

Diagnosics Evaluation Series

No.1

SDi

Report

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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www.who.int/std_diagnostics

Laboratory-based evaluation of rapid syphilis diagnostics

Results from 8 SDI Sites



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

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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 8 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 presents 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 cofactors 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.

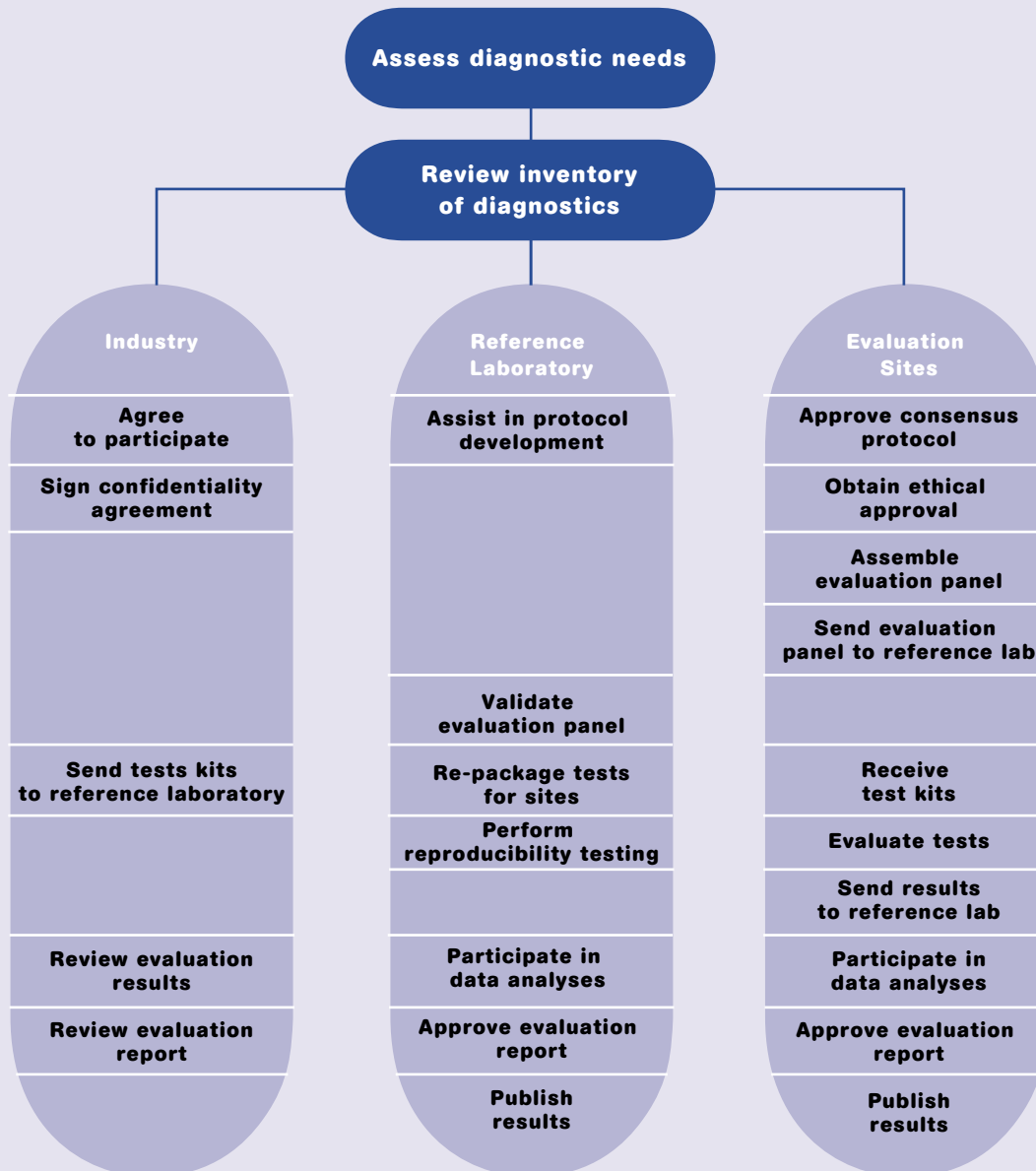
This report describes the results from the first phase of the evaluation only.

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	DIESE 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. Chungalucha
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-

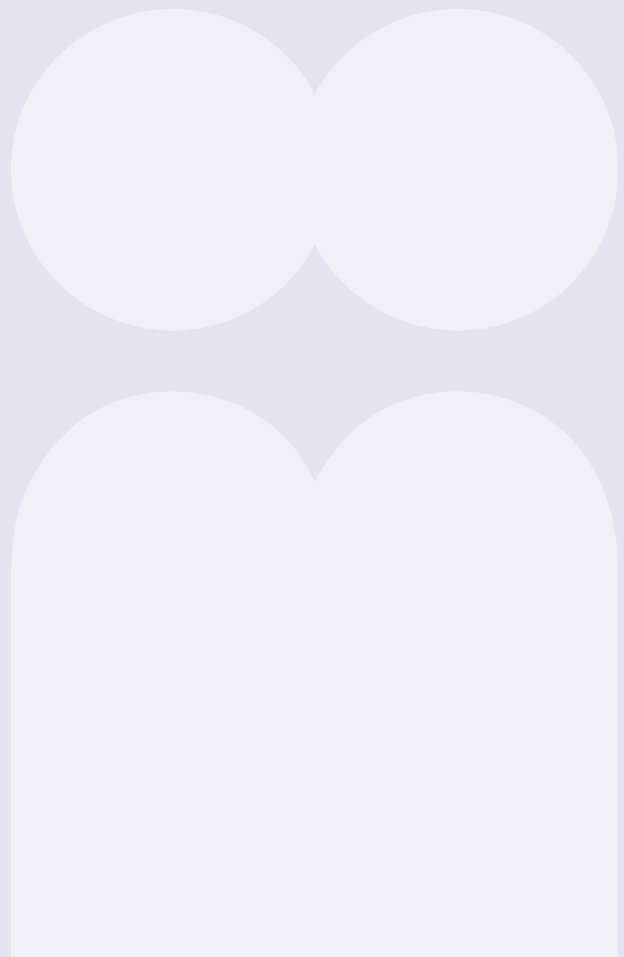
Each site complied with the request as much as possible but not all sites had sufficient quantities of characterised serum specimens to comply with the request. The evaluations in this report were undertaken using 789 serum samples available from the 8 sites, 399 of which were TPHA or TPPA positive.

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 would be 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.

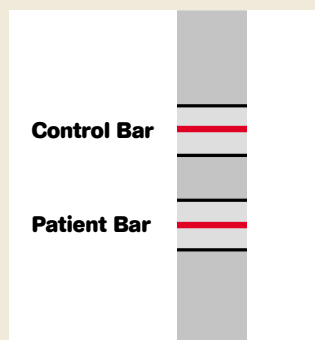
1. Abbott Laboratories Determine Syphilis TP

**EQUIPMENT REQUIRED
BUT NOT SUPPLIED:**

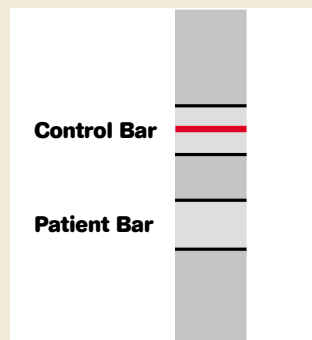
- micropipette and tips,
volume 50 µl

STANDARD OPERATING PROCEDURE:

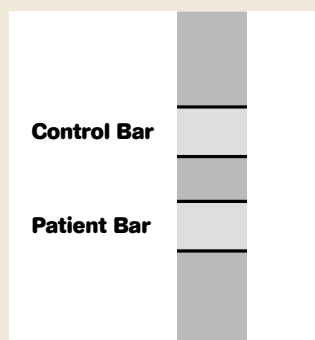
- 1) Remove the protective foil cover from each test
- 2) Using a micropipette, apply 50 µl of serum to the sample pad (marked by arrow symbol)
- 3) Wait a minimum of 15 minutes before reading the test result
- 4) Interpret test results as follows:



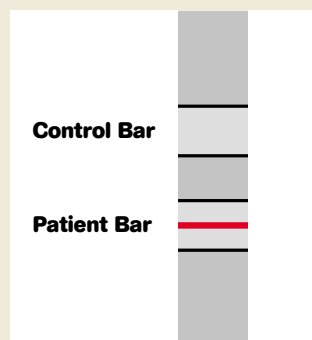
Positive



Negative



Invalid



Invalid

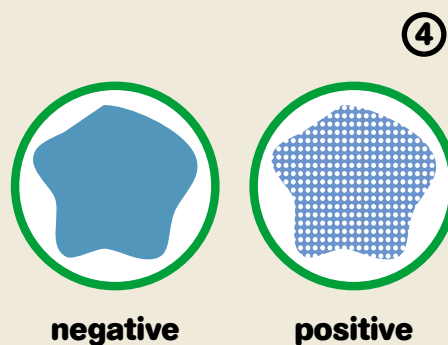
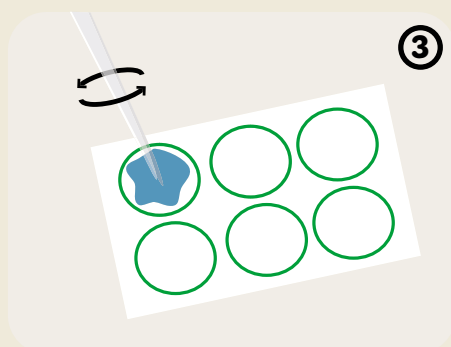
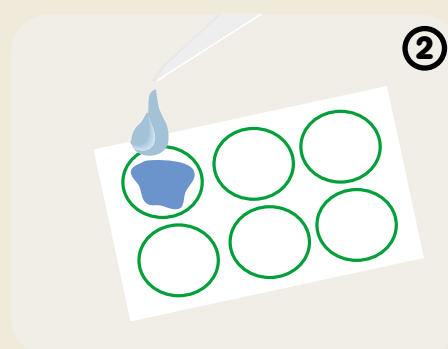
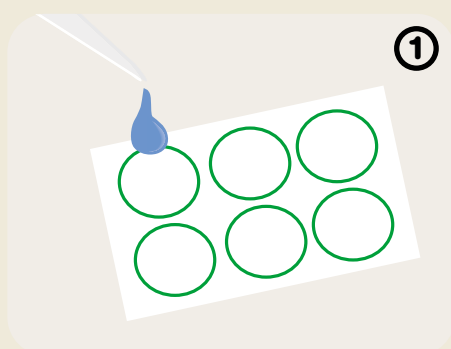
2. Dienes Diagnostica Syphilis Fast

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

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

STANDARD OPERATING PROCEDURE:

- 1) Place 40 µl serum into a circle on the card
- 2) Add 20µl 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



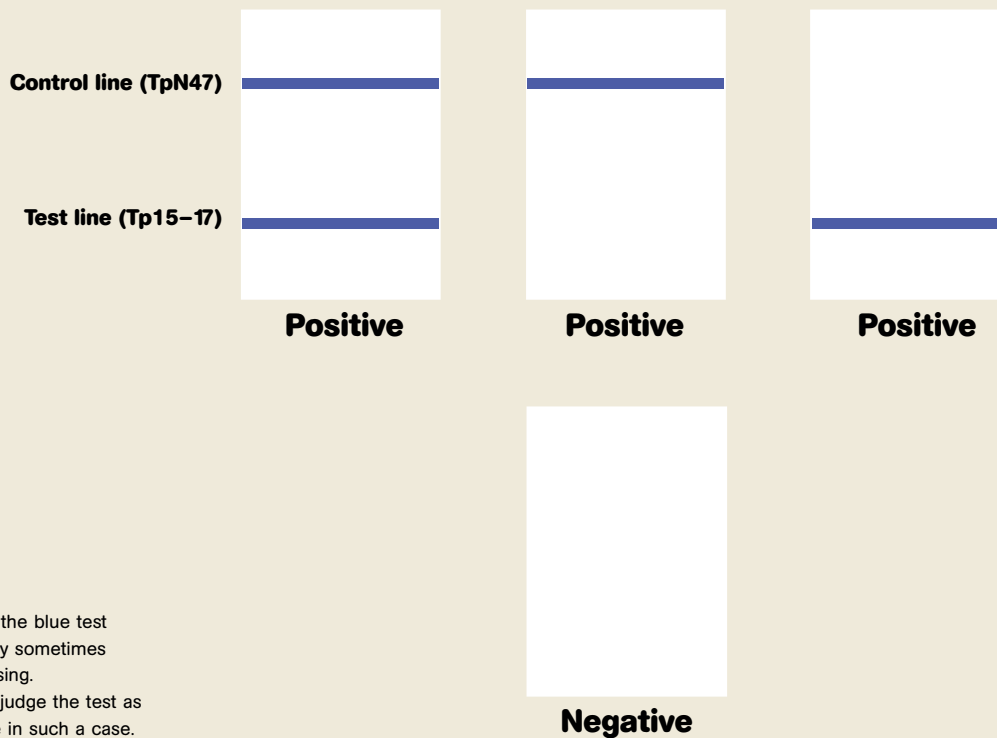
3. Fujirebio Inc Espline TP

**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:



Note:

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

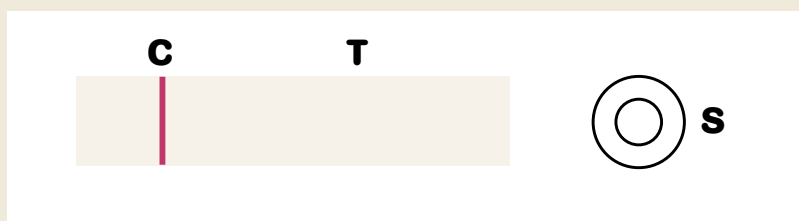
4. Omega Diagnostics VISITECT Syphilis

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

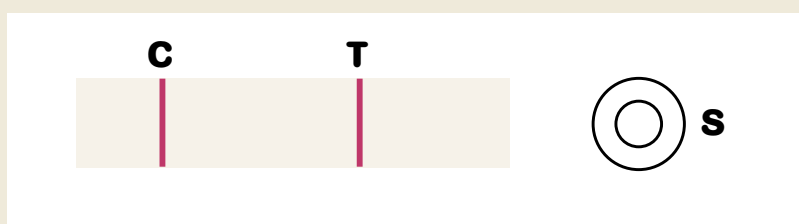
None

STANDARD OPERATING PROCEDURE:

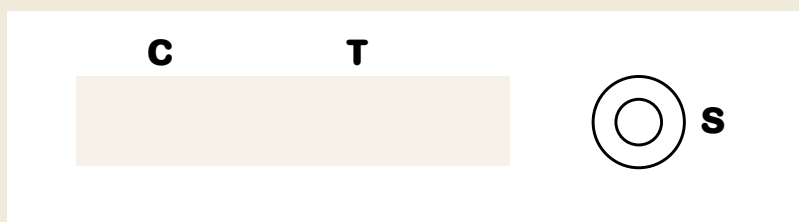
- 1) Use dropper provided, dispense 1 drop of serum to sample well S
- 2) Add 2 drops of diluent buffer from the buffer bottle to sample well S
- 3) Read results after 15 minutes



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



Positive: In addition to the control band, 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

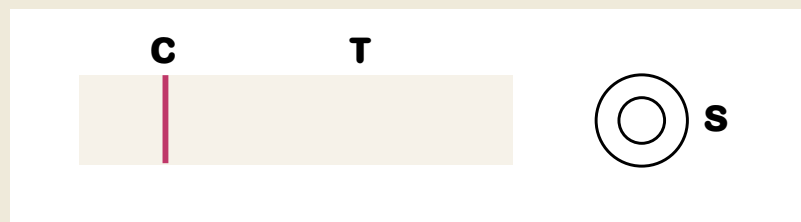
5. Qualpro Diagnostics Syphicheck-WB

**EQUIPMENT REQUIRED
BUT NOT SUPPLIED:**

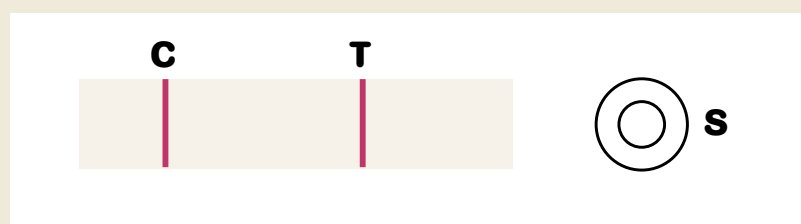
None

STANDARD OPERATING PROCEDURE:

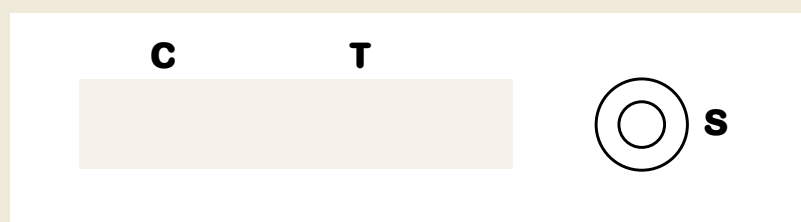
- 1) Remove the test from the pouch and place on a flat surface
- 2) Using the dropper provided, add 1 drop of serum to the sample well S
- 3) Add 2 drops of diluent buffer from the diluent bottle to sample well S
- 4) Read the results in 15 minutes as follows:



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



Positive: In addition to the control band, 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 μ l

STANDARD OPERATING PROCEDURE:

- 1) Remove the test from the foil pouch and place on a flat, dry surface
- 2) Slowly add 10 μ l 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:

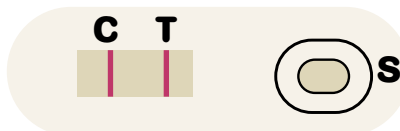
Syphilis



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 note books 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	
		+	-
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

c = false-negative result

b = false-positive 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 $\times 100$ /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 $\times 100$ /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 $\times 100$ /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 Dienes, 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 Dienes 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 Dienes 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		Espine 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	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 Dैसे 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%
Dैसे (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	Dैसे	Fujirebio	Standard	Omega	Qualpro
Abbott						
Dैसे	<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 indicated 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%
Dैसे (Syphilis Fast)	92.8%

*Compared against the reference standard tests: TPHA/TPPA

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

	Abbott	Dैसे	Fujirebio	Standard	Omega	Qualpro
Abbott		0.4889	0.6647	0.6317	0.006	0.0115
Dैसे			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 indicated a significant difference in specificity between the tests

Table 6. Test reproducibility

Parameter	Determine Syphilis TP Abbott Laboratories	Syphilis Fast DIESSE Diagnostica	Espine 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		Espiline TP Fujirebio Inc		Syphicheck-WB Qualpro Diagnostics		SD Bioline Syphilis 3.0 Standard Diagnostics		Visitect Syphilis Omega Diagnostic	
	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	1.875	3	1.875	2.125	2.125	3	2.125	3	2.5
	BI	2										
	PO	3										
	NA	3										
	MW	3										
	CO	2										
	DU	2										
	FA	3										
	MO	3										
BI	2											
Technical complexity	PO	3	2.875	1.125	3	2.625	1.875	2.375	3	2.375	2	2
	NA	3										
	MW	3										
	CO	3										
	DU	3										
	FA	3										
	MO	3										
	BI	2										
	PO	3										
NA	2	2	1.25	3	2.125	1.875	2	3	1.625	1		
MW	1											
CO	3											
DU	1											
FA	2											
MO	3											
BI	2											
PO	2											
NA	2											
MW	1	0	0	0	0	1	0	0	1	1		
CO	3											
DU	1											
FA	2											
MO	0											
BI	0											
PO	0											
NA	0											
MW	0											
CO	0											
DU	0											
FA	0											
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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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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