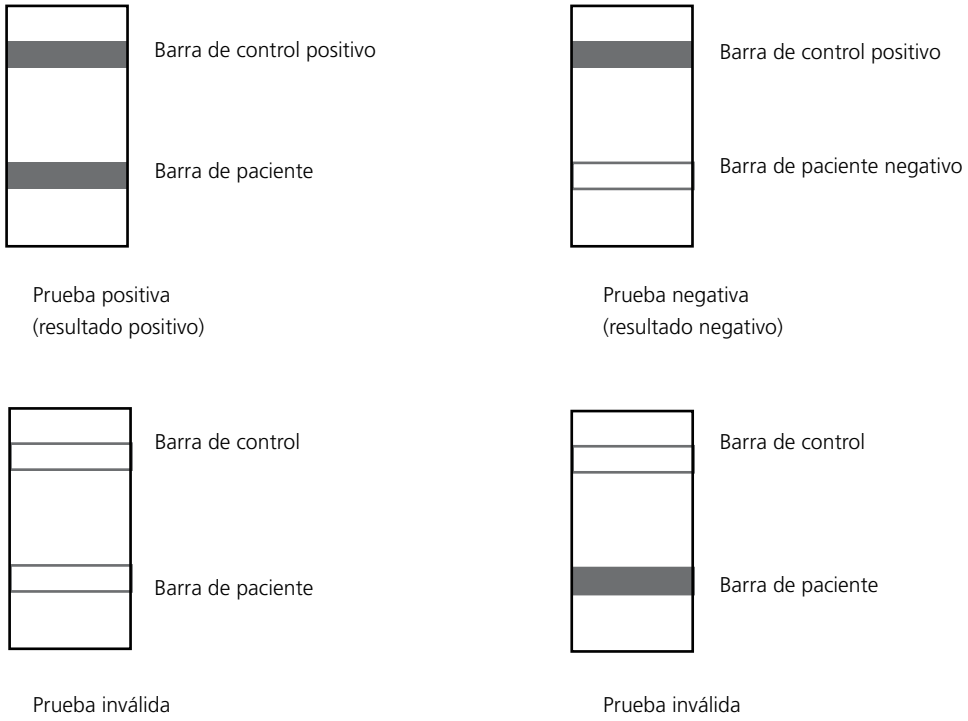
Accesibilidad: ¿cuántas de las personas con riesgo y de mujeres en embarazo tienen acceso a las pruebas de sífilis? Las pruebas rápidas permiten claramente la tamización antenatal, independientemente de la facilidad de acceso a los servicios de salud.

Calidad de la prueba: ¿cuál es la calidad de su prueba? ¿Se cuenta con un programa de control de calidad que garantice que los resultados son confiables? Este control se evidencia simultáneamente con el montaje de las pruebas rápidas (figura 1).

Tratamiento del paciente reactivo: ¿cuántas de las personas que se hacen la prueba reciben el resultado y el tratamiento de forma inmediata (idealmente en la misma visita)? Esto se puede conseguir con la utilización de las pruebas rápidas.

Rapidez: ¿la introducción de las pruebas rápidas ayudaría a mejorar las coberturas y el acceso a programas eficientes? Pregunta que nos debemos hacer para montar las pruebas rápidas teniendo en cuenta sus características y costos.

Figura 1. Interpretación de una prueba rápida



¿Qué aspectos se deben tener en cuenta cuando se va a escoger una prueba rápida?

Cuando se va escoger una prueba rápida se deben tener en cuenta los cinco aspectos que se describen y, de acuerdo con el análisis que se haga, se escoge la prueba que se requiera.

1. *Características de la prueba:* debido a las consecuencias que puede traer un error diagnóstico y los escasos efectos de un tratamiento excesivo, es mejor escoger una prueba de mejor sensibilidad que especificidad. Si el uso que se le va a dar a la prueba rápida es como prueba confirmatoria, es mejor una prueba más específica.

2. *Facilidad de uso:* se refiere al número de pasos de la prueba, si se puede usar la sangre total y la facilidad de realizar la prueba.

3. *Condiciones de uso:* se deben evaluar las condiciones de humedad. Lo recomendado es seleccionar pruebas rápidas que vengan empacadas de forma individual y con empaques a prueba de humedad.

4. *Condiciones de almacenamiento:* la mayoría de las pruebas rápidas para sífilis se pueden conservar entre 4 °C y 30 °C. Si la temperatura del ambiente es mayor de 30 °C, se debe controlar periódicamente la prueba para garantizar el resultado.

5. *Vida media:* se recomiendan pruebas con una muy buena vida media, especialmente en lugares donde se deben recorrer grandes distancias para llegar a un centro de salud. La vida media de 18 meses es la recomendada.

La sífilis congénita y el papel de las pruebas rápidas

El impacto de la sífilis materna en el embarazo: *T. pallidum* en la sangre de una mujer embarazada puede ser trasmitido al feto, particularmente en el estadio temprano de la infección (sífilis menor de un año). La mayoría de las mujeres con sífilis de menos de un año de duración transmitirán la infección a su niño por nacer [9, 19]. Aunque la mujer en embarazo puede transmitir la infección al feto tan temprano como a las nueve semanas de gestación, la transmisión normalmente toma lugar entre las semanas 16 y 28 del embarazo. La probabilidad de infección está directamente relacionada con el estadio clínico de la sífilis materna durante el embarazo o el estado del embarazo cuando se adquiere la infección. En la sífilis temprana materna, la tasa de transmisión materno-fetal puede alcanzar tasas de transmisión hasta de 80%, mientras que en la sífilis tardía la transmisión disminuye considerablemente (20). La concentración de espiroquetas en la sangre es más alta en los dos primeros años después de la infección y disminuye lentamente como resultado de la inmunidad adquirida. Por lo tanto, el riesgo de infección al compañero sexual es más alto en los dos primeros años y luego disminuye, aunque el riesgo de infección materno-fetal persista. El curso de la infección materna no parece alterarse con el embarazo [20].

Como se sabe, la detección en sangre de la infección de sífilis puede demorarse entre 10 a 45 días después de la exposición; por lo tanto, una prueba negativa inicial no garantiza la ausencia de infección. La mujer embarazada cuya prueba inicial sea negativa, debe tamizarse nuevamente durante el embarazo o al momento del parto. Los datos de incidencia de sífilis congénita entre infantes nacidos vivos son limitados por muchas razones, incluyendo dificultad en el diagnóstico, casos de infección asintomática y un sistema de vigilancia ineficiente tanto en los diagnósticos (oportunidad de tamización y su eficacia) como en el reporte de casos. Es claro que durante el embarazo la sífilis causa una considerable variedad de efectos, entre los cuales se mencionan: aborto espontáneo, muerte perinatal, niños con bajo peso al nacer (incluyendo prematuros) e infección neonatal con sífilis [21-25], lo que obliga, sin ninguna duda, a tener un buen sistema de vigilancia y control que detecte y prevenga todas estas posibles complicaciones, a sabiendas de que se trata de una enfermedad curable.

Carga de la sífilis y su impacto durante el embarazo: como se mencionó antes, la OMS estima que se presentan 12 millones de casos de sífilis anualmente en el mundo [1,24]. En países en desarrollo, 3% a 15% de las mujeres en edad reproductiva tienen sífilis. Cerca de 30% de las mujeres embarazadas con sífilis tendrá un bebe muerto a causa de la sífilis (mortinato por sífilis) y otro 30% tendrá un bebe vivo, pero nacerá con sífilis congénita, una complicación con una mortalidad de hasta 50% [1].

Las pruebas rápidas juegan un papel clave en el diagnóstico y tratamiento de la mujer embarazada [25]. Toda mujer en embarazo con una prueba positiva debe ser tratada independientemente de su historia de tratamiento en embarazos anteriores. Esto es importante debido al riesgo potencial de una nueva infección y las consecuencias que ésta traería [1].

Conclusiones

Las pruebas rápidas para sífilis son una excelente alternativa para impactar la pandemia de sífilis, especialmente en regiones o países don-

de no se cuenta con los recursos adecuados para hacer el diagnóstico y el manejo propios de la enfermedad.

Nuestro país, y especialmente las áreas rurales, cumplen con las condiciones ideales para su implementación y adopción como estrategia gubernamental, especialmente enfocados a hacia la eliminación de la sífilis congénita y el diagnóstico rápido en grupos considerados como "reservorios" de la infección, tales como: trabajadores sexuales y sus clientes, hombres que tienen sexo con hombres y usuarios de drogas intravenosas, en los cuales el impacto de un diagnóstico y un tratamiento acertados no deja la menor duda de su utilidad.

Es hora, pues, de que se adopten estas pruebas y se implementen sin ninguna reserva.

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Abstract

Sex Transm Dis. 2015 Jan;42(1):48-52. doi: 10.1097/OLQ.0000000000000222.

Point-of-care treponemal tests for neurosyphilis diagnosis.

Ho EL[1], Tantalo LC, Jones T, Sahi SK, Marra CM.

Author information

Abstract

BACKGROUND: The laboratory diagnosis of neurosyphilis rests upon identifying cerebrospinal fluid (CSF) abnormalities, including CSF-Venereal Disease Research Laboratory (VDRL) reactivity. The CSF-VDRL may not be available in the parts of the world where neurosyphilis is most common. Treponemal immunochromatographic strip tests (ICSTs) have been developed as point-of-care tests on blood for syphilis diagnosis in resource-limited settings.

METHODS: We optimized 3 commercial ICSTs for performance on CSF and tested CSF samples from 217 patients with syphilis. The Syphicheck-WB test (Qualpro Diagnostics, Goa, India; "Syphicheck") was chosen for further study based on agreement with CSF-VDRL test results. We determined CSF-Syphicheck titers for 152 samples. We modified the CSF-Syphicheck for point-of-care testing in a US sexually transmitted diseases clinic and compared results on 102 paired centrifuged and uncentrifuged CSF samples obtained in the laboratory to the results obtained at point of care; results of samples diluted 1:4 were compared in a subset.

RESULTS: The diagnostic sensitivity of a reactive CSF-Syphicheck (62%-64%) and the diagnostic specificity of a CSF-Syphicheck titer at or above 1:4 (79%-81%) were equivalent to the CSF-VDRL (54%-69% sensitivity, 73%-75% specificity) for laboratory and clinical neurosyphilis diagnoses. The CSF-Syphicheck normalized after neurosyphilis therapy similarly to the CSF-VDRL. The modified CSF-Syphicheck performed well at the point of care, albeit with better performance on cell-free compared with uncentrifuged CSF.

CONCLUSIONS: Cerebrospinal fluid treponemal ICSTs hold promise for point-of-care neurosyphilis diagnosis in regions where the CSF-VDRL is not available. Further study should address the performance of CSF ICSTs in resource-limited settings.

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Author	Jafari, Yalda
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URL	http://digitool.library.mcgill.ca/thesisfile104812.pdf
Publication Date	2011
Degree	MS
Discipline/Department	Department of Epidemiology & Biostatistics
Degree Level	masters
University/Publisher	McGill University
Abstract	Background: The World Health Organization estimates there were 12 million new cases of syphilis in 2006. . In developing countries there is often a lack of proper screening due to limited laboratory services. In contrast, in developed countries there is often limited access to care among hard-to-reach populations. In context of these healthcare system disconnects, point-of-care (POC) tests have proven to be an invaluable resource, yet in order to justify their use, their diagnostic accuracy and implementation outcomes must first be established. Methods: We searched six electronic databases from 1 January 1980 to 24 September 2010 for articles evaluating syphilis POC tests. Data was extracted and a second reviewer independently reviewed a subset of the articles. Subgroups of studies were created according to index test, sample, and reference standard employed. Pooled sensitivity and specificity estimates were calculated using Hierarchical Summary Receiver Operating Characteristic (HSROC) curves. Adjustments were made to account for imperfect reference standards. A narrative

review of implementation outcomes was undertaken.Results: The most frequently evaluated kits were Determine® (29%), Bioline® (18%), Syphicheck® (15%), and Visitect® (14%). After adjustment for imperfect reference standard, in serum samples, using a TP (Treponemal Pallidum) specific reference standard (e.g. TPPA), Bioline® had the highest pooled sensitivity, 99.67% (95% credible interval 97.65, 100) , followed by Determine® , 99.14% (96.93, 100), Visitect®, 98.18% (93.53, 100) and Syphicheck®, 88.46% (73.54, 99.87). Syphicheck® had the highest pooled specificity, 99.98% (99.64, 100), followed by Visitect®, 99.89% (99.19, 100), Determine®, 99.68% (98.70, 100) and Bioline®, 99.56% (98.55, 100). In whole blood, Bioline® had the highest pooled sensitivity, 91.47% (87.06, 96.12), followed by Determine®, 89.49% (79.88, 98.15), Visitect®, 82.93% (94.50, 100) and Syphicheck®, 81.99% (71.84, 91.99). Determine® had the highest pooled specificity, 99.91% (99.44, 100) followed by Visitect®, 99.87% (99.58, 100) followed by Syphicheck®, 99.81% (99.46, 100), and Bioline®, 99.61% (99.04, 100). Acceptability, feasibility, and impact of POC tests were demonstrated in various studies. Preference was not well established and economic evaluations were too heterogeneous to be conclusive. Conclusion: Bioline® and Determine® had the highest estimates of pooled sensitivity and specificity respectively. Higher parameter estimates in serum warrant the use of these tests in serum, rather than whole blood where feasible. Comparing our findings to current strategies in place, it is appropriate to use POC tests to screen for syphilis where access to laboratories and laboratory based serological tests are limited or where patients do not return for results. Further research into implementation outcomes is warranted and a framework for evaluating these outcomes is urgently needed.

Contexte: L'organisation mondiale de la santé (OMS) estimait à 12 millions le nombre de nouveaux…

Subjects/Keywords Health Sciences - Epidemiology

Contributors Lawrence Joseph (Supervisor2); Nitika Pai (Supervisor1)

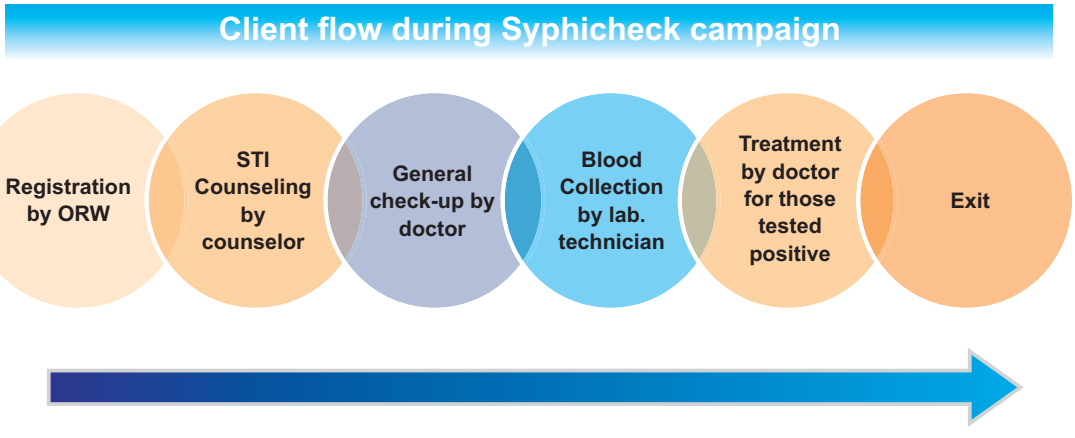
3. CAMPAIGN TO SCALE UP SYPHILIS SCREENING

3.1 Point of care test for syphilis (Syphicheck campaign)

The World Health Organization (WHO) estimates that 12 million new cases of syphilis occur every year globally. The reported syphilis prevalence varies widely among female SWs (FSWs) in India, ranging from 3.1 percent to 51.0 percent, depending on geographic location, sex worker typology and laboratory definition of syphilis.

Despite the widespread availability of rapid plasma regain (RPR) tests through Aastha-supported clinics and recommendation for semi-annual screening, project monitoring data indicates that only 10–15 percent SWs in project catchment areas accept routine syphilis screening each quarter. Results from a qualitative assessment pointed to the fear of venepuncture (both the needle stick and the amount of blood drawn) and the reluctance to wait one hour for results in fixed clinic settings as the main reasons for the low screening coverage. SWs often failed to follow-up on results and treatment. Health care providers also reported operational difficulties in RPR testing at mobile clinics, citing inadequate illumination for blood drawing and the need to transport samples to distant laboratories after late night sessions.

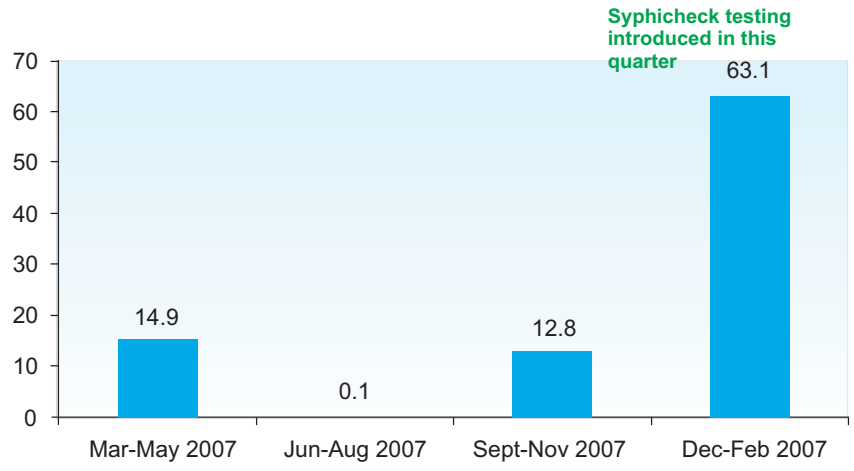
In response, a health campaign, known as Syphicheck campaign, was designed and implemented during a three-month period, from December 2007 through February 2008, in Mumbai and Thane in Maharashtra. Expanded health check-ups were offered at all project-supported clinic sites, including static clinics, satellite clinics (scheduled times at a private practitioner's clinic space) and mobile clinics (conducted at houses, bars, and rooms in brothels and lodges). In addition to the routinely offered STI check-up services and general health care, SWs were also offered check-ups for blood pressure and hemoglobin level, blood type determination, and syphilis screening (point of care with RPR confirmation) during the campaign period. All these services were provided free of charge.



The test results were recorded on a health card that was given to the client/patient. Stakeholders, such as brothel owners, bar managers, pimps and members of the SW community, were engaged in the planning process for the campaign. Before the launch, clinic-based staff, outreach staff and PEs distributed promotional materials and IPC messages about the importance of syphilis screening and the availability of a new test that used finger prick blood and made results available within 15 minutes. Additional mobile clinics were planned to ensure maximum geographic coverage in the areas of active sex trade, as determined by prior mapping exercises.

Syphilis screening was carried out on the whole blood obtained through a finger prick, using an immunochromatographic rapid test (point of care [POC]) to detect antibodies to T. pallidum (Syphicheck-WB). Positive rapid test results were confirmed by RPR testing (Agappe Diagnostics Ltd, Kerala, India) in accordance with the recommended WHO algorithm. RPR testing was performed on-site at fixed clinics. Samples from satellite and mobile clinics were transported to the fixed clinic laboratory in cold boxes and stored at 2°C to 8°C. RPR testing was conducted within 24 hours of sample collection. Internal quality controls were ensured for Syphicheck-WB and RPR kits, and an external quality assurance system was established for RPR testing, with the Topiwala National Medical College in Mumbai as the reference laboratory. SWs who tested positive on both Syphicheck-WB and RPR were treated for syphilis with oral doxycycline (100mg) t.d.s. for 14 days and azithromycin (1g OD STAT), as per national guidelines. All positive cases were followed up through PEs to ensure compliance to treatment.

% clinic attendees screened for Syphilis



A sharp increase in the percentage of clinic attendees screened forsyphilis after the introduction of Syphicheck

In total, 31,395 SWs attended clinic services during the three-month intervention period. The uptake of POC rapid syphilis screening among SWs attending clinic services during the three-month intervention period stood at 63.1 percent, which was more than four times higher than the monthly average uptake of 14.3 percent at clinic sites during the preceding nine-month period.

Among the 19,809 SWs who accepted rapid syphilis testing, 598 tested positive (3.0 percent prevalence of lifetime syphilis infection). Of those who tested positive with the rapid test, two-thirds accepted a confirmatory RPR test, with a reactivity rate of 85.3 percent (any titer) and 40.5 percent for active syphilis (RPR titer 1:8).

With its innovative approach, Aastha pioneered large-scale application of rapid diagnostic tests for syphilis screening of high-risk populations in mobile settings outside of a research environment.

Rapid POC treponemal tests have been evaluated for performance in multiple geographic areas and have shown to be a cost-effective tool for syphilis screening in antenatal clinic settings, at STI clinics and in field conditions when carried out by low-skilled paramedics. These tests are particularly useful for SW populations in developing countries because they can be performed by trained non-laboratory personnel using whole blood specimens obtained by a finger prick, with results available within minutes. In addition, the storage of test kits at room temperature (up to 30°C) and lack of need for electricity or laboratory equipment make them ideally suited to mobile clinic conditions. As the rapid treponemal test kits directly addressed the main barriers to syphilis screening identified by both SWs and health care providers during the pre-intervention assessment, they were introduced along with a general health-screening package to increase attendance at clinics and increase uptake of syphilis screening.

The introduction of rapid tests dramatically increased - by more than fourfold - the uptake of syphilis screening among female, male and TG SWs at STI service sites during the intervention period. Using the POC tests (rapid syphilis tests) at STI clinics helped overcome the barriers of limited laboratory capacity for syphilis screening, logistical difficulties of blood transport, and the high-risk groups' fear of blood being drawn. As the intervention was planned with a broad range of stakeholders in a participatory manner, it increased the subsequent support and buy-in from the community. Also, with results generally available within 15-30 minutes, SWs were more likely to agree to the test. Nevertheless, only two-thirds of the SWs who tested positive on the initial rapid test agreed to RPR confirmatory testing.

Performance Evaluations

AS A REFERENCE PRODUCT

SYPHICHECK®-WB

Rapid test for Syphilis (Modified TPHA)

Tulip Group

BMJ open accessible medical research

Seroprevalence of syphilis among HIV-infected individuals in Addis Ababa, Ethiopia: a hospital-based cross-sectional study

Begna Tulu Eticha,[1] Zufan Sisay,[2] Addisu Alemayehu,[3] Techalew Shimelis[3]

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ABSTRACT

Objective: To determine the prevalence of syphilis and its risk factors among people with HIV at a hospital in Ethiopia.

Design: A hospital-based cross-sectional study.

Setting: This study was conducted at one of the largest public hospitals in Addis Ababa , Ethiopia.

Participants: A consecutive 306 HIV-positive patients were recruited prospectively from January to March 2010. For comparative purposes, 224 HIV-negative consecutive attendees at the voluntary counselling and testing centre in the same period were also included. Participants under 15 years of age and treated for syphilis and with a CD4 T-cell count below 50 cells/mm^3 were excluded.

Outcome measures: Blood samples and data on sociodemographic and risk factors for syphilis were collected. Sera were screened for syphilis using rapid plasma reagin (RPR) test, and those positives were retested using *Treponema pallidum* haemagglutination assay (TPHA) test.

Results: The seroprevalence of syphilis among HIV-infected individuals was 9.8% compared with 1.3% among HIV-uninfected individuals, OR 8.01 (95% CI 2.4 to 26.6; p=0.001). A comparable rate of syphilis was found among men (11%) and women (8.9%) with HIV infection. Syphilis prevalence non-significantly increased with age, with the highest rate in 40–49 years of age (16.9%). Except a history of sexually transmitted infections, which was associated with syphilis OR 2.25 (95% CI 1.03 to 4.9; p=0.042), other risk factors did not raise the odds of infection.

Conclusions: The high prevalence of syphilis among people with HIV infection highlights the need to target this population to prevent the transmission of both infections. Screening all HIV-infected people for syphilis and managing those infected would have clinical and epidemiological importance.

ARTICLE SUMMARY

Article focus

- To determine the prevalence of syphilis among HIV-infected people.
- To compare the prevalence of syphilis by HIV status.
- To assess the risk factors for syphilis in HIV-infected people.

Key messages

- High prevalence of syphilis among HIV-positive individuals was observed.
- Syphilis prevalence is significantly higher among HIV positives than among HIV-negative people.
- Syphilis prevalence is not significantly influenced by age and gender.

Strengths and limitations of this study

- This hospital-based cross-sectional study provided preliminary data that would support future research.
- The study did not use stronger statistical power to detect the differences in risk factors of syphilis status.
- No clinical features of syphilis-positive patients were assessed.

INTRODUCTION

Sexually transmitted infections (STIs) are the major public health problems in most parts of the world. Based on the WHO estimate, STIs and their complications are among the top five disease categories for which adults seek health care in developing countries.[1] Syphilis is one of the most important STIs, caused by the bacterium *Treponema pallidum*.[2] It has been estimated that, annually, about 12 million new infections occur worldwide; of which, almost two-thirds are in sub-Saharan Africa and south/southeast Asia.[3] Unprotected sex, blood transfusion, needle sharing and vertical transmission from mother to the child are major modes of syphilis transmission.[2 4]

Syphilis, as a cause of ulcerative genital lesions, presents site for HIV entry and shading. Moreover, by activating immune cells and raising viral load, syphilis could facilitate HIV transmissibility.[5] On the

contrary, concurrent HIV infection may adversely affect the natural history, clinical manifestations and treatment response of syphilis.[3 6]

In Ethiopia, studies reported syphilis prevalence ranging from 1% to 10.9% in diverse risk groups such as pregnant women, blood donors, street dwellers and elderly people.[7–10] Moreover, according to the antenatal care (ANC)-based sentinel surveillances, syphilis prevalence increased from 1.8% in the year 2003 to 2.7% in 2005, and then stabilised at 2.3% in 2007 and 2009. The rates of syphilis-HIV coinfection among ANC attendees had also been rising from 4.1% in the year 2003 to 4.9% in 2005 and 5.3% in 2007, but dropped to 3.9% in 2009.[11–14] However, because of the limitations that the aforementioned risk groups consist of smaller size of HIV-infected individuals, and the sex and age composition of ANC attendees is limited to female gender and reproductive age group, the generated data may not reflect the true picture of syphilis among HIV-infected population. Therefore, this study was conducted to determine the prevalence and risk factors of syphilis among HIV-infected clients at St Paul's General Specialized Hospital.

METHODS AND MATERIALS

This cross-sectional study was conducted at St Paul's General Specialized Hospital, Addis Ababa from January to March 2010. The hospital is among the largest public hospitals in Ethiopia and provides HIV voluntary counselling and testing (VCT) as a routine service. Clients who are tested HIV positive are registered at the antiretroviral therapy (ART) clinic and assessed for their disease status. Clinical and immunological assessments (CD4 T cell count) at enrollment and at 6 monthly follow-up visits help determine patients' eligibility for ART. Those receiving ART are also monitored for clinical progress on a regular basis. Services including HIV counselling and testing, clinical and immunological assessments as well as ART are provided free of charge. HIV-infected patients are not routinely screened for syphilis and only those with clinical indications are tested.

Consecutive HIV-infected individuals with and without ART status, and who had immunological and biochemical testing were recruited prospectively. Clients tested HIV negative at the VCT centre during the study period were also recruited for comparative purposes. In total, 306 HIV positive and 224 HIV negative clients were considered for analysis. In either HIV serogroups, participants less than 15 years of age, and those who took syphilis treatment were excluded, as reactive non-treponemal test result may not remain after treatment. In HIV-positive clients, those found with a CD4 T cell count below 50 cells/mm^3 were excluded from the study owing to the unreliability of serological tests in a state of severe immunosuppression.

Counsellor nurses interviewed the study participants using structured questionnaire on sociodemographic and other risk factors such as history of blood transfusion, unsafe injection, multiple sexual partners, STIs, and syphilis family history. Blood samples were collected and screened for syphilis using the non-treponemal serologic test, rapid plasma reagin (RPR) test (Human, Germany). Sera found to be positive by RPR tests were further tested using treponemal test, modified *T pallidum* haemagglutination assay (TPHA) (Syphicheck-WB, Qualpro Diagnostics, India). Laboratory testing was carried out according to the directions of the manufacturers and all tests were run against the positive and negative controls. Only those samples positive by both RPR and TPHA were considered to have syphilis infection.

The study was approved by the Ethics Review Committee of Aklilu Lemma Institute of Pathobiology, Addis Ababa University and the St Paul's Hospital management body. Participation was entirely voluntary, and written consent was obtained from the study participants. Any information obtained during the study was kept with utmost confidentiality. Syphilis screening was performed free of charge, and those tested positive were managed by the physicians.

Data entry and analysis was performed using SPSS V.16. Results were summarised using descriptive statistics. Pearson's χ^2 test was used to evaluate differences between proportions; χ^2 for linear trend was also calculated using Epi Info V.7. Binary logistic regression analysis was used to assess the effect of sociodemographic and other risk factors on syphilis seropositivity. The OR was used as a measure of association.

RESULTS

Of 312 HIV-positive and 228 HIV-negative individuals approached during the study period, 6 and 4 individuals were excluded owing to refusal to participate, and insufficient serum sample and incomplete questionnaire, respectively. Thus, 306 HIV positive and 224 HIV negative clients were considered for analysis. A total of 188 (61.4%) participants with HIV received ART and the rest were ART naïve (38.6%). Majority of HIV-infected participants were urban dwellers (95.4%) and married (53.3%; table 1). HIV-infected respondents had a mean age 35.8 years (SD = 8.7, range 19–73 years) compared with 28.2 years (SD=9.8, range 15–73 years) in HIV non-infected groups. The male to female ratios in participants with and without HIV infection were 0.71 : 1 and 0.96 : 1, respectively.

The prevalence of syphilis infection was 9.8% in HIV positive participants compared to 1.3% in HIV negative participants; OR 8.01 (95% CI 2.4 to 26.6, p=0.001). The distribution of syphilis was similar among HIV-infected clients with and without ART (11.2% vs 7.6%, respectively; p=0.31). Sera reactive by RPR test were more likely found TPHA positive among HIV positives (54.5%) than in HIV-negatives (10%, p<0.001; table 2).

Syphilis occurred exclusively among urban dwellers in either of the HIV serogroups. Seropositivity of syphilis was comparable between men (11%) and women

Table 1 Syphilis infection in relation to sociodemography in HIV-positive and HIV-negative individuals at St Paul's Hospital, 2010

Characteristics	HIV positive			HIV negative		
	Number (%) tested	Number (%) positive for syphilis	Crude OR (95% CI)	Number (%) tested	Number (%) positive for syphilis	Crude OR (95% CI)
Residence						
Rural	14 (4.6)	0		18 (8)	0	
Urban	292 (95.4)	30 (10.3)	–	206 (92)	3 (1.5)	–
Sex						
Female	179 (58.5)	16 (8.9)	1	114 (50.9)	1 (0.9)	1
Male	127 (41.5)	14 (11)	1.26 (0.59 to 2.69)	110 (49.1)	2 (1.8)	2.1 (0.18 to 23.4)
Age (years)						
<19	2 (0.7)	0	–	52 (23.2)	1 (1.9)	1.84 (0.11 to 30.1)
20–29	65 (21.2)	4 (6.2)	1	95 (42.4)	1 (1.1)	1
30–39	156 (51)	13 (8.3)	1.39 (0.41 to 4.42)	47 (21)	0	–
40–49	59 (19.3)	10 (16.9)	3.11 (0.92 to 10.5)	19 (8.5)	1 (5.3)	5.2 (0.31 to 87.4)
>50	24 (7.8)	3 (12.5)	2.18 (0.45 to 10.5)	11 (4.9)	0	–
Marital status						
Single	60 (19.6)	5 (8.3)	1.41 (0.39 to 5.1)	146 (65.2)	3 (2.1)	–
Married	163 (53.3)	20 (12.3)	2.2 (0.79 to 6)	60 (26.8)	0	
Divorced/widowed	83 (27.1)	5 (6)	1	18 (8)	0	
Religion						
Orthodox	228 (74.5)	24 (10.5)	2.1 (0.47 to 9.1)	170 (75.9)	3 (1.8)	–
Protestant	41 (13.4)	4 (9.8)	1.9 (0.32 to 10.9)	25 (11.2)	0	
Muslim	37 (12.1)	2 (5.4)	1	29 (12.9)	0	
Educational status						
Illiterate	41 (13.4)	9 (22)	4.78 (0.96 to 23.8)	13 (5.8)	0	
Primary school	95 (31)	8 (8.4)	1.56 (0.32 to 7.74)	51 (22.8)	0	
Secondary school	134 (43.8)	11 (8.2)	1.52 (0.32 to 7.19)	124 (55.4)	2 (1.6)	–
Certificate and above	36 (11.8)	2 (5.6)	1	36 (16.1)	1 (2.8)	
Occupation						
Government employee	41 (13.4)	3 (7.3)	1	29 (12.9)	1 (3.4)	1
Private employee	82 (26.8)	8 (9.8)	1.37 (0.34 to 5.46)	71 (31.7)	1 (1.4)	0.4 (0.02 to 6.62)
Housewife	63 (20.6)	6 (9.5)	1.33 (0.31 to 5.66)	21 (9.4)	0	–
Student	5 (1.6)	0	–	41 (18.3)	1 (2.4)	0.7 (0.04 to 11.67)
Merchant	35 (11.4)	4 (11.4)	1.63 (0.34 to 7.86)	19 (8.5)	0	
Housemaid	11 (3.6)	3 (27.3)	4.75 (0.81 to 27.9)	7 (3.1)	0	
No work	69 (22.5)	6 (8.7)	1.21 (0.29 to 5.11)	36 (16.1)	0	
Ethnicity						
Amhara	156 (51)	14 (9)	1	117 (52.2)	2 (1.7)	1
Oromo	87 (28.4)	9 (10.3)	1.2 (0.49 to 2.83)	64 (28.6)	1 (1.6)	0.9 (0.08 to 10.3)
Others	63 (20.6)	7 (11.1)	1.3 (0.49 to 3.3)	43 (19.2)	0	–

Table 2 Syphilis serological tests in HIV-positive and HIV-negative individuals at St Paul's Hospital, 2010

			HIV positive						HIV negative	
			ART users		ART naïve		Total			
Syphilis test	Total tested	Number (%) of positive	Tested	+ve (%)	Tested	+ve (%)	Tested	+ve (%)	Tested	+ve (%)
RPR	530	85 (16)	188	36 (19.1)	118	19 (5.6)	306	55 (18)	224	30 (13.4)
TPHA	85	33 (38.8)	36	21 (58.3)	19	9 (47.4)	55	30 (54.5)	30	3 (10)
Syphilis seropositivity	530	33 (6.2)	188	21 (11.2)	118	9 (7.6)	306	30 (9.8)	224	3 (1.3)

ART, antiretroviral therapy; RPR, rapid plasma reagin; TPHA, *Treponema pallidum* haemagglutination; +ve, positive.

(8.9%) with HIV infection. Syphilis prevalence seems to increase with increasing age, with the highest rate in the age range 40–49 years (16.9%), though χ^2 for linear trend analysis showed no statistical significance (χ^2=2.46, p=0.117). A decreasing rate of syphilis was observed with increasing educational level, where illiterate HIV-positive participants (22%) had higher odds of infection compared with those having at least a certificate (5.6%); OR 4.78 (95% CI 0.96 to 23.8, p=0.056). Similarly, the association between occupation and syphilis was marginally non-significant where housemaids (27.3%) were affected compared with government employees (7.3%); OR 4.75 (95% CI 0.81 to 27.9, p=0.085; table 1).

The exposure of HIV-infected and HIV-non-infected participants to various risk factors of syphilis is summarised in table 3. Except syphilis family history, which occurred in a comparable rate in either of the HIV serogroups, other risk factors such as history of blood transfusion (10.5%), having multiple sexual partners (36.9%) and unsafe injection (12.7%) and a history of STIs (45.4%) were more frequently reported by HIV-infected participants. However, it was only a history of STIs, which was significantly associated with syphilis among HIV-infected participants; OR 2.25 (95% CI 1.03 to 4.9, p=0.042).

DISCUSSION

This study showed that the prevalence of syphilis among HIV positives was 9.8%, with no significant difference between those receiving ART (11.2%) and ART naives (7.6%). The finding appears to be compatible with rates of syphilis–HIV coinfection among street dwellers (7.9%)[8] and elderly people (6%)[9] in northwest Ethiopia (Gondar) and in Nigeria (14%).[15] However, contrasting our result, the coinfection rate was lower among ANC attendees in Ethiopia (3.9%)[14] and higher among sexually transmitted disease (STD) clinic attendees in Argentina (59.7%).[16] The observed inconsistencies may be because of the composition of the investigated subpopulation, where ANC attendees, for instance, have apparently lower risk of syphilis compared with STD clinic attendees. In view of the adverse impact syphilis has to facilitate the transmission of coexisting HIV, intervention measures targeting this particular risk group has greater importance to prevent both infections.

In the present study, syphilis was significantly associated with HIV infection, where HIV-infected individuals had about eightfold higher risk of syphilis compared with HIV-non-infected people. This result was in line with findings that revealed the existence of association between HIV and syphilis in different localities and subpopulations. A consistent twofold increase in syphilis–HIV coinfection rates among ANC attendees[11–14] and fourfold among street dwellers in Ethiopia,[10] as well as eightfold in HIV-infected population in Nigeria[15] may be because of the fact that HIV and syphilis shares routes of transmission. These reports also indicated the

Table 3 Syphilis infection in relation to syphilis risk factors in HIV-positive and HIV-negative individuals at St Paul's Hospital, 2010

	HIV positive			HIV negative		
Characteristics	Number (%) tested	Number (%) positive for syphilis	Crude OR (95% CI)	Number (%) tested	Number (%) positive for syphilis	Crude OR (95% CI)
Blood transfusion						
No	274 (89.5)	28 (10.2)	1	216 (96.4)	3 (1.4)	–
Yes	32 (10.5)	2 (6.2)	0.59 (0.13 to 2.58)	8 (3.6)	0	
Multiple sexual partner						
No	193 (63.1)	19 (9.8)	1	195 (87.1)	2 (1)	1
Yes	113 (36.9)	11 (9.7)	0.99 (0.45 to 2.16)	29 (12.9)	1 (3.4)	3.45 (0.3 to 39.2)
Unsafe injection						
No	267 (87.3)	27 (10.1)	1	219 (97.8)	3 (1.4)	–
Yes	39 (12.7)	3 (7.7)	0.71 (0.21 to 2.57)	5 (2.2)	0	
Syphilis family history						
No	281 (91.8)	26 (9.3)	1	200 (89.3)	2 (1)	1
Yes	25 (8.2)	4 (16)	1.87 (0.60 to 5.86)	24 (10.7)	1 (4.2)	4.3 (0.38 to 49)
STIs						
No	167 (54.6)	11 (6.6)	1	182 (81.2)	2 (1.1)	1
Yes	139 (45.4)	19 (13.7)	2.25 (1.03 to 4.9)	42 (18.8)	1 (2.4)	2.2 (0.19 to 24.8)

STIs, sexually transmitted infections.

varying strength of association between HIV and syphilis in diverse risk groups. However, none of these studies pointed out whether syphilis and HIV were contracted concurrently or one infection preceded another to explain the causal nature of such epidemiologic synergy between HIV and syphilis.

The seroprevalence of syphilis was not significantly affected by gender in either HIV serogroups, similar to findings elsewhere.[10 15] However, Griemberg *et al*[16] reported men had a higher risk of HIV, syphilis and syphilis–HIV coinfection compared with women. This report is also in contrast to the established higher rate of HIV among women in our region,[17] which may be because of the difference in risk behaviour by gender in various geographical regions. We also found increasing syphilis prevalence with age among HIV-infected individuals, with the highest rate reported in the age group 40–49 years (16.9%), followed by age group above 50 years (12.5%), though no statistically significant linear trend was observed. A raising syphilis prevalence with age was consistently reported by others,[8 10 14 15] which might be because of the increased risk of exposure to syphilis with time. Moreover, our data showed that illiterate and housemaid HIV-infected participants were disproportionately affected by syphilis, which point the significance of education to prevent syphilis transmission.

In Ethiopia, where HIV and syphilis has strong association, and transmission of the former is primarily through heterosexual exposure,[17] people with multiple sexual partners would obviously be at higher risk of contracting syphilis as well. Of course, the significance of such risk behaviour to influence syphilis prevalence was documented in our context, where having more than two sexual partners increased odds of syphilis infection sixfold compared with those with no sexual partner.[10] However, the lack of association between a history of multiple sexual partners and syphilis in our study deserves further investigation for possible explanation. Syphilis prevalence was about twofold higher among HIV-infected participants who reported a history of STIs compared with those with no history of STIs.

Findings in this study need to be interpreted in light of its methodological limitations. First, absence of association between various risk factors and syphilis might be because of the fact that the study did not use stronger statistical power to detect the differences. Second, the reduced sensitivity of non-treponemal tests in primary as well as late latent syphilis and the potential for false-negative results owing to prozone reactions might lead to underestimation of syphilis infection rate. Moreover, the limitation of possible false-positive reaction with non-treponemal and treponemal tests needs to be given attention, as positive results may not necessarily indicate disease activity. Finally, this study overlooked the importance of including clinical data, which would have been a good opportunity to describe the clinical presentation of syphilis among HIV-infected patients.

In conclusion, this study showed high prevalence of syphilis among HIV-infected people compared with HIV-non-infected people. Thus, intervention measures targeting HIV-infected individuals would have paramount importance to prevent transmission of syphilis as well as HIV. As part of this effort, screening all HIV-infected people for syphilis and managing those infected is critically needed. Further studies using a longitudinal design with stronger statistical power would reliably investigate the possible interaction between HIV and syphilis.

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Seroprevalence of syphilis among HIV-infected individuals in Addis Ababa, Ethiopia: a hospital-based cross-sectional study

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DIAGNOSTICS

A smartphone dongle for diagnosis of infectious diseases at the point of care

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This work demonstrates that a full laboratory-quality immunoassay can be run on a smartphone accessory. This low-cost dongle replicates all mechanical, optical, and electronic functions of a laboratory-based enzyme-linked immunosorbent assay (ELISA) without requiring any stored energy; all necessary power is drawn from a smartphone. Rwandan health care workers used the dongle to test whole blood obtained via fingerprick from 96 patients enrolling into care at prevention of mother-to-child transmission clinics or voluntary counseling and testing centers. The dongle performed a triplexed immunoassay not currently available in a single test format: HIV antibody, treponemal-specific antibody for syphilis, and nontreponemal antibody for active syphilis infection. In a blinded experiment, health care workers obtained diagnostic results in 15 min from our triplex test that rivaled the gold standard of laboratory-based HIV ELISA and rapid plasma reagin (a screening test for syphilis), with sensitivity of 92 to 100% and specificity of 79 to 100%, consistent with needs of current clinical algorithms. Patient preference for the dongle was 97% compared to laboratory-based tests, with most pointing to the convenience of obtaining quick results with a single fingerprick. This work suggests that coupling microfluidics with recent advances in consumer electronics can make certain laboratory-based diagnostics accessible to almost any population with access to smartphones.

INTRODUCTION

Smartphones are being adopted at a breathtaking pace, including in developing countries (*1*, *2*). They offer fast computing, a friendly user interface, and connectivity to data stored in the cloud (that is, servers accessible wirelessly), all at falling prices. Although smartphones are increasingly being adapted for health diagnostics, the most common applications have leveraged individual components and functions, such as cameras (*3*), data communication (*4*), and data processing (*5*), rather than replicating any complete diagnostic assay performed in clinical laboratories (*6*, *7*).

We sought to build on previous work in miniaturizing diagnostics hardware (*6*, *8*, *9*) for the rapid point-of-care (POC) diagnosis of HIV, syphilis, and other sexually transmitted diseases. Early diagnosis and treatment of such diseases in pregnant mothers have been shown to reduce adverse health consequences to both mothers and their children (*10*). Treponemal antibodies appear earlier than nontreponemal antibodies in syphilis infection (*11*) and have been used in syphilis rapid diagnostic tests (RDTs). Recently, some manufacturers (Chembio, SD Bioline, and MedMira) have developed dual HIV/treponemal-syphilis tests, but these tests rely on lateral-flow or immunofiltration technologies, which could limit their performance and ability to multiplex (*12*). We therefore sought to engineer all the capabilities of a benchtop enzyme-linked immunosorbent assay (ELISA) instrument into a small diagnostic accessory—a "dongle"—that attaches to a smartphone.

The dongle was designed to be small and light enough to fit in one hand and to run assays on disposable plastic cassettes with preloaded reagents, where disease-specific zones would provide an objective readout, similar to an ELISA microplate assay. The assay would be similar to an ELISA, but with gold nanoparticles and silver ions performing the amplification step instead of enzymes and substrate (*13*). For our assay targets, we chose HIV and treponemal syphilis antibody tests from our previous work (*13*) while detecting a third target, anti-cardiolipin antibody, as a nontreponemal syphilis marker. In addition, we added immunoglobulin M (IgM) as a secondary antibody for early syphilis detection (*11*). The U.S. Centers for Disease Control and Prevention (CDC) recommends a nontreponemal [for example, rapid plasma reagin (RPR)] test on all anti-treponemal enzyme immunoassay reactive specimens (*14*). However, a triplex test with HIV, treponemal syphilis, and nontreponemal syphilis results is not currently available commercially and clinically. As such, our device is advantageous in that it would help to characterize the infection as active or inactive [because treponemal syphilis antibody level remains high for life (*11*)], thus saving diagnostic time and simplifying treatment workflow (*11*, *14*, *15*).

Testing in the field can exhibit markedly different performance from tests run in a laboratory owing to variations in clinical specimens, local environmental conditions (including temperature and humidity), and variations in how the tests are run by individual users. In the field, sensitivities have been reported to be as low as 82% and specificities as low as 85% for the widely used HIV RDTs (*16*–*18*), 64 to 96% sensitivity and 97 to 99% specificity for treponemal syphilis antibody tests (Determine, SD Bioline, Syphicheck, VisiTect, and Chembio) (*19*–*21*), and 85% sensitivity and 96% specificity for nontreponemal

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syphilis antibody test (Chembio) (*21*) on whole blood (fingerprick or venipuncture) performed by trained staff. RDTs are also limited in number of markers in a single test, subjective user interpretation of band intensities, and lower sensitivity and reproducibility than laboratory-based tests.

Here, we demonstrate the field performance of three POC ELISA quality tests run simultaneously on a smartphone dongle under the following conditions: (i) the tests were run by health care workers (HCWs); (ii) the tests were run on fingerprick whole blood collected fresh from intended end users (patients); and (iii) the tests were run in a blinded manner where the reference laboratory results were unknown until testing of all subjects had concluded. In a 15-min assay, the dongle provided accurate diagnostic results on triplexed markers for HIV and syphilis, suggesting that laboratory-quality diagnostic services could be made accessible to any population with smartphones.

RESULTS

Dongle design for extremely low power consumption

The dongle (Fig. 1A) consists of two main innovations to achieve low power consumption. First, we eliminated the power-consuming electrical pump (*6*) by using a "one-push vacuum," where a user mechanically activates a negative pressure chamber (Fig. 1B) to move a sequence of reagents prestored on a cassette (Fig. 1C). The simple vacuum chamber was created with a rubber bulb, with one port connected to the assay cassette outlet and one port to a silicone one-way valve. When the bulb is depressed, air exits out the one-way valve, and a spring aids the bulb in reexpansion, creating a negative pressure within the chamber that pulls liquids through the channels. The total flow times for a six-wash sequence were consistent between three users, with an average of 119 s (fig. S1). By contrast, our setup mechanically generates the vacuum source at the time of assay; this procedure is durable (similar to a bulb for a manual sphygmomanometer), requires little user training, and does not require maintenance and additional manufacturing to prepackage a vacuum source (which can also leak over time). Other components in the dongle, including robust and low-cost light-emitting diodes (LEDs), photodetectors (*6*, *13*), and a microcontroller, consumed very low power. Using commercially available electronic components with an injection-moldable case, our device would have a manufacturing cost of $34, in comparison to $18,450 for typical ELISA equipment (Fig. 1D and table S1).

The dongle measured the optical density (OD) (absorbance) of silver enhancement on each assay (Fig. 2A), as described previously (*13*). Briefly, each detection zone was treated with capture proteins, and whole-blood sample flowed over each zone, followed by signal development reagents (gold-labeled antibodies, washes, and silver development solution), such that the concentration of analyte captured on each zone corresponded to the OD of silver enhancement. We designed the dongle so that power was only consumed during OD readings (8.5 mW, 8 s) and during information transfer back to the smartphone (1.5 mW, 8 min) (Fig. 2B). No power was consumed by the dongle while the sample and washes were flowing. Over a 15-min assay, the dongle had an average power consumption of 1.6 mW, or 0.22 mWh per test. By comparison, a typical smartphone uses 751 mW on a 3G network and 17.5 mW on standby mode (*6*), and a laser pointer uses about 1 to 5 mW (*22*). Using an Apple iPod touch (4th generation) to power the dongle, we found that each run consumed about 2.4% of the battery, allowing 41 runs to be made on a single charge (note that newer generations of smartphones can hold more charge).

With such low power consumption by the dongle, we were able to implement a second innovation to remove the need for a battery: using the audio jack for transmitting power and for data transmission, as demonstrated by Kuo *et al.* (*23*). The audio jack connection (3.5 mm, 4 lead) has remained ubiquitous and standardized among smartphones, which allowed our dongle to be compatible with the growing variety of mobile phones and tablets. Here, a 19-kHz audio signal was sent from the iPod touch through the audio jack to the dongle and converted into a stable DC 3.0 V (fig. S2A). The dongle had no internal battery and used only the power delivered by the audio signal. The power harvested from the audio jack was stable and sufficient for reliable OD measurements compared with a benchtop analyzer (fig. S2B). For the target and positive control zones, there was no significant difference in OD measurements taken by the dongle and benchtop analyzer. The negative control zone showed a small but significant difference ($P = 0.03$, Student's t test). The dongle produced OD readings that correlated with a serial dilution of a strongly RPR-positive (1:128 titer) syphilis sample ($R^2 = 98.9\%$) (Fig. 2C).

We programmed a microcontroller that performed FSK by converting a decimal integer into binary, and each bit was sent as a high-frequency (1632 Hz, or "1") or low-frequency (816 Hz, or "0") signal and transmitted the photodiode readings through the audio jack and back to the phone (Fig. 1B and fig. S2A). Our initial implementation was focused on fidelity of signal, but signal transmission can be sped up in the future. To test the accuracy of the signal, we programmed the microcontroller to send a pattern of alternating 1 and 0, or high- and low-frequency signals. We observed 100% accuracy for 12,160 bits tested. During the test, the custom smartphone application ("app") on the phone converted the photodiode signals to absorbance units, which could be reported as "positive" or "negative" when above or below, respectively, a preset cutoff value.

Device function and operation

We built on our previous work for HIV/syphilis detection (*13*) in five major ways. First, we expanded the detection zones in the disposable microfluidic cassette from four to five zones, for detecting HIV, treponemal syphilis, and nontreponemal syphilis antibodies simultaneously with internal negative and positive controls (Fig. 1C). Second, gold-labeled IgM antibodies were added to the assay (Fig. 2D). Anti-cardiolipin antibodies are commonly found in IgM antibodies, and therefore, the addition of gold-labeled IgM offers enhanced sensitivity of nontreponemal syphilis. Third, to improve long-term stability in shipping and storage, we lyophilized gold-conjugated secondary antibodies inside the antibody holder, along with a stabilizer and anticoagulant, and packed the holder in an individual moisture barrier bag before shipping them to Rwanda. Lyophilized antibodies demonstrated comparable performance to gold-conjugated antibodies freshly diluted in buffer (Fig. 2E).

Fourth, to mimic field testing conditions, we prepared the test cassettes ahead of time at Columbia University before transporting them to Rwanda. By using the stabilizing agent StabilCoat during physisorption of capture proteins, the protein retained its function over 3 weeks at 60°C (fig. S3) [equivalent to roughly 28 weeks at 25°C according to Arrhenius-like approximations (*24*)]. This stabilizer was also found to be as effective as other blocking agents (fig. S4). Fifth, the wash buffers and silver reagents were preloaded on the reagent cassette each day before

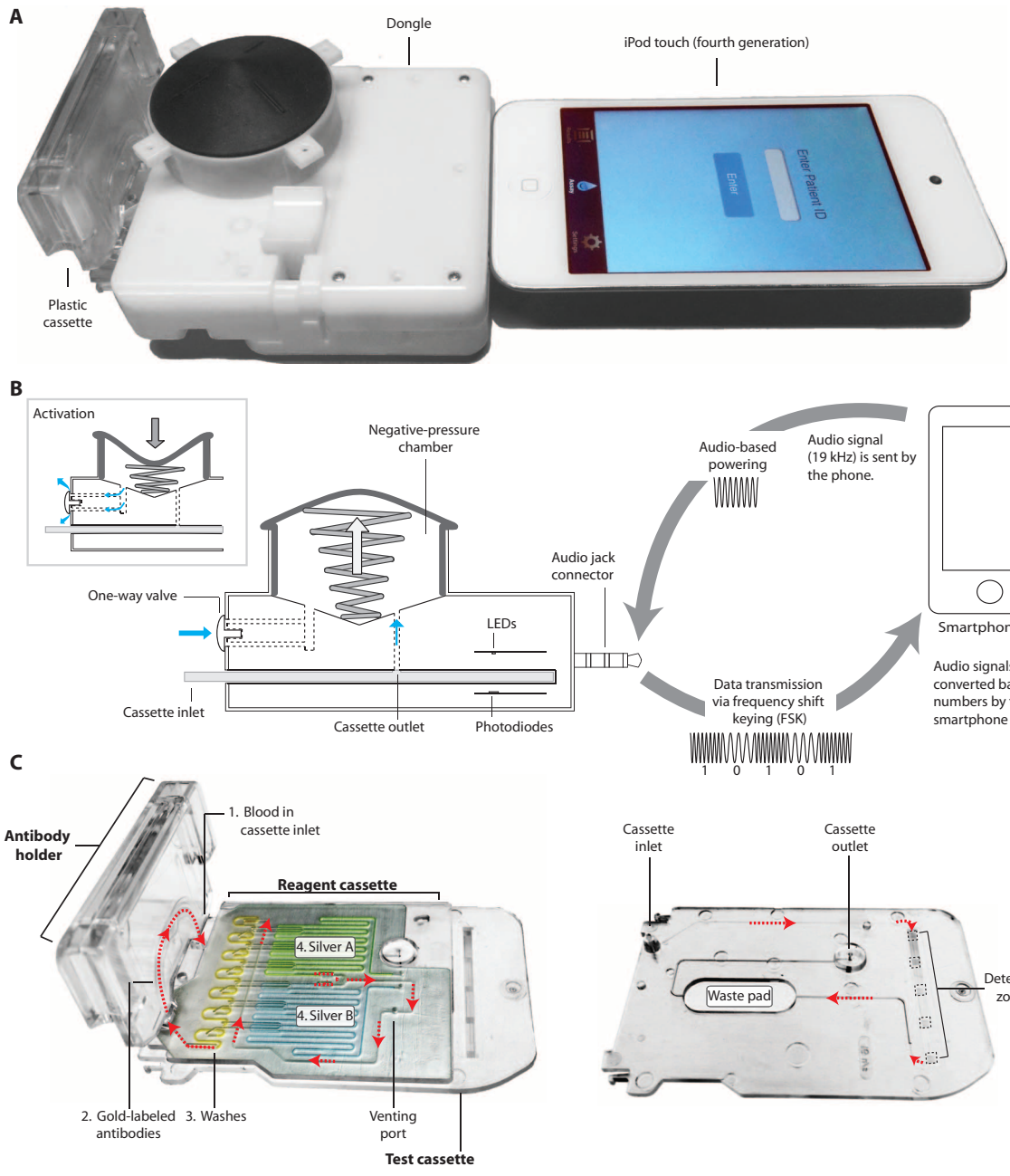

Fig. 1. Overview of the dongle. (**A**) An image of the dongle with a microfluidic cassette connected to an iPod touch. (**B**) Schematic diagram of dongle highlighting a power-free vacuum generator using the audio jack connector for audio-based powering and frequency shift keying (FSK) data transmission to a smartphone or other smart-enabled device. Subfigure shows vacuum activation. (**C**) Left: A reagent cassette (top layer) that contains prestored reagents [washes (yellow) and silver A and B (blue and green)] and the test cassette (bottom layer) that contains five detection zones. Reagents are numbered in the order they flow through the test cassette. First, blood in the inlet flows through the channels, followed by gold-labeled antibodies resolubilized in 9 μl of 1% bovine serum albumin (BSA)/0.05% Tween 20 in phosphate-buffered saline (PBS) and two 2-μl 0.05% Tween 20 in PBS and four 2-μl water washes with air gaps in between. Once the venting port is closed, silver A and B mix and flow through the channels. Right: Sequence of flow through test cassette. From the inlet, fluids move through each detection zone sequentially, then flowing into a waste pad where blood sample and reagents are collected without any fluids exiting the chip. The power-free vacuum chamber connects to the cassette outlet, drawing fluids from the inlet toward the waste pad. (**D**) Comparison of features of conventional ELISA (*6*) versus the dongle in terms of methods and cost for each main module required for the diagnostic test.

D

	ELISA		Smartphone dongle	
	Method	Cost (USD)	Method	Cost (USD)
Electronics	Plate reader	$8,850	Custom PCB (outsource manufacturing in China)	$31
Communication	Data acquistion cable	—	FSK via audio jack	—
Mechanical	Plate washer	$9,600	Power-free vacuum pump (injection moldable case)	$3
Total		$18,450 *+ computer*		$34 *+ smartphone*

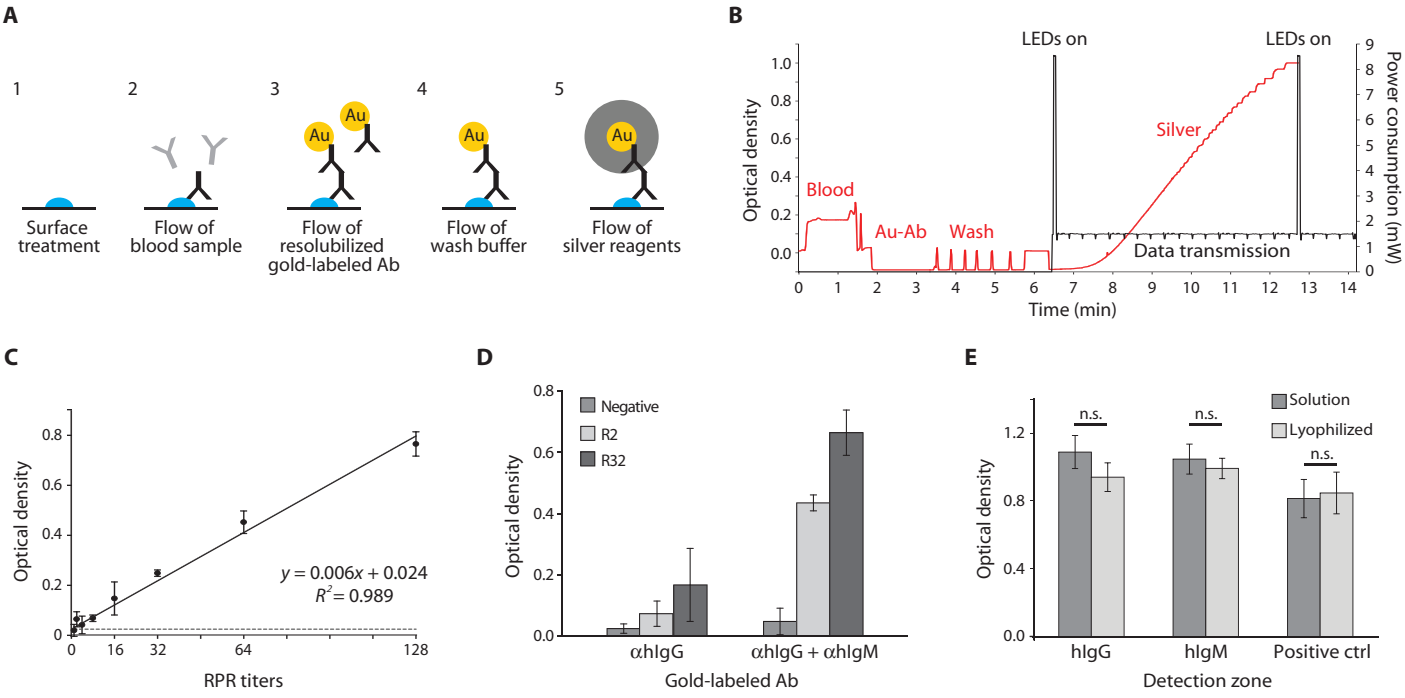

Fig. 2. Assay and field readiness. (**A**) Schematic diagram of assay reactions: (1) Each zone is individually treated with proteins, or none for negative control (ctrl). (2) Whole-blood sample is flowed through the channel, followed by (3) gold (Au)–labeled antibodies (Ab), (4) washes, and (5) silver reagents. (**B**) Power consumption of dongle (black) and OD of the HIV zone (red) during the assay. (**C**) Serial dilution of RPR-positive (1:128) serum to mimic lower RPR titers. Data are averages ± SD (n = 3) and plotted with a linear regression fit and correlation. (**D**) Comparison of signal measurements obtained by addition of gold-labeled anti-human IgM (αhIgM) to gold-labeled anti-human IgG (αhIgG) and gold-labeled anti-human IgG alone as detection antibodies for negative, weak positive nontreponemal syphilis [RPR titer, 1:2 (R2)], and strong positive nontreponemal syphilis [RPR titer, 1:32 (R32)] plasma samples. Data are averages ± SD (n = 4 anti-human IgG; n = 3 anti-human IgG/anti-human IgM). (**E**) Comparison of signal from gold-labeled anti-human IgG and anti-human IgM antibodies lyophilized in a plastic antibody holder and freshly prepared in solution. Detection zones were functionalized with human IgG, human IgM, and rabbit anti-goat antibodies (positive ctrl). Data are averages ± SD (n = 3). n.s., not significant; Student's t test.

testing. These conditions replicated real shipping and transportation conditions, minimized user steps, and increased field readiness to enable a "plug-and-play" operation for the user.

To perform the test, the user mixed 1 μl of whole-blood sample with 9 μl of diluent, placed ~2 μl of the mixed sample into the cassette, attached the antibody holder into the cassette, inserted the cassette into the dongle, pressed the bulb fully to initiate vacuum, and pressed "start assay" on the app to start phase 1 of the test (Figs. 1 and 3A and fig. S5, A to D). After 5 min, all reagents downstream of the venting port (gold-labeled antibodies and washes) will have passed through the chip. For silver development, or phase 2, the user was then prompted to slide the toggle to close a venting port, to initiate flow and mixing of silver A and B (Fig. 1C). The two silver reagents were stable (and stored) separately; the venting port design allowed mixing of the silver reagents immediately before use, minimizing silver autocatalysis. To prevent exposure of chemicals to the user, sample and reagents were contained in a membrane filter within the cassette, and the antibody holder was securely connected to the cassette. OD readings were taken before and after silver development, and at the end of the assay (15 min), results for all markers are available and displayed on the app interface (fig. S5, E and F).

Field testing with target end users

The dongle app presented a user-friendly interface to aid the user through each test, step-by-step pictorial directions, built-in timers to alert the user to next steps, and records of test results for later review (Fig. 3A and fig. S5). Given the simplicity of running the test, training of HCWs (laboratory technicians with no experience in ELISAs) took about 30 min. Five HCWs tested fresh fingerprick whole blood from 96 patients, whose disease statuses were unknown until the reference laboratory results were unblinded at the end of the study.

At three health centers in Kigali, Rwanda, HCWs recruited patient volunteers enrolled in preventing mother-to-child transmission (PMTCT) and voluntary counseling and testing (VCT) programs for the study, with our research team providing further information about the study to participants as needed. Consent forms were translated to Kinyarwanda (the principal local language) and obtained by a third-party translator fluent in English and Kinyarwanda. Fingerprick whole-blood specimens were collected and coded with a study ID number with no link to access other health information, to protect patient privacy. In parallel, venipuncture was performed on the same patients. For reference tests, HIV RDTs were completed at each site, whereas HIV ELISA, syphilis *Treponema pallidum* hemagglutination (TPHA), and syphilis RPR reference tests were performed at the Rwanda National Reference Laboratory using plasma.

The test results for detection of each marker were compared with the gold standard readout from laboratory-based HIV ELISA, TPHA, and RPR and are presented in terms of signal to cutoff of each target relative to its reference test (Fig. 3B and table S2). Cutoff values to determine if a sample was positive or negative for each marker were

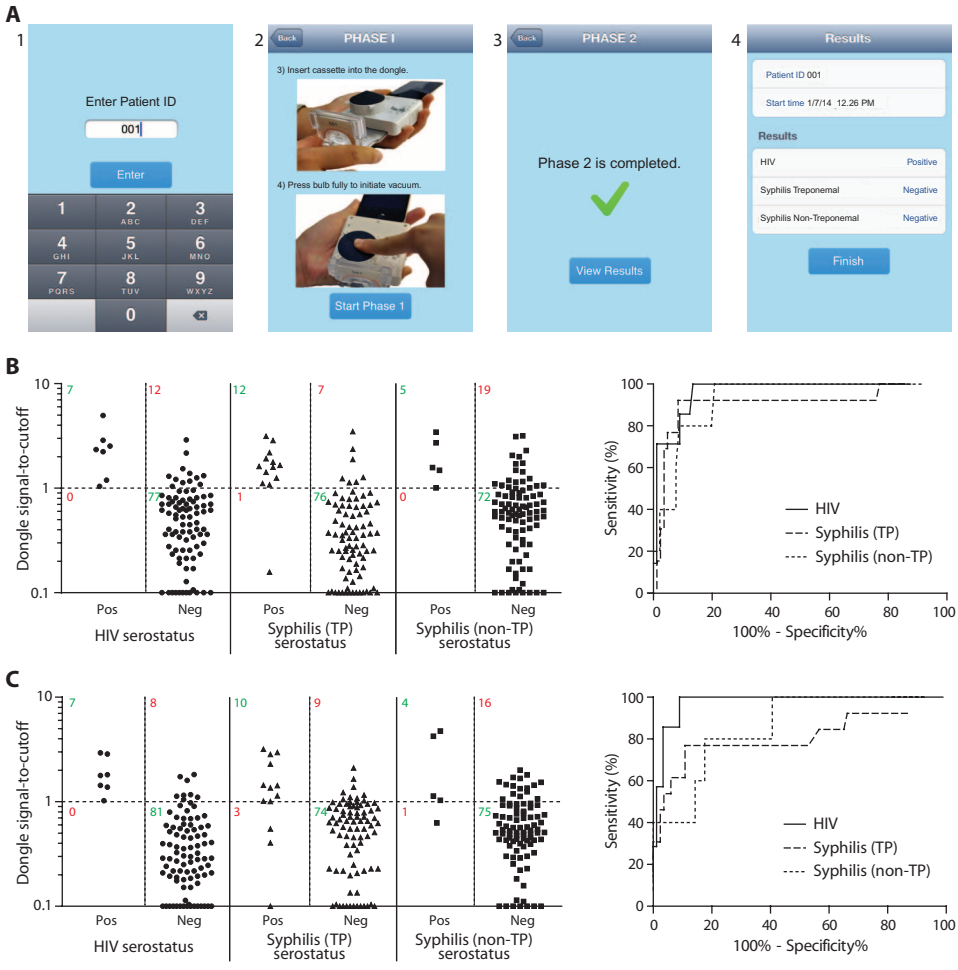

Fig. 3. Field trial in Rwanda. (**A**) User interface on a smartphone shows steps of dongle operation: (1) enter "Patient ID"; (2) step-by-step pictorial instructions starting from sample collection; (3) assay waiting time and status; and (4) results for each disease marker in format of "Positive," "Negative," or "Indeterminate." (**B**) Third-party field testing of the dongle using clinical fingerprick whole-blood specimens. A vertical scatterplot shows dongle device signal-to-cutoff ratios of samples positive (Pos) or negative (Neg) for HIV, treponemal (TP) syphilis, and nontreponemal (non-TP) syphilis as determined by gold standard tests (HIV ELISA, TPHA, and RPR). An ROC curve is provided for each disease marker. (**C**) Field testing by the development team of the dongle using venipuncture whole-blood specimens. Vertical scatterplots and ROC curves for each disease marker.

selected by using receiver-operating characteristic (ROC) curves. Although a final product will offer preset cutoff values, in this development work, we identified cutoff values retrospective to data collection that maximize sensitivity (minimize false negatives) because our test is targeted toward screening applications. Cutoff values for internal negative and positive controls were also applied to verify validity of test results; no tests were excluded on the basis of these criteria. An indeterminate range (for example, if OD is within 10 to 20% of cutoff) (*25*) can be implemented for future tests to indicate the need to rerun the test.

The detection of HIV antibodies had a sensitivity of 100% [95% confidence interval (CI), 59 to 100%] and specificity of 87% (95% CI, 78 to 93%). Sensitivity for detection of treponemal antibodies was 92% (95% CI, 64 to 100%) with specificity of 92% (95% CI, 83 to 97%). Sensitivity for detection of anti-cardiolipin antibodies was 100% (95% CI, 48 to 100%) with specificity of 79% (95% CI, 69 to 87%). ROC curve shows area under the curve of 0.96 for HIV, 0.90 for treponemal syphilis, and 0.92 for nontreponemal syphilis (Fig. 3B). Specifically, table S2 shows results from the dongle that differed from reference tests, with one false-negative result for the treponemal syphilis test, and 12, 7, and 19 false-positive results for HIV, treponemal syphilis, and nontreponemal syphilis, respectively.

On venipuncture whole blood, the dongle yielded a sensitivity and specificity of 100% (95% CI, 59.0 to 100) and 91% (95% CI, 83.0 to 96.0) for HIV, 77% (95% CI, 46.2 to 95.0) and 89% (95% CI, 80.4 to 95.0) for treponemal syphilis, and 80% (95% CI, 28.4 to 99.5) and 82% (95% CI, 73.0 to 89.6) for nontreponemal syphilis (Fig. 3C). There was no significant difference of dongle assay performance on fingerprick and venous whole blood for HIV ($P = 0.45$), treponemal syphilis ($P = 1.0$), and nontreponemal syphilis ($P = 0.33$), using McNemar test.

Subsequent to the field trial, we found that cassettes with an increased concentration of coated proteins could take neat (undiluted) whole blood and produce accurate results (fig. S6 and table S3). The HIV antigen (10 μg/ml) spotting concentration yielded 100% sensitivity (95% CI, 78.20 to 100) and specificity (95% CI, 29.24 to 100), compared to those spotted with antigen (2 μg/ml), which yielded 86.67% sensitivity (95% CI, 59.94 to 98.34) and 33.33% specificity (95% CI, 0.84 to 90.57).

Patient survey

To assess user feedback, a third-party interviewer fluent in English and Kinyarwanda conducted the surveys to reduce any biases from the study team. The questions posed to the participants focused on dongle/fingerprick preference versus traditional venipuncture (and why), desired test time, and whether the dongle would be recommended (and why) (Fig. 4). A vast majority of patients (97%) would recommend the dongle to others because of the fast turnaround time (57%), potential to offer results for multiple diseases (44%), and simplicity of procedure (29%). Fingerprick blood collection was preferred to conventional venipuncture by 95% of patients because it is less painful (98%), it takes shorter time (60%), the HCW had trouble with the needle collection (55%), the patient is scared of a needle (43%), and a fingerprick takes less volume of blood (42%). However, 2% of patients preferred venipuncture because they trust the result more with venous blood (Fig. 4). HCWs appreciated the lack of user interpretation to read the result or external power to operate and also noted in surveys that the dongle could be useful in low-volume testing sites (for example, VCT or mobile visit) or serve as a backup test for high-volume

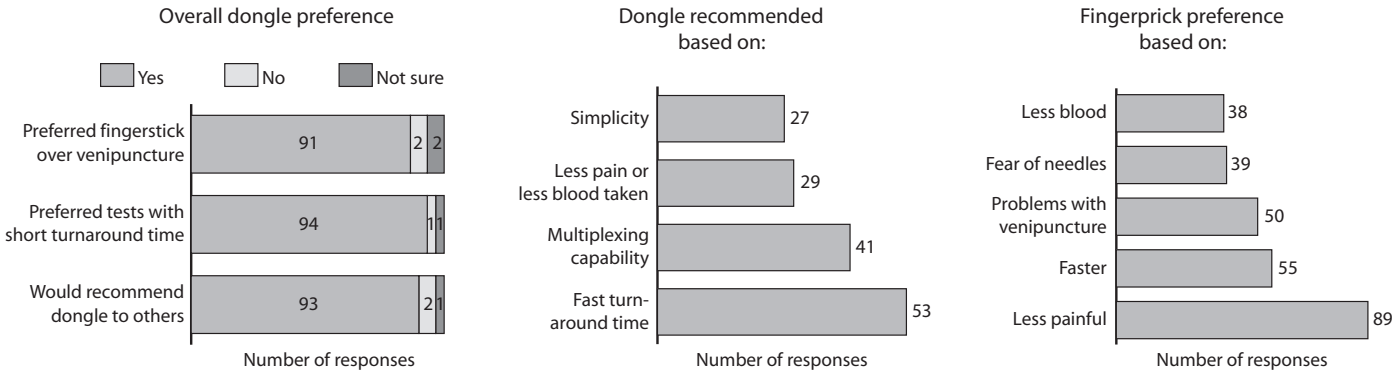

Fig. 4. Satisfaction survey from participants in this study.

patients' clinics in case of power outage, which we experienced during testing.

DISCUSSION

This dongle presents new capabilities for users ranging from health care providers to consumers. For HCWs, management of infectious diseases could be improved with access to multiplexed test at the POC. For example, for syphilis, health impact modeling has suggested that a test with only 70 to 80% sensitivity and specificity, but performed at the POC, can reduce deaths by 10-fold over a hypothetically perfectly accurate laboratory-based test (*26*) by increasing detection of infections. For large-scale screening, high sensitivity (with few false negatives) is most important, as is achieved by the dongle. For HIV, scaling up HIV testing at the community level with immediate antiretroviral therapy could nearly stop HIV transmission and approach elimination of the virus (*27*).

The dual test for syphilis holds clinical value for HCWs. The current procedure in many developing countries calls for a single laboratory-based qualitative RPR test for syphilis, such that all patients with positive nontreponemal results are recommended for treatment. Because this procedure can lead to overtreatment, given the intrinsic lower specificity of nontreponemal assays (*14*, *15*), several countries are adopting treponemal-specific RDTs (*28*). However, blanket treatment of patients testing positive with a treponemal-specific RDT can lead to overtreatment and penicillin resistance, because treponemal antibodies remain even after infection has cleared. A dual nontreponemal and treponemal syphilis test would empower HCWs to follow guidelines (*14*, *15*) that recommend treatment under two scenarios: if both tests are positive, or if treponemal-specific results are positive and the patient either had no previous infection or exhibits signs of new infection. Differentiation between active and past infection is particularly valuable in endemic areas, including Rwanda (*29*). In addition, compared to syndromic management (*11*), POC syphilis tests also help HCWs avoid overtreatment and improve cost-effectiveness; among our subject group, 13 patients exhibited positive TPHA results—although only 4 exhibited positive RPR results—and would all be treated under guidelines with rapid treponemal-specific tests.

It is challenging to use RDTs to detect multiple markers. The dongle is versatile in that it can detect selectively IgM and/or IgG antibodies on multiple markers. This capability supports early detection of syphilis, because anti-treponemal IgM antibodies appear earlier than anti-treponemal IgG by 2 weeks (*11*). Addition of a nontreponemal test and detection of IgM—performed alongside HIV—moves another step toward a complete POC multiplex antenatal-care panel. Performing three individual commercially available tests can cost up to $8.50 [$0.80 to $5 for HIV RDT (*30*), $1 to $3 for treponemal RDT (*15*), and $0.50 for RPR (*31*)]. By comparison, material and reagent cost per test for our triplex test is $1.44, leaving room for a substantially lower anticipated market price. In addition, compared to RDTs, the dongle offers automation of assay, objective readout of signal, and quantitation, although it requires more instrumentation than RDTs. RDTs present challenges for untrained users to execute precise liquid handling and metering, particularly in multistep tests. Our system contains precise injection-molded cassettes, preloaded reagents, optimized optics, and exact alignment that can reduce user variability. Add-on optical readers are becoming available for RDTs but still present challenges to the user (for example, in positioning and illumination accuracy) because RDTs are originally designed to be single-step stand-alone tests (*32*).

Two practical challenges to deploying POC diagnostic devices are specimen collection and infrastructure of facilities. This dongle test required one fingerprick or 2 μl of whole blood for all three tests (avoiding multiple fingerpricks, a point appreciated by the patients in the survey), where HIV and syphilis RDTs require anywhere from 5 to 60 μl each. Another challenge to blood analysis in resource-poor settings is power outages, which is obviated with our audio jack–powered dongle. We show that when so little power is needed for diagnostics, the audio jack connection can power and record an entire laboratory-level immunoassay with amplification and washing reagents (Fig. 2B). Although the audio jack connection has been used for data communication in devices, including for heart rate monitoring (*33*), measuring exhaled carbon monoxide in smokers (*34*), and electrochemical sensing (*8*), it has been less adapted for powering health diagnostics devices, typically owing to large energy consumption.

Consumer adoption of health-monitoring devices is increasing rapidly. This dongle takes a step toward coupling microfluidics with advances in consumer electronics. The hardware of the dongle exhibits characteristics similar to familiar consumer electronics devices through a number of technical innovations: low power (using a power-free, continuous-flow vacuum and requiring no power), durable components (using LEDs and photodetectors), and portability. Our system generates a reliable, repeatable vacuum at the time of the assay while keeping the consumables simple to manufacture, building on previous methods to

simplify the fluid movement steps, such as vacuum (*13*) and degas-driven flow (*35*). We also considered the manufacturability, shipping, and storage of the tests by manufacturing the cassettes and precoating them with proteins and stabilizing agents before shipping to the use location.

In addition, we lyophilized gold-labeled antibodies and anticoagulant onto the antibody holder to increase storage time. The most important feature behind a plug-and-play experience is the reduction of manual steps, enabled by integrating advances in fluidics and mechanical, optical, and electronic components. In the future, robot-assisted loading of reagent cassettes could be implemented, because the reagents were stable for over 6 months at room temperature (*13*, *36*) and wash plugs have been shown to stay separated after airborne shipping (*37*). To enhance the user experience, we built a touch-activated pictorial software interface that allowed for training in 30 min, a step-by-step software guide on the smartphone contemporaneous with assay operation, and reporting of results (yes/no or quantitative titers) without user interpretation. As a result, patients expressed satisfaction with the dongle, citing the 15-min turnaround time as the biggest benefit, because a third of patients wait more than 2.5 hours to receive their results (personal communication with patients). Overall, the combined hardware, software, and microfluidic specifications suggest that new consumer-oriented medical devices are on the precipice of moving beyond glucose monitoring, vital signs, and wellness into clinical diagnostics for endemic diseases, including HIV and syphilis.

MATERIALS AND METHODS

Study design

The goal of this study was to develop a power-free lab-on-a-chip device for HIV and syphilis diagnosis in resource-limited settings. To evaluate dongle performance, the device was deployed in Rwanda, into the hands of HCWs who did not have ELISA training, for prospective diagnosis of patients already scheduled to receive HIV and syphilis testing. This study was approved by Columbia University Institutional Review Board (IRB) and Rwanda National Ethics Committee. Study sites were selected from the highest HIV/syphilis prevalence in the PMTCT group, based on 2013 routine data from the HIV division at Rwanda Biomedical Center. We selected three community-level health centers (Kimironko, Biryogo, and Gahanga) in Kigali with guidance from HIV/AIDS and STIs Diseases Division, Rwanda Biomedical Center, based on incidence of disease and willingness to participate in the study. HCWs involved in the study were laboratory technicians at the health centers and selected on the basis of willingness to participate in the study (Supplementary Materials and Methods). All patients who were enrolled in PMTCT or VCT programs over a 2-week period and who provided consent participated in this study. We did not perform power analysis; instead, we chose a sample size of ~100, which allowed us to focus on incorporating device testing with clinic flow, getting user feedback, and assessing patient reception, in resource-limited settings. Additional patient information is in Supplementary Materials and Methods.

After sample collection, patients were given a short survey about their experiences by a third-party translator. Patients were compensated 1000 RWF (Rwandan Franc) ($1.54) for their participation. Disease statuses were blinded to the Columbia team and HCWs who conducted the test. HIV RDTs (Colloidal Gold, Determine, and Uni-Gold) were completed at each site. HIV ELISA (Vironostika), syphilis TPHA (Spinreact), and RPR (Spinreact) as reference tests were performed at Rwanda National Reference Laboratory using plasma separated from venipuncture blood. At the end of the trial, the results were unblinded to the study team, and reference test results were compared to the results obtained by the dongle.

Dongle design and manufacture

Custom-printed circuit boards were designed in Altium and printed from PCB Universe. A bill of materials and the circuit diagram are provided (table S1 and fig. S7). LEDs and photodiodes were precisely aligned with the cassette slot so that testing zones aligned without manual effort. One-millimeter pinholes made of 1-mm-thick black Delrin (McMaster-Carr) were aligned above each photodiode to prevent stray light. The dongle casing was designed in SolidWorks and printed in-house (Objet24 3D Printer, Stratasys). Vacuum chamber was created with a one-way umbrella valve (Minivalve), a rubber bulb from a 140-ml syringe (Becton Dickinson), and a conical spring (Century Spring Corp.) inside to aid reexpansion. Silicone rubber O-rings and sheets (McMaster-Carr) were used to connect to outlet and seal the venting port. Power-free fluid flow, signal readout, and audio jack powering and signal transmission are provided in Supplementary Materials and Methods.

Cassette preparation

All cassettes were prepared at Columbia University before transporting to Rwanda. We added disease-specific proteins to the cassette surface by direct physisorption with a stabilizing agent (StabilCoat, SurModics), except cardiolipin, which was covalently attached to the plastic surface using EDC-sulfo-NHS reaction (Supplementary Materials and Methods). We used robot-assisted manufacturing (adapted from OPKO Diagnostics) for reproducible and high-throughput cassette preparation. We selected recombinant multiepitope chimeric antigens (gp41, gp36, and O-IDR) for an HIV 1/2 (BioLink International) marker, a 17-kD recombinant outer membrane protein TpN17 (Lee Laboratories) (*6*, *13*) for a treponemal syphilis marker, synthetic cardiolipin prepared from plant source (*38*) provided by CDC for a nontreponemal syphilis marker, and rabbit anti-goat IgG (Life Technologies) for a positive control. For an internal negative control (provide background signal), the surface was not functionalized with any protein but treated with blocking agent. Gold-labeled anti-human IgG and IgM antibodies were lyophilized inside the antibody holder by OPKO Diagnostics.

Each day before testing at the clinic site, two 2-μl PBS–0.05% Tween 20 and four 2-μl water washes as well as 60 μl each of silver nitrate (silver A) and silver reducing agent (silver B) were loaded to the reagent cassette manually by pipetting and sealed using an adhesive tape (OPKO Diagnostics) to mimic prepackaged reagents (Fig. 2A).

Device operation

To perform the test, the user (HCW) collected fingerprick whole blood using conventional methods. One microliter of whole blood was then diluted with 9 μl of 1% BSA–0.05% Tween 20 in PBS, and 2 μl of the mixed sample was pipetted onto the disposable test cartridge (fig. S5B). Cassette preparation is described in Supplementary Materials and Methods. The user inserted the antibody holder (prefilled with 9 μl of 3% BSA–0.05% Tween-PBS) into a microfluidic cassette, inserted the cassette into the dongle, and pressed the bulb fully to initiate vacuum (fig. S5, C and D). After 5 min, the user was prompted to move a toggle to close a venting port and to initiate silver development for 9 min.

Afterwards, results for all markers were displayed on the screen (fig. S5F), and raw absorbance values were recorded along with the study ID.

Each patient venipuncture whole blood was tested by the Columbia team in parallel with fingerprick blood testing. An aliquot of venipuncture whole blood (~50 μl) was made from each whole blood collected. Venipuncture whole blood was processed in the same manner as the fingerprick blood.

Post–field test optimization in the laboratory is described in Supplementary Materials and Methods.

Pilot field trial

Study sites, HCWs, populations, and confidentiality agreements, as well as dongle operation training and testing are described in Supplementary Materials and Methods.

Statistics

Averages, SDs, linear fit, and two-sided Student's t tests ($\alpha = 0.05$) were calculated with Microsoft Excel. Student's t test was chosen to compare two small sets of quantitative data when data in each sample set were related. Vertical scatterplots, sensitivity, specificity, 95% CIs, ROC curves, and McNemar test were created in GraphPad Prism.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/7/273/273re1/DC1
Materials and Methods
Fig. S1. User-activated negative pressure–driven flow.
Fig. S2. Smartphone-dongle interface and comparison with benchtop analyzer.
Fig. S3. Stability of functionalized protein on microfluidic cassette.
Fig. S4. Comparison of blocking agents.
Fig. S5. Step-by-step illustration of dongle testing.
Fig. S6. Optimization of dongle HIV assay using undiluted whole-blood samples.
Fig. S7. Circuit diagram of dongle.
Table S1. Bill of dongle materials and cost per component.
Table S2. Raw data from field testing in three Rwandan clinics with reference results.
Table S3. Raw data from venipuncture whole blood from Columbia University Medical Center (CUMC) with reference results for optimization of HIV antigen concentration.

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A smartphone dongle for diagnosis of infectious diseases at the point of care

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Editor's Summary

Dongle + app = mobile test for sexually transmitted diseases

There are thousands of health-related "apps" for smartphones, from tracking sleep patterns to recording heart rate to logging caloric intake. The power of such apps in connecting resource-limited communities to health care workers and, in turn, to proper and immediate care is now emerging. In this issue, Laksanasopin and colleagues describe a microfluidic-based diagnostic test for HIV and syphilis that attaches to (and is powered by) the iPod's headphone jack. The mobile test also comes complete with an easy-to-use app, flashing test results on-screen in under 15 min. The test is based on the standard immunoassay but uses gold-labeled antibodies to detect HIV and syphilis antigens in only 2 μl of whole blood, and then silver reagents to amplify the resulting signal. The authors deployed the dongle in Rwanda, testing its sensitivity and specificity on 96 patients. Evaluated side by side with the gold standard tests for HIV and syphilis, the dongle produced results with a sensitivity and specificity needed for making treatment decisions in the field. In a survey, a vast majority of patients reported satisfaction with dongle performance. After a few next-generation tweaks, including reducing the size of the dongle, the entire diagnostic package is ready for adoption in resource-poor clinics and communities, to improve detection of HIV and syphilis and empower health care workers to administer timely and appropriate treatments.

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The Sexually Transmitted Diseases Diagnostics Initiative (SDI)

UNDP/World Bank/WHO
Special Programme for Research and Training in Tropical Diseases (TDR)

TDR/SDI/DE/03.1

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Laboratory-based evaluation of rapid syphilis diagnostics

Results from 8 SDI Sites

The Sexually Transmitted Diseases Diagnostics Initiative (SDI)

UNDP/World Bank/WHO
Special Programme for Research and Training in Tropical Diseases (TDR)

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Table of contents

Executive summary

Evaluation of the performance and utility of simple rapid tests for sexually transmitted diseases in primary health care settings in developing countries is a priority for SDI. Over 20 rapid treponemal tests for the diagnosis of syphilis are commercially available but reliable information on their performance characteristics is limited. This report is the first in a series of laboratory-based evaluations to assess the performance characteristics of rapid syphilis tests and to identify promising candidates for further evaluations in field settings. Six rapid treponemal tests were evaluated in eight SDI laboratory sites, using 789 archived serum samples. The sensitivity of the rapid tests ranged from 85-98% and the specificity ranged from 93-98%, compared against *Treponema pallidum* Haemagglutination Assays (TPHA) or *Treponema pallidum* Particle Agglutination Assays (TPPA) as reference standards. In general, tests with higher sensitivities tend to have lower specificities and vice versa. All tests showed reasonable reliability and were considered by site staff as easy to use. Four of these tests have been selected for further evaluations of test performance and utility in field settings. Evaluations in the field will provide an opportunity to assess test performance using whole blood specimens and to conduct operational research studies to assess the sustainability and impact of using these rapid tests in a disease control programme. It is hoped that rapid syphilis tests will prove to be useful tools in the control of syphilis and in the elimination of congenital syphilis.

1. Background

The Sexually Transmitted Diseases Diagnostics Initiative (SDI) is a programme within the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

The mission of the SDI is to promote the development, evaluation and application of affordable, rapid, simple point-of-care sexually transmitted infections (STI) diagnostics appropriate for primary health care settings in developing countries. The placement of SDI in the Product Research and Development group of TDR allows the initiative to benefit from the considerable expertise in product development, evaluation and implementation in the group and to exploit synergies in the development of diagnostics for other communicable diseases.

According to the latest WHO global estimates, 386 million new cases of curable STI occur every year, of which 80-90% are in the developing world, where there is poor or no access to diagnostics. A large proportion of STIs present with little or no symptoms, but undiagnosed and untreated infection often lead to serious reproductive sequelae and adverse outcome of pregnancy. In particular, STI diagnostics are urgently needed for HIV endemic areas, as studies in sub-Saharan Africa have shown that STIs are important co-factors in the transmission of HIV infection.

At a joint SDI-Wellcome Trust meeting on rapid STI diagnostics for primary health care settings in developing countries, evaluation of the performance and utility of rapid syphilis diagnostics in preventing congenital syphilis was identified as an important priority for SDI.

Serologic tests are important tests for the diagnosis of all stages of syphilis, and are the only means of identifying infection in asymptomatic individuals and in patients whose lesions cannot be tested for *Treponema pallidum*, the causative agent of syphilis. Flocculation type tests, such as the rapid plasma reagin (RPR) test, are widely used for syphilis screening. Although rapid and simple to use, these flocculation type tests require equipment, training and are non-specific for syphilis, as the tests detect antibodies to cardiolipin. Positive non-treponemal test results usually require confirmation with treponemal-specific tests such as the *Treponema pallidum* Haemagglutination Assay (TPHA) and *Treponema pallidum* Particle Agglutination Assay (TPPA). However, these confirmatory tests are technically demanding and not widely available in most developing country settings outside of reference laboratories. Simple, rapid, point-of-care treponemal specific tests are now commercially available. These tests may be suitable for use in primary health care settings to identify patients for presumptive treatment or for confirming non-treponemal test results, but there is limited data on their performance characteristics and utility in primary health care settings.

Over 20 rapid syphilis tests are currently commercially available. Given the high cost of field trials, a triage step is necessary to select a limited number of the most promising tests for field trials. Hence the SDI rapid test evaluations will be carried out in two phases:

1) a laboratory-based evaluation of test performance and reliability using archived serum specimens from diverse geographic locations;

2) field trials of test performance, acceptability to patients and care providers, and utility for disease control and prevention.

This report describes the results from the first phase of the evaluation only.

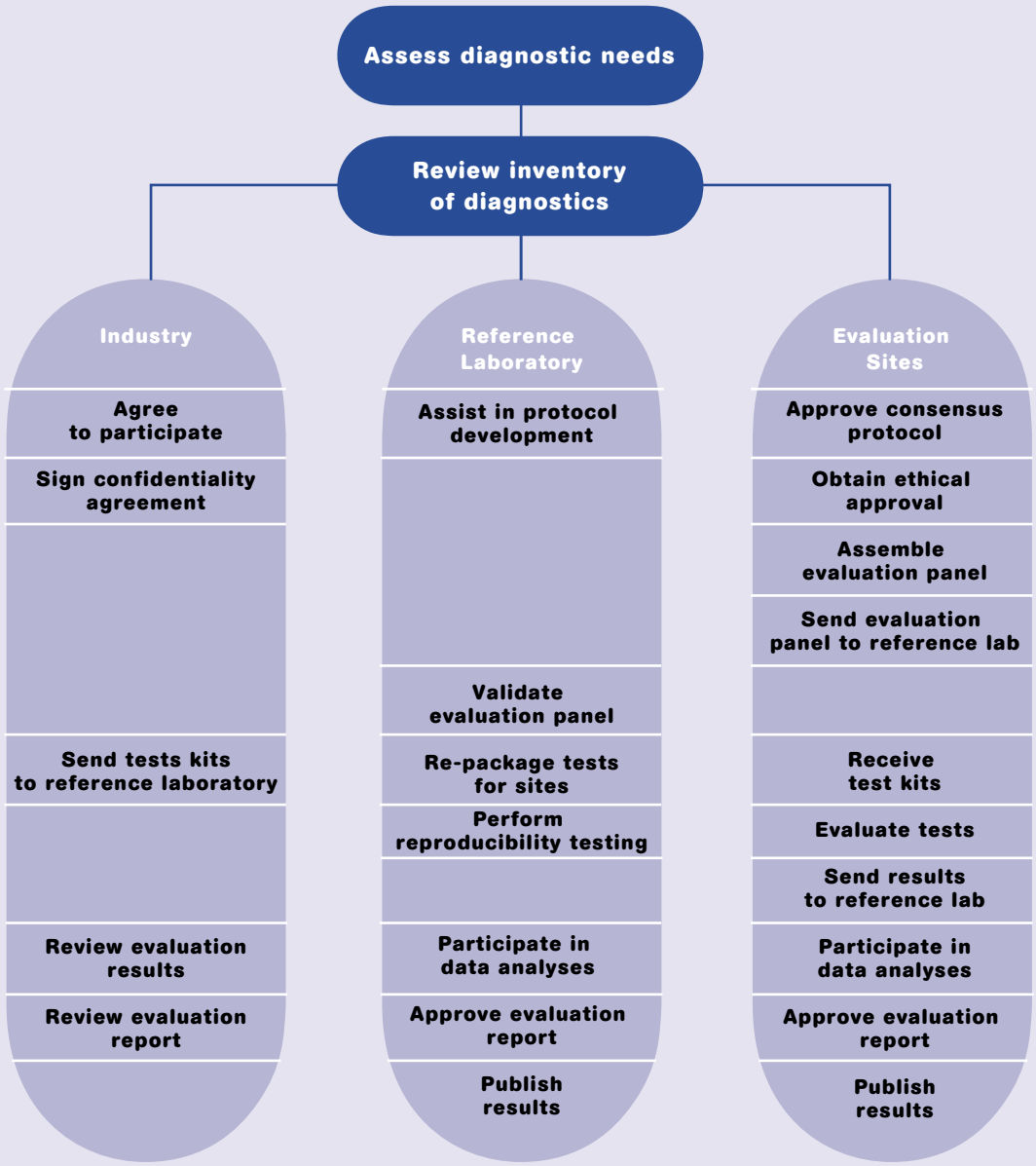
2. Objectives

1. To compare the performance of rapid treponemal tests against current reference standard tests such as the *Treponema pallidum* Haemagglutination Assay (TPHA)

2. To assess the operational characteristics of rapid treponemal tests, including the ease of use, technical complexity and inter-reader variability

3. Evaluation plan

Figure 1. Laboratory-based evaluation of rapid syphilis diagnostics



SDI coordinated the various components of the evaluation according to the plan as shown in Figure 1 (see facing page). Communications amongst sites and with reference laboratories were encouraged. Contact details for all the sites and the reference laboratories are contained in Annex 1.

3.1. Tests under evaluation

At the first meeting of the ad hoc SDI Expert Working Group for laboratory-based evaluations, it was agreed that the tests to be included in this evaluation should have the following operational characteristics:

- **Rapid** – Test result is available in less than 30 minutes.
- **Simple** – Test can be performed in 3-4 steps, requiring minimal training and equipment
- **Easy to interpret** – Card or strip format with visual readout.

A letter was sent from SDI to companies that manufacture and/or sell tests that fit the above inclusion criteria, to inform them of the SDI rapid diagnostics evaluation scheme. Companies which agreed to participate in the evaluation were asked to donate tests for the evaluation and to sign an agreement for the results of these evaluations to be published in a WHO report and made available to health departments of WHO member states.

Six companies agreed to participate in the first of these evaluations:

1. **Abbott Laboratories** USA
2. **Diesse Diagnostica** Italy
3. **Fujirebio Inc.** Japan
4. **Omega Diagnostics** Scotland
5. **Qualpro Diagnostics** India
6. **Standard Diagnostics** South Korea

A table summarizing the characteristics of these tests can be found on the following page.

At the conclusion of the evaluation, companies were sent a courtesy draft of the report prior to publication. Companies could review the data from each site and data analyses and send their comments to SDI, but they were not in a position to modify any of the conclusions, in accordance with the terms of the Confidentiality Agreement they signed with WHO.

Table 1. Rapid syphilis diagnostics under SDI evaluation

Name	Determine Syphilis TP	Syphilis Fast	Espline TP	Syphicheck-WB	SD BIOLINE Syphilis 3.0	VISITECT Syphilis
Company	Abbott Laboratories Chicago, USA www.abbottdiagnostics.com	DIESSE Diagnostica Senese SpA, Milan, Italy www.diesse.it	Fujirebio Inc. Tokyo, Japan www.fujirebio.co.jp	Qualpro Diagnostics Goa, India www.tulipgroup.com	Standard Diagnostics,Inc. Kyunggi-do, Korea www.standardia.com	Omega Diagnostics Ltd. Scotland, UK www.omegadiagnostics.co.uk
Assay type	Immuno-Chromatography	Latex particle agglutination	Immuno-chromatography	Immuno-chromatography	Immuno-chromatography	Immuno-chromatography
Antigen	TpN47	TpN15, TpN17	TpN15-17, TpN47	TpN17, TpN47	TpN15, TpN17, TpN47	TpN17, TpN47
Solid phase	membrane strip	card	membrane strip in cassette	membrane strip in cassette	membrane strip in cassette	membrane strip in cassette
Specimen type	whole blood plasma serum	serum	plasma serum	whole blood plasma serum	whole blood plasma serum	whole blood plasma serum
Number of tests per package	10 tests/card	50	10x5	10 or 25/pack	30	25
Shelf life	24 months at 2-30°C	18 months reagents stable for 6 months at 2-8°C after reconstitution	9 months at 2-10°C	18 months at 4-30°C	18 months at room temperature	24 months at 4-30°C
Volume of sample	50 µl whole blood or 50 µl serum/plasma	20 µl serum	25 µl serum/ plasma	50 µl whole blood or 25 µl serum/plasma	20 µl whole blood or 10 µl serum/plasma	50 µl whole blood or 25 µl serum/ plasma
Supplies required but not provided	micropipette and tips for 50 µl	micropipette and tips for 20 µl and 40 µl	micropipette and tips for 25 µl	none	micropipette and tips for 10 µl and 20 µl	none
Results available	5-20 minutes	8 minutes	15 minutes for reading	15 minutes	5-20 minutes	15 minutes
Price/test (US$) (from company)	< 2.00	not available	3.30	0.75	0.90	< 1.00

3.2. Study sites

A request for applications from laboratories interested in participating in the evaluation of rapid syphilis diagnostics was posted on the WHO/TDR web site and distributed through the SDI mailing list. Applicants were asked to respond to a questionnaire regarding laboratory capacity and experience with diagnostics evaluation. Laboratories with relevant experience, facility and capacity for test kit evaluations were asked to send a qualifying panel of 20 sera, along with the corresponding test results, to one of the SDI Reference Laboratories for validation. The syphilis reference laboratories at the U.S. Centers for Disease Control and Prevention (CDC), and the Public Health Laboratory Service (PHLS) in the United Kingdom agreed to act as SDI Reference Laboratories.

Of the 26 laboratories that applied, eight laboratories were selected for the SDI network, based on their proficiency at performing syphilis serology, access to populations of moderate to high disease prevalence, capacity and ability to carry out evaluations in a timely manner and links to field sites for evaluation. Sites were selected from diverse geographic locations to determine if test performance is influenced by endemic conditions. Each site signed a Technical Services Agreement with WHO. The evaluation data is jointly owned by WHO and the sites.

Table 2. SDI sites for laboratory-based evaluations of rapid syphilis diagnostics

Site location	Institution	Principal applicant
AFRICA		
Durban, South Africa	University of Natal	W. Sturm
Fajara, The Gambia	MRC Laboratories	B. West
Mwanza, Tanzania	National Institute for Medical Research	J. Changalucha
ASIA		
Nanjing, China	National Center for STD and Leprosy Control	Y.P. Yin
Colombo, Sri Lanka	National STD/AIDS Control Programme	S. Mananwatte
AMERICAS		
Port au Prince, Haiti	Les Centres GHESKIO (Groupe Haitien d'Etude du Sarcome de Kaposi et des Infections Opportunistes)	J.W. Pape D.W. Fitzgerald
Birmingham, USA	University of Alabama	E. Hook III
EUROPE		
Moscow, Russian Federation	Central Institute for Skin and Venereal Diseases	A. Kubanova E. Filatova

3.3. Sources of sera for evaluation

Each laboratory site was asked to assemble an evaluation panel from their collection of archived serum specimens as follows:

1) 50 TPHA/TPPA positive specimens including:
- 40 RPR+, TPHA/TPPA+
- 10 RPR-, TPHA/TPPA+

2) 50 TPHA/TPPA negative specimens including:
- 40 RPR-, TPHA/TPPA-
- 10 RPR+, TPHA/TPPA-

Each site complied with the request as much as possible but not all sites had sufficient quantities of characterised serum specimens to comply fully. The evaluations in this report were undertaken using 789 serum samples available from the 8 sites, 399 of which were TPHA or TPPA positive.

It was of interest to include in the evaluation panel serum specimens that represented biological false positive results (RPR+, TPHA/TPPA-) and past treated infections (RPR-, TPHA/TPPA+). Since the numbers are limited, RPR results were not used in the data analyses.

The evaluations in this report were undertaken using only serum samples as specified above.

3.4. Sample size calculations

Each rapid test was evaluated at each of the eight laboratories using a locally assembled panel of 100 sera. For each test under evaluation, the use of 800 sera, of which 50% are positive, is an adequate sample size for a precision of +3% around the point estimate for test sensitivity, if the sensitivity is around 50%. For tests with greater sensitivity and for specificity, the precision will be even greater.

4. Evaluation site preparation

4.1. Consensus protocol

A standardised protocol, entitled the "Manual of Operations for Rapid Syphilis Test Evaluations", was developed with the SDI ad hoc Expert Working Group and the site principal investigators prior to the evaluation. The consensus protocol was used by all the sites for the evaluation.

4.2. The Evaluation Team

The evaluation team at each site consisted of the principal applicant, a technical supervisor and two technicians. The responsibilities of each evaluation team member were as follows:

Principal applicant:

- Participate in the development of the consensus protocol
- Obtain ethical committee approval for the evaluation protocol and for the use of archived sera for the evaluation
- Ensure the evaluation is conducted according to the consensus protocol as approved
- Send data to SDI for collation with data from other sites
- Participate in the overall analyses of the evaluation results

Technical supervisor:

- Ensure all personal identifiers and data are unlinked from the serum specimens selected for rapid test evaluation
- Ensure that both technicians are blinded to the reference test results for the evaluation panel by assigning the sera a study code (sera numbered 1 to 100 with each number preceded by the first two letters of the site location e.g. the evaluation panel from the Durban site coded DU 1-100)
- Supervise the pilot run and the performance of the rapid test evaluations
- Ensure that the results of the rapid tests are read independently by technicians 1 and 2
- Sign off the laboratory books of technicians 1 and 2 at the end of each day
- Collate the results from the two technicians and enter them into the spreadsheet provided by SDI

Technician 1:

- Perform rapid tests in accordance with manufacturers' directions
- Record results in a laboratory record book
- Place completed tests in a folder for technician 2 to read
- Assess the operational characteristics of each rapid test according to the scheme provided

Technician 2:

- Read results of rapid tests independently of technician 1 for assessment of inter-reader variability
- Record results in a separate laboratory record book from that used by technician 1
- Read results again after one hour

4.3. Ethical considerations

Each evaluation site obtained institutional review board or ethics committee approval for performing the evaluations in accordance with the consensus evaluation protocol and for the use of unlinked archived sera in the evaluation panel. Each site documented, to the satisfaction of the local ethics committee, the mechanism whereby all personal identifiers and patient information were unlinked from the serum specimens so that the sera could not be traced to individual patients.

4.4. Preparation of evaluation panels

Each site assembled 100 sera according to the characteristics described in section 3.3. Each serum sample was divided into two aliquots, one of which was stored frozen on site and the other sent to the SDI Reference Centres for further testing if necessary.

4.5. Blinding to reference standard results

The laboratory supervisor at each site ensured that the specimens were coded with the first two letters of the site location and numbered 1 to 100. All personal identifiers and data were unlinked from the serum specimens selected for evaluation. Both technicians were blinded to the reference test results.

4.6. Piloting the study protocol

At each site, the technicians performed each of the tests under evaluation with two positive and one negative sera from the evaluation panel under the supervision of the technical supervisor. The tests were read by both technicians. If the results were invalid, the tests were repeated. The supervisor and technicians proceeded with the evaluation only when they were confident regarding every aspect of the evaluation.

5. The evaluations

5.1. Performing the rapid tests

5.1.1. General guidelines on the use of test kits:

1) Note lot number and expiry date: a kit should not be used beyond the expiry date
2) Ensure correct storage conditions: if a desiccant is included in the package, do not use the kit if the desiccant has changed colour
3) If test kits are stored in the refrigerator, they should be brought to room temperature (about 30 minutes) before use. The use of cold test kits may lead to false-negative results
4) Damaged kits should be discarded
5) Once opened, a test kit should be used immediately
6) Reagents from one kit should not be used with those of another kit
7) Test should be performed exactly as described in the product insert/instructions.

5.1.2. Biosafety guidelines:

- Treat all specimens as potentially infectious
- Wear protective gloves and laboratory gown while handling specimens
- Do not eat, drink or smoke in the laboratory
- Do not wear open-toe footwear in the laboratory
- Clean up spills with appropriate disinfectants e.g. 1% bleach
- Decontaminate all materials with an appropriate disinfectant
- Dispose of all waste, including test kits in a biohazard container.

5.1.3. Preparing serum samples for testing:

All serum samples should be brought to room temperature before use. If a precipitate is visible, the serum should be clarified by centrifuging at 12,000g for 5 minutes prior to testing.

5.1.4. Order of testing:

For the evaluation of multiple rapid tests, to avoid comparison of results between tests for each serum sample, each rapid test should be evaluated with the entire panel of 100 sera before evaluating another test. The order in which the various kit brands are evaluated is left up to each site. The dates of each evaluation for each serum sample should be noted.

For each rapid test, it was recommended that the evaluation should be conducted in batches of 25 sera each. The evaluation was conducted with the entire panel of 100 sera before any repeat testing was carried out if invalid results were obtained.

5.2. Standard Operating Procedures (SOPs)

5.2.1. SOPs for test kits under evaluation:

The following pages contain an illustrated summary of the test procedure for each of the tests covered in this report. For full details and any questions regarding the SOPs, please refer to the product insert for each test kit.

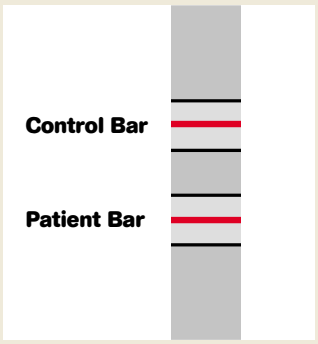
1. Abbott Laboratories
Determine Syphilis TP

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

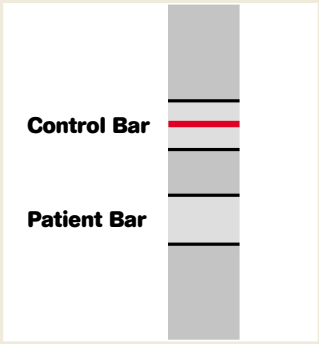
- **micropipette and tips, volume 50 µl**

STANDARD OPERATING PROCEDURE:

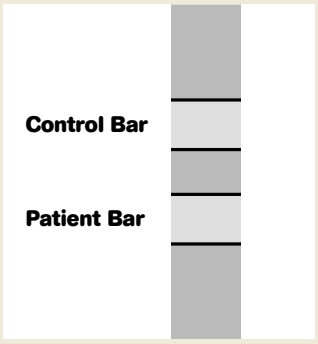
1) Remove the protective foil cover from each test
2) Using a micropipette, apply 50 µl of serum to the sample pad (marked by arrow symbol)
3) Wait a minimum of 15 minutes before reading the test result
4) Interpret test results as follows:



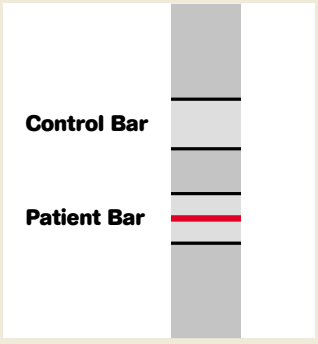
Positive



Negative



Invalid



Invalid

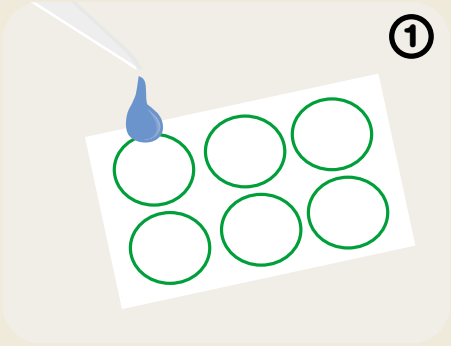
2. Diesse Diagnostica Syphilis Fast

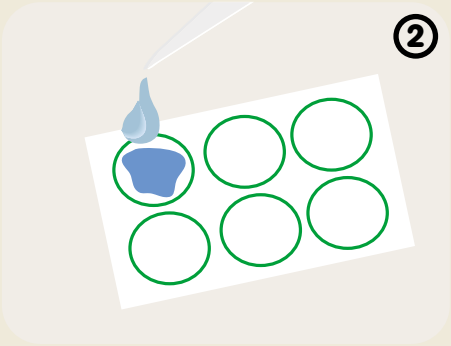
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

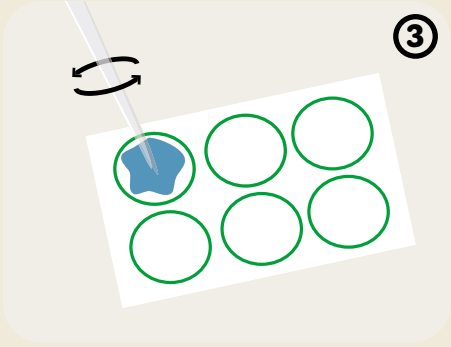
- **micropipette and tips, volumes 20 µl and 40 µl**
- **automatic rotator (optional)**

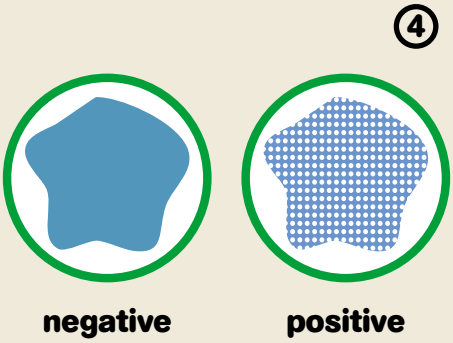
STANDARD OPERATING PROCEDURE:

1) Place 40 µl serum into a circle on the card
2) Add 20 µl coated latex (R1) into the same circle
3) Mix with stick provided and rotate for 8 minutes. When using an automatic rotator, set at 100 rpm
4) Read test after 8 minutes. The presence of a flocculation pattern in the circle indicates a positive test









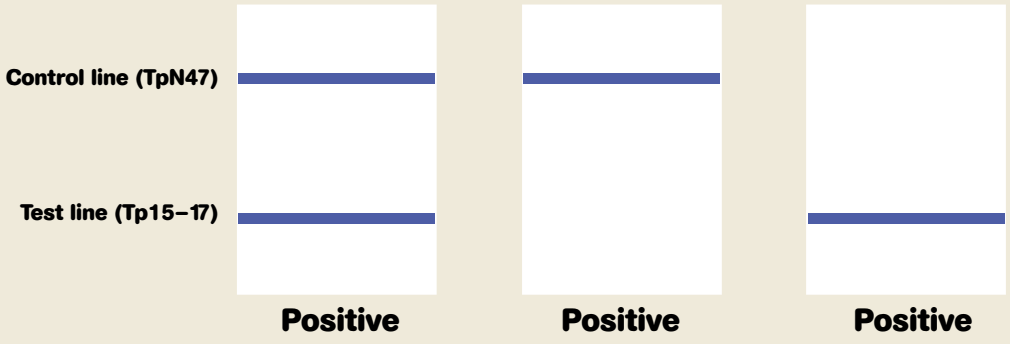
3. Fujirebio Inc
Espline TP

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- **micropipette and tips, volume 25 µl**

STANDARD OPERATING PROCEDURE:

1) **Allow the test cassettes to warm up to room temperature in the aluminum pouch (30 minutes)**
2) **Remove the test cassette from the aluminum pouch**
3) **Using a micropipette, add 25 µl of serum to the sample window of the cassette**
4) **Quickly push on the protruding part of the cassette marked with 3 lines, to release the developing solution inside the cassette**
5) **Let the cassette stand in a horizontal position for 15 minutes**
6) **Interpret the results as follows:**



Positive **Positive** **Positive**

Negative

Note:
Part of the blue test line may sometimes be missing.
Please judge the test as positive in such a case.

4. Omega Diagnostics VISITECT Syphilis

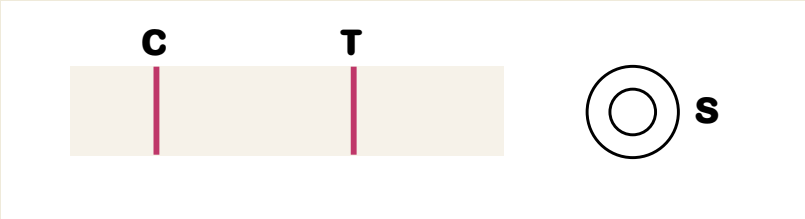
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

None

STANDARD OPERATING PROCEDURE:

1) Use dropper provided, dispense 1 drop of serum to sample well S
2) Add 2 drops of diluent buffer from the buffer bottle to sample well S
3) Read results after 15 minutes

Negative: Appearance of only one pink to deep purple coloured line at the control region "C"



Positive: In addition to the control line, a distinct pink to deep purple coloured line also appears in the test region "T"

Invalid: If no coloured line appears within the result window after performing the test, the result is considered invalid

5. Qualpro Diagnostics Syphicheck-WB

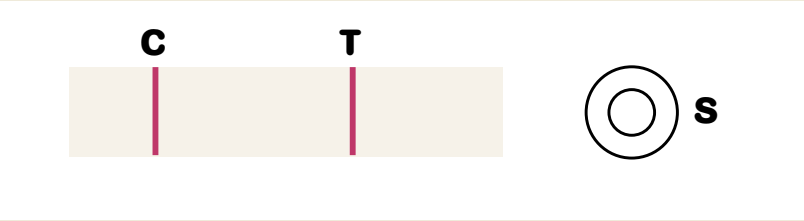
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

None

STANDARD OPERATING PROCEDURE:

1) Remove the test from the pouch and place on a flat surface
2) Using the dropper provided, add 1 drop of serum to the sample well S
3) Add 2 drops of diluent buffer from the diluent bottle to sample well S
4) Read the results in 15 minutes as follows:

Negative: Appearance of only one pink to deep purple coloured line at the control region "C"



Positive: In addition to the control line, a distinct pink to deep purple coloured line also appears in the test region "T"

Invalid: If no coloured line appears within the result window after performing the test, the result is considered invalid

6. Standard Diagnostics, Inc
SD BIOLINE Syphilis 3.0

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- **micropipette and tips, volume 10 µl**

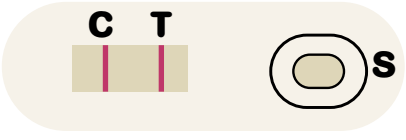
STANDARD OPERATING PROCEDURE:

1) Remove the test from the foil pouch and place on a flat, dry surface
2) Slowly add 10 µl of serum to the sample well S
3) Add 3 drops of assay diluent to the sample well S
4) Read the test at 5-20 minutes as follows:

Syphilis

Negative result:
The presence of only one band at "C" within the result window

Syphilis



Positive result:
The presence of two colour bands ("T" and "C") within the result window, no matter which band appears first

Syphilis

Invalid result:
If the purple colour band is not present within the result window after performing the test

5.2.2. Determining inter-reader variability and stability of test results

In many clinic settings, staff may be unable to read the test at the designated time of 15-20 minutes after the specimen was added. It is therefore of interest to determine if test results are stable after one hour.

1) Each test was performed and read by technician 1 according to the instructions described and the results recorded in a laboratory record book

2) The test was then placed in a numbered folder and handed to technician 2

3) Technician 2 interpreted the test result independently immediately on receipt of the folder and repeated the reading after one hour

4) Technician 2 recorded the results in a separate laboratory record book

5) At the conclusion of the evaluation, results were entered into the data collection form as rapid test results under "reader 1, reader 2 designated time, and reader 2 designated time + 1 hour." (see Annex 2)

5.2.3. Handling of indeterminate results

Results that were not clearly positive or negative were recorded as indeterminate. The test may be repeated if there are sufficient test kits available after the first round of evaluation.

5.2.4. Assessing operational characteristics

Each rapid test was assessed for the following operational characteristics by technician 1 after completing the testing of the first 25 specimens from the evaluation panel:

- **Clarity** of kit instructions (maximum possible score of 3)
- **Technical complexity or ease of use** (maximum possible score of 3)
- **Ease of interpretation** of results (maximum possible score of 3)

(For details see Annex 2).

In addition, a score of 1 was given to the rapid tests that do not require any additional equipment or supplies. The total possible score is 10. The higher the score, the more suitable the test is for use in primary health care settings in developing countries

5.2.5. Test reproducibility (performed by reference laboratories only)

The objectives of this type of testing are to answer the following questions:

1) Lot-to-lot variability: will the test give the same results with tests of different manufacturing lots using the same specimens?

2) Run-to-run variability: will tests performed on the same specimen on different days give the same results? (this is a rapid test variation of the in-run and between-run precision for ELISA or other assays).

3) Operator-to-operator variability: will the test give the same results on the same specimen if it is performed by two different operators?

These three aspects of test reproducibility were determined as follows:

1) Lot-to-lot variability: 25 serum specimens to be run on 2 lots of each rapid test

2) Run-to-run variability: 9 serum specimens to be tested on 5 successive days for each rapid test.

3) Operator-to-operator variability: 2 technicians each performing the test using the same 20 specimens

5.3. Quality Assurance

All the laboratory sites demonstrated their proficiency at performing standard reference tests for syphilis by scoring 90% or better on their qualifying panel of 20 sera.

The laboratory notebook for each technician was signed off by the supervisor at the end of each day. The data entry into the spreadsheet was double-checked against the notebooks from both Technician 1 and 2.

6. Evaluation data

6.1. Data entry

The evaluation results were recorded in the laboratory notebooks of technicians 1 and 2. The results were entered into the Laboratory Data Collection Form provided as a spreadsheet by SDI. If a test was repeated for any reason, all results were entered into the spreadsheet and the reason for repeating noted.

The score sheet for the operational characteristics of each rapid test was filled out by technician 1 and entered in the Excel file provided by SDI. For Examples of the Laboratory Data Collection Form and the scoring scheme for evaluation of operational characteristics see Annex 2.

All laboratory notebooks and electronic records of study data were kept until the conclusion of the study.

6.2. Data analyses

The reference or "gold" standard is the TPPA or TPHA results previously obtained for each serum specimen at each site, validated by the reference centres.

Sensitivity and specificity: For each rapid test compared to the validated reference test results obtained at each site were analysed as follows:

		Reference test results +	Reference test results -
Rapid test results	+	a	b
	-	c	d
		a+c	b+d

Rapid test sensitivity = a/(a+c)
Rapid test specificity = d/(b+d)

a = true-positive result
b = false-positive result
c = false-negative result
d = true-negative result

The sensitivity and specificity of the rapid tests compared to the reference test were calculated. No discrepant analyses were performed. The overall performance (i.e. sensitivity and specificity) of each rapid test against the reference standard for all the sites was summed up as the kappa value for the test. A kappa value of 0.75 or greater is excellent.

To determine the extent of site-to-site variations for each rapid test, the Breslaw-Day test for homogeneity of odds ratios was calculated. The variation in test specificity in malaria endemic versus malaria free sites was also determined.

Test reproducibility: The variability of each rapid test was calculated as follows:

1) Lot-to-lot variability = the number of test results which differ between 2 lots x100/total number of tests performed on the 2 lots using the same 25 serum specimens
2) Run-to-run variability = the number of test results which differ between days x100/total number of tests performed on the same 9 serum specimens on 5 successive days
3) Operator-to-operator variability: the number of test results which differ between 2 readers of rapid test results x100/total number of tests performed using the same 20 serum specimens

Operational Characteristics at each site: The suitability of the rapid test for use in primary health care settings in developing countries was assessed qualitatively based on the operational characteristics as described in Annex 2. The score was based on a total of 10.

7. Evaluation results

A total of 789 sera from 8 geographically diverse laboratory sites were used to evaluate the 6 rapid syphilis tests. 399 of these sera were reference standard positive and 390 were reference standard negative. Table 3 overleaf shows the sensitivity and specificity of each test for each site compared to the reference standard of either TPHA or TPPA at that site. For each test, the mean sensitivity and specificity with 95% confidence intervals are shown at the bottom of Table 3.

Test Performance

Test Sensitivity

The Fujirebio Espline, Abbott Determine, and Standard BIOLINE tests showed the highest sensitivity (97.7%, 97.2% and 95% respectively) [Table 4a]. The sensitivities of these 3 tests were not significantly different from each other, but are significantly different from those of the Diesse, Omega and Qualpro tests (p values <0.03).

Test Specificity

The Omega VISITECT and the Qualpro Syphicheck tests showed the highest specificity (98% and 97.7% respectively) [Table 5a]. These are not significantly different from each other but differed from the other four tests (see p values in Table 5b).

For estimation of overall test performance, the kappa value is used to determine the combined correlation of test sensitivity and specificity for all the sites against the reference standard (a kappa value of 0.75 is considered excellent). Thus all the rapid tests had excellent correlation with the reference standard tests at each site, with kappa values for the 6 tests ranging from 0.84 to 0.95 (see Table 3).

Variation among evaluation sites

The Breslaw-Day test for homogeneity of odds ratios is a measure of site-to-site variation for each test. The three tests that gave the most variation were the Omega VISITECT, the Abbott Determine, and the Diesse Syphilis Fast tests, with p values of 0.03, 0.0086 and 0.0002 respectively. The variation can be due to a number of factors including differences in the sera selected for each panel, local variation on how the test was performed or read, and the margin for subjective interpretation of results. There was no significant difference between malaria endemic and malaria-free sites with respect to test specificity.

Reproducibility

For the 6 rapid tests, reproducibility was measured by determining lot-to-lot, operator-to-operator and run-to-run variation. The results are summarized in Table 6. Overall, the variability ranged from 0-10%.

Stability of test results

To anticipate the use of these tests in a clinic setting where providers may not be able to read the tests at the designated time of 15-20 minutes after the specimen was added, it was felt that it would be useful to determine if the tests can be read reliably after 1 hour even though this is not recommended by the test manufacturer. Test results were stable after one hour for the Abbott Determine, Fujirebio, Qualpro Syphicheck and Omega VISITECT tests with less than five results different from the original results. The Diesse Syphilis Fast appeared to dry after an hour making the reading difficult. By the second reading, 22 results were different from the original test result, turning from negative to false-positive results.

Table 3. Performance of rapid diagnostics tests for syphilis

Sites	Determine Syphilis TP Abbott Laboratories		Syphilis Fast DIESSE Diagnostica		Espline TP Fujirebio Inc		Syphicheck-WB Qualpro Diagnostics		SD BIOLINE Syphilis 3.0 Standard Diagnostics		VISITECT Syphilis Omega Diagnostics	
	sensitivity*	specificity*	sensitivity*	specificity*	sensitivity*	specificity*	sensitivity*	specificity*	sensitivity*	specificity*	sensitivity*	specificity*
Moscow, Russian Federation	100	100	72	98	100	83	80	100	92	98	76	100
Birmingham, USA	88	92	57	92	98	88	82	94	94	90	80	94
Port au Prince, Haiti	100	98	100	92	98	100	90	98	100	100	90	100
Nanjing, China	98	93	79	89	94	93	77	95	89	96	81	93
Mwanza, Tanzania	96	94	94	90	98	100	80	100	94	94	82	100
Colombo, Sri Lanka	100	98	96	100	98	96	88	100	96	100	92	100
Durban, South Africa	96	90	94	96	96	94	82	100	94	98	86	100
Fajara, The Gambia	100	88	94	86	100	92	96	94	100	84	92	96
Overall results	sensitivity*	specificity*	sensitivity*	specificity*	sensitivity*	specificity*	sensitivity*	specificity*	sensitivity*	specificity*	sensitivity*	specificity*
	97.2 95.6,98.8	94.1 91.8,96.4	86 82.5,89.4	92.8 90.3,95.4	97.7 96.3,99.2	93.4 90.9,95.8	84.5 80.9,88.0	97.7 96.2,99.2	95 92.8,97.1	94.9 92.7,97.1	85 81.4,88.5	98 96.5,99.4
Homogeneity**	p=0.0086		0.0002		0.2529		0.1427		0.1132		0.03	
kappa & 95% CI*** (confidence intervals)	0.95 0.93,0.97		0.87 0.84,0.90		0.95 0.93,0.97		0.84 0.80,0.87		0.9 0.87,0.94		0.85 0.82,0.89	

* Sensitivity and specificity are listed as percentages.

** The Breslaw-Day test for homogeneity of odds ratios was calculated to determine the extent of site-to-site variation of test performance.

*** The kappa value for each test is a summation of the overall performance of each test against the reference standard for all sites. A value of 0.75 or greater is considered excellent.

The Standard BIOLINE had 12 results different from the original, with most of the results becoming false-positive after 1 hour.

Operational characteristics

The Abbott test obtained the best score (7.5 out of 10) in the questionnaire on ease of use with the Omega VISITECT, Qualpro Syphicheck, Fujirebio Espline and Standard BIOLINE all less than 10% different from each other (6.5-7.1 out of 10). The Diesse Syphilis Fast test scored lowest (4.3 out of 10) on technical complexity and ease of interpretation (for details see Table 7).

In general, none of the tests were technically complex to perform and are all appropriate for use in primary health care settings in developing countries.

Table 4. Comparative sensitivity performance of rapid syphilis tests

4a.

Tests	Sensitivity*
Fujirebio (Espline)	97.7%
Abbott (Determine)	97.2%
Standard (BIOLINE)	95.0%
Diesse (Syphilis Fast)	86.0%
Omega (VISITECT)	85.0%
Qualpro (Syphicheck)	84.5%

*Compared against the reference standard tests: TPHA/TPPA

4b. Comparative differences in test sensitivity (p values)

	Abbott	Diesse	Fujirebio	Standard	Omega	Qualpro
Abbott						
Diesse	<0.0001					
Fujirebio	0.6547	<0.0001				
Standard	0.0977	<0.0001	0.0374			
Omega	<0.0001	0.6879	<0.0001	<0.0001		
Qualpro	<0.0001	0.5496	<0.0001	<0.0001	0.844	

The highlighted boxes indicate a significant difference in sensitivity between the tests

Table 5. Comparative specificity performance of rapid syphilis tests

5a.

Tests	Specificity*
Omega (VISITECT)	98.0%
Qualpro (Syphicheck)	97.7%
Standard (BIOLINE)	94.9%
Abbott (Determine)	94.1%
Fujirebio (Espline)	93.4%
Diesse (Syphilis Fast)	92.8%

*Compared against the reference standard tests: TPHA/TPPA

5b. Comparative differences in test specificity (p values)

	Abbott	Diesse	Fujirebio	Standard	Omega	Qualpro
Abbott		0.4889	0.6647	0.6317	0.006	0.0115
Diesse			0.7704	0.2297	0.0006	0.0014
Fujirebio				0.3618	0.0016	0.0034
Standard					0.0213	0.038
Omega						0.8063
Qualpro						

The highlighted boxes indicate a significant difference in specificity between the tests

Table 6. **Test reproducibility**

Parameter	Determine Syphilis TP Abbott Laboratories	Syphilis Fast DIESSE Diagnostica	Espline TP Fujirebio Inc	Syphicheck-WB Qualpro Diagnostics	SD BIOLINE Syphilis 3.0 Standard Diagnostics Inc	VISITECT Syphilis Omega Diagnostics
Lot-to-lot Variation[1]	1/50	4/50	3/50	0/50	3/50	1/50
Day-to-day Variation[2]	0/45	3/45	1/45	1/45	0/45	3/45
Operator-to-Operator at reference labs[3]	1/40	0/40	0/40	3/40	1/40	4/40
Operator-to-Operator at sites	4/789	20/789	0/789	6/789	0/789	5/789

Values given as number of discordant results/total number of tests performed

1) 2 lots of rapid tests performed using the same 25 sera
2) 9 sera tested on 5 days
3) 10 sera run by 2 operators at each of 2 reference laboratories

Table 7. Operational characteristics of rapid diagnostic test for syphilis

Operational characteristic		Determine Syphilis TP Abbott Laboratories		Syphilis Fast DIESSE Diagnostica		Espline TP Fujirebio Inc		Syphicheck-WB Qualpro Diagnostics		SD Bioline Syphilis 3.0 Standard Diagnostics		Visitect Syphilis Omega Diagnostisc	
	Site	Site score	Mean score	Site score	Mean score	Site score	Mean score	Site score	Mean score	Site score	Mean score	Site score	Mean score
Clarity of kit instructions	MO	3	2.625	2	1.875	3	1.875	3	2.125	3	2.125	3	2.5
	BI	2		2		2		1		1		2	
	PO	3		2		1		2		2		3	
	NA	3		1		2		2		3		3	
	MW	3		3		2		3		2		3	
	CO	2		2		1		2		2		2	
	DU	2		2		3		2		3		2	
	FA	3		1		1		2		1		2	
Technical complexity	MO	3	2.875	0	1.125	3	2.625	1	1.875	3	2.375	2	2
	BI	2		1		2		1		1		2	
	PO	3		0		2		2		2		2	
	NA	3		2		3		3		3		3	
	MW	3		3		3		3		3		2	
	CO	3		1		3		2		2		2	
	DU	3		1		3		1		3		1	
	FA	3		1		2		2		2		2	
Ease of interpretation of results	MO	3	2	0	1.25	2	2.125	2	1.875	3	2	2	1.625
	BI	2		1		2		3		2		0	
	PO	2		1		2		2		2		2	
	NA	2		1		3		3		3		3	
	MW	1		2		3		2		1		2	
	CO	3		1		1		1		2		2	
	DU	1		3		2		0		2		0	
	FA	2		1		2		2		1		2	
Equipment required but not provided	MO	0	0	0	0	0	0	1	1	0	0	1	1
	BI	0		0		0		1		0		1	
	PO	0		0		0		1		0		1	
	NA	0		0		0		1		0		1	
	MW	0		0		0		1		0		1	
	CO	0		0		0		1		0		1	
	DU	0		0		0		1		0		1	
	FA	0		0		0		1		0		1	
Total score		7.5/10		4.3/10		6.6/10		6.9/10		6.5/10		7.1/10	

MO = Moscow, **BI** = Birmingham, **PO** = Port au Prince, **NA** = Nanjing, **MW** = Mwanza, **CO** = Colombo, **DU** = Durban, **FA** = Fajara

8. Conclusions

The 6 rapid syphilis tests evaluated all showed excellent overall performance compared to the reference standard tests of TPHA or TPPA using archived serum specimens. They were considered easy to use and interpret by trained laboratory technicians. Their performance in field settings and utility in a disease control programme remain to be determined.

Most of these rapid tests utilize one or more recombinant treponemal antigens in formats that confer varying test sensitivity and specificity. The test sensitivity may also be influenced by the volume of serum used for each particular brand of rapid test.

Since treponemal antibodies tend to be retained for years, treponemal tests may be less useful in areas of high disease prevalence as these tests cannot be used to distinguish between a new infection and a prior infection which has been successfully treated. It may be possible to use the rapid tests as a screening test and then perform quantitative non-treponemal tests to determine if the patient has an active infection.

In areas of low disease prevalence, it may be possible to use these tests as screening tests to identify patients for presumptive treatment. By selecting combinations of these rapid tests, it may also be possible to use one rapid test as a screening test and another as a confirmatory test.

In considering which tests should be further evaluated in field settings, the SDI ad hoc Expert Working Group felt that it was difficult to select one or two tests based on test performance characteristics alone. The final consensus was that all four rapid tests that can use whole blood and do not require refrigeration should be taken forward to SDI field trials.

Given the simplicity and low cost of these rapid tests, it is hoped that they may prove to be effective tools in the control of syphilis and for screening of pregnant women to prevent congenital syphilis in primary health care settings.

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Annex 2. Data forms

Laboratory Data Collection Form

Name of test: .....
Manufacturer: .....
Lot number: .....
Expiry date: .....
Evaluation site: .....

Study #	Date of testing	Rapid test results			Reference test results	
		reader 1	reader 2		RPR (titer)	TPHA/ TPPA
			designated time	designated time + 1 h		
ES ##						
ES ##						
ES ##						
ES ##						
ES ##						
ES ##						
ES ##						
ES ##						
ES ##						
ES ##						
ES ##						
ES ##						
ES ##						
ES ##						
ES ##						
ES ##						
ES ##						
ES ##						

Evaluation of Operational Characteristics of Rapid Syphilis Test

Name of test: .....

Manufacturer: .....

Evaluation site: .....

1. Clarity of kit instructions

difficult to follow	0	○
fairly clear	1	○
very clear	2	○
excellent	3	○

2. Technical complexity

complex	0	○
fairly easy	1	○
very easy	2	○
excellent	3	○

3. Ease of interpretation of results

difficult	0	○
fairly easy	1	○
very easy	2	○
unambiguous	3	○

4. Equipment required but not provided e.g. micropipette

yes	0	○
no	1	○

Comments: .....

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