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Dual elimination of mother-to-child transmission of HIV and congenital syphilis

DIAGNOSTIC TECHNOLOGY LANDSCAPE

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World Health Organization

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Abbreviations

CDC	United States Centers for Disease Control and Prevention
CI	confidence interval
dL	decilitre
EDTA	ethylenediaminetetraacetic acid
FTP	File Transfer Protocol
g	gram
GPS	global positioning system
GPRS	global position radio satellite
GSM	global system for mobile communication
HIV	human immunodeficiency virus
ID	identification
ISO	International Organization for Standardization
mL	millilitre
MSM	men who have sex with men
MTCT	mother-to-child transmission
PMTCT	prevention of mother-to-child transmission
POC	point of care
RDT	rapid diagnostic test
TP	*Treponema pallidum*
TPHA	*Treponema pallidum* hemagglutination assay
TPP	target product profile
TPPA	*Treponema pallidum* particle agglutination
µL	microlitre
VCT	voluntary counselling and testing
WHO	World Health Organization

Background

The mother-to-child transmission (MTCT) of human immunodeficiency virus (HIV) and syphilis are significant causes of death in infants and young children globally each year. Of the approximately 2.7 million new HIV infections among adults and children in 2011, for example, it is estimated that about 900 babies per day (or more than 325 000 babies per year) were born to HIV-positive mothers, mostly in sub-Saharan Africa *(1,2)*. Without treatment, the life expectancy of these children is low, with more than half of them dying before the age of two *(3)*.

Another significant cause of infant mortality is maternal syphilis. The World Health Organization (WHO) has estimated that about 1.86 million cases of syphilis occur worldwide among pregnant women each year, many of whom are either untreated or inadequately treated *(4)*. It is estimated that more than 500 000 perinatal deaths (i.e. deaths that occur from 22 weeks gestation through the first 7 days of life) occur each year as a result of untreated maternal syphilis *(5)*.[1] Without universal testing and treatment of syphilis in pregnancy, as many as 50% of pregnancies in women with syphilis will result in adverse outcomes, including perinatal death, prematurity and low birth weight *(6)*.

Yet, there are effective interventions to prevent these adverse outcomes for infants and young children who are at risk of HIV and/or syphilis caused by MTCT. The United States Centers for Disease Control and Prevention (CDC) estimates that key interventions, including HIV testing and counselling of all pregnant women and provision of antiretroviral drugs to all HIV-positive women during pregnancy, among other interventions, can reduce MTCT of HIV from 35% to 5% *(7)*. It also has been demonstrated that programmes that include syphilis testing along with appropriate and timely penicillin treatment for pregnant women who test positive for *Treponema pallidum* (TP) (the bacteria that causes syphilis) infection can reduce adverse pregnancy outcomes *(8–10)*.

WHO has long supported screening of all pregnant women for HIV *(11)*, and many countries have greatly expanded their HIV screening over the years. Furthermore, as a result of the ongoing perinatal mortality caused by syphilis and the cost-effectiveness of antenatal screening and treatment, even in settings where the prevalence of syphilis in pregnant women is low to moderate *(12–15)*, WHO launched a global initiative for the elimination of congenital syphilis in 2007 *(16)*. Yet, despite this call and the launch of the Global Congenital Syphilis Project (GCSP) to advocate for, and invest in, the fight against congenital syphilis, syphilis screening programmes for pregnant women still have not been widely implemented in resource-limited settings.

In summary, in the case of MTCT of both HIV and syphilis, testing pregnant women is a critical intervention for prevention, care and treatment of both mother and child. There are a number of combined HIV/syphilis (treponemal) tests emerging that could be effective tools in the dual elimination of MTCT of HIV and syphilis. This document reviews the current testing landscape for such diagnostic tools.

1 This compares to annual death rates for other important infections in pregnancy, such as HIV, which is estimated to cause between 250 000 and 290 000 perinatal deaths worldwide, and malaria in pregnancy, which is estimated to cause about 200 000 perinatal deaths *(5)*.

Technology landscape

There are more than one hundred HIV rapid tests commercially available today, all of which can be used for screening pregnant women for the virus. These tests, which are generally antibody tests, have been widely adopted, especially in resource-limited settings, where they can be used in decentralized facilities, including prevention of mother-to-child transmission (PMTCT) and voluntary counselling and testing (VCT) centres, to provide same-day results to patients. In general, the technical performance of these HIV rapid tests, as reported by manufacturers, is strong. Sensitivities usually range from 99.3% to 100% and specificities range from 99.7% to 99.9% *(17)*.

Similarly, there are a smaller number of rapid diagnostic tests for syphilis screening, which are antibody tests that detect TP. Among these are rapid tests from Alere, Inc. (Determine™), Standard Diagnostics (SD Bioline), the Tulip Group/Qualpro (Syphicheck) and Omega Diagnostics (Visitect®). Although the performance of these tests has been questioned, a recent meta-analysis on their performance demonstrates that rapid TP tests for syphilis report sensitivity and specificity estimates comparable to laboratory-based tests, for which there is no gold standard *(18)*.

However, given the difficulties of test delivery in most resource-limited settings, for the purpose of screening for the dual elimination of HIV and syphilis in pregnant women, it has been suggested that a combination rapid test for simultaneous detection of HIV and syphilis would be simpler and also may be more cost effective than using separate tests. As with the existing HIV rapid tests and TP rapid tests, for use in these settings it is essential that the combined tests meet the WHO ASSURED criteria for an ideal rapid test in that they are: affordable, sensitive, specific, user friendly, robust and rapid, equipment-free and deliverable to those who need the test *(19)*.

Target product profile (TPP)

Building on the WHO ASSURED criteria and in order to give guidance to developers with respect to market needs for performance and operational characteristics for combined HIV/syphilis tests for use at or near the point of patient care, the Bill & Melinda Gates Foundation commissioned the development of a TPP for such an assay. The TPP has been reviewed by key stakeholders who are members of the GCSP, including WHO, the CDC and the London School of Hygiene & Tropical Medicine (Annex A).

The TPP was developed for a combined HIV/syphilis assay for use in resource-constrained countries in a variety of decentralized target settings. These include health centres, health posts, PMTCT centres and VCT sites as well as for community outreach. Stakeholders envision a device-free assay that can be used with or without a separate reader; however, a simple, easy-to-use device-based platform with a self-contained reader also could be used.

Each assay must demonstrate that it performs favourably when compared to an appropriate reference technology. In addition, it will need to meet certain operational standards. Research by the author, and others, have identified certain weaknesses in the health-care systems in resource-limited settings that affect successful delivery of diagnostics, including: (i) shortages of human resources and lack of training for staff; (ii) supply chain challenges; (iii) lack of diagnostic equipment and equipment breakdowns; and (iv) lack of robust quality assurance and quality control systems. These weaknesses suggest that the following operational specifications for point-of-care (POC) diagnostic assays/platforms should be prioritized.

> **Ease-of-use.** Sample preparation should be simple, with the ability to use unprocessed sample specimens; and only a small number of operator steps, especially timed steps, should be required to perform the test. Test kits (i.e. the reagents and disposables required to perform an assay on a single patient) should be self-contained.
>
> **Training.** The assay should be simple enough that its use can be explained to a health-care worker in one day of training or less, including its methods of sample collection and preparation.

High tolerance to difficult environmental conditions. Test kits must be stable at high temperatures and humidity and must be able to survive extreme fluctuations in temperature; no cold chain should be required during transport and/or storage.

Self-contained quality control. There should be a procedural control internalized in the cartridge for each individual test as well as an indicator of instability or test expiration.

Data capture, connectivity and data export. If combined with a reader, the reader must store patient results and its output needs to be compatible with centralized data aggregation and analysis. In order to monitor test performance, a GPS/GPRS modem, preferably internal to the reader, should be incorporated, and full data export capabilities over mobile phone networks should be a minimal standard.

Biosafety. To enhance biosafety, operational specifications should include the requirement for closed, self-contained systems with no biosafety cabinet required and unprocessed sample transfer only.

Waste disposal. Since medical waste is frequently stored for long periods of time before incineration, diagnostic consumables, such as test kits, must be rendered non-toxic after use and must not release toxic compounds when burned. Furthermore, as an optimal standard, compostable plastics for test kits and other materials would be preferred.

In addition to the high-priority product standards summarized above, the following specifications are also important.

Cost. The cost of assays will be a critical factor in implementation and uptake of new POC diagnostics. Funding for diagnostics is limited, both at the global level and in-country, where cost-effectiveness will be assessed.

Sample capacity, throughput and time to result. These are important specifications for new POC diagnostic assays, but there is no single specification for capacity, throughput and turnaround time that will fit all settings. Rather, these specifications will depend on the volume of testing and turnaround time for each assay at the health centre/health post. The ability to give same-day results is critical and must be considered with respect to each assay; otherwise, the value of a POC test is substantially diminished. The working day in many health-centre settings is greatly abbreviated and the turnaround time for a diagnosis must also allow time for the pre-analytic activities (e.g. patient registration) and post-analytic activities (e.g. clinical interpretation and treatment) necessary to provide a complete service to the patient within one working day.

Combined HIV/syphilis tests: currently developed

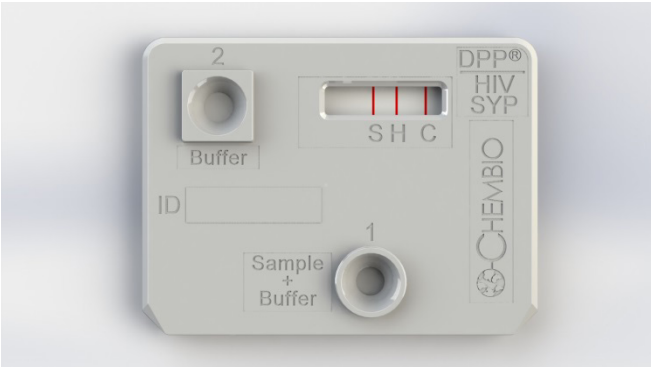
HIV/Syphilis Duo Rapid Test (Standard Diagnostics)

One of three HIV/syphilis rapid diagnostic tests currently on the market is the HIV/Syphilis Duo Rapid Test from Standard Diagnostics (pictured below).

The Standard Diagnostics HIV/Syphilis Duo Rapid Test is an easy-to-use, rapid lateral flow assay for the simultaneous detection of HIV-1, including subtype O, and HIV-2 and/or syphilis TP from whole blood (venous or fingerstick), serum or plasma samples with results in approximately 15–20 minutes.

DPP® HIV-Syphilis Assay (Chembio Diagnostic Systems)

Also on the market, the DPP® HIV-Syphilis Assay from Chembio Diagnostic Systems (pictured below) is a single-use immunochromatographic, rapid screening test for the detection of antibodies both to HIV types 1 and 2 (HIV-1/2) and to syphilis TP in fingerstick whole blood, venous whole blood, serum or plasma samples. The test, which requires only 10 µL of blood, includes the Chembio Diagnostic Systems Sample-Tainer™ specimen collection bottle, which is a safe closed system for handling potentially infectious blood samples. Time to results is about 10 minutes.



Multiplo Rapid TP/HIV-Syphilis Antibody Test (MedMira, Inc.)

The Multiplo Rapid TP/HIV-Syphilis Antibody Test from MedMira, Inc. (pictured below) is now commercially available in Colombia, and the company is pursuing product registration in a number of other Latin American countries. The combination assay is in the same format as the company's HIV antibody test.

The Multiplo Rapid TP/HIV-Syphilis test combines qualitative detection of HIV-1 and HIV-2 with qualitative detection of TP in an immunofiltration format. Time to results is about three minutes.

The detailed performance (sensitivity and specificity) and operational characteristics of the three assays described above are detailed in Annex B. All of these tests have been submitted to WHO for prequalification, but none of them has been prequalified yet.

Combined HIV/syphilis tests: in development

In addition to the three assays referenced above, there are several additional combined HIV/syphilis assays in the pipeline, including the following tests.

INSTI Combined HIV/Syphilis Test (Biolytical Laboratories)

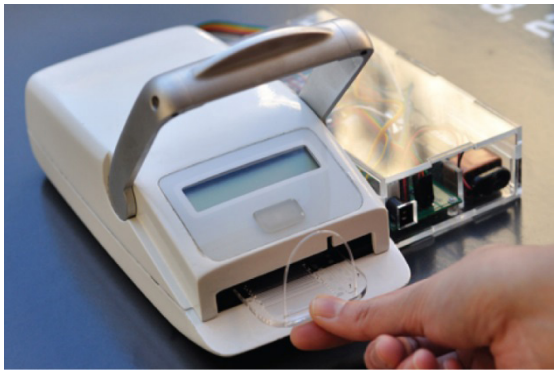
The INSTI Combined HIV/Syphilis Test from Biolytical Laboratories will likely be commercially available in 2014. Pictured below is the company's current HIV rapid test; the new HIV/Syphilis Test will contain the identical elements.

Comparable to the tests already on the market, the INSTI test is designed to provide rapid qualitative detection of HIV-1 and HIV-2 as well as syphilis TP in a rapid test format using immunofiltration. Time to results is about 60 seconds. See Annex C for detailed operational characteristics.

Several assays could become available in 2015 and beyond. These include assays from Junco Labs and Columbia University in collaboration with OPKO Health, Inc., Trinity Biotech and MBio Diagnostics.

mChip Assay (Junco Labs and Columbia University in collaboration with OPKO Health, Inc.)

The first of these assays, the mChip Assay (pictured below) from Junco Labs and Columbia University in collaboration with OPKO Health, Inc., will go beyond existing combination HIV/syphilis TP assays and may include qualitative detection of non-treponemal syphilis and the quantitative detection of anaemia (haemoglobin) in a device-based format that utilizes a reusable microfluidic mChip and a smart phone for read-out of results. The assay is expected to be available in 2015. See Annex C for detailed operational characteristics.



Uni-Gold™ HIV/Syphilis Assay (Trinity Biotech)

Trinity Biotech, which manufactures two Uni-Gold™ HIV assays and a separate Uni-Gold Syphilis Assay, which was launched in July 2013, is expected to develop a combination HIV/syphilis assay using its Uni-Gold rapid test platform format (operational characteristics are detailed in Annex C). The company indicates that it would take about 15–16 months to develop a combined HIV/syphilis assay, but development has not yet begun. Therefore, such an assay is not likely to be commercially launched before 2015.

PreventIt (Research Consortium)

A combination HIV/syphilis assay being funded by the European Union is under development by a team, including the Royal Tropical Institute (the Netherlands), IMAccess (France), Sirigen (United Kingdom), CCM (the Netherlands) and the London School of Hygiene & Tropical Medicine (United Kindgom). The assay, called PreventIt, which is still in the relatively early stages of development, will simultaneously diagnose the HIV antigen and syphilis antibody status of individuals.

The proposed test device will consist of a circular plate coated at one or more points with immobilized specific analyte and a stabilized reagent (similar to the device pictured below).

The test is performed by spotting a droplet of the sample next to the reagent, which is thereby dissolved, and the mixture is then allowed to react with each of the analytes. Because the plate will have a smooth hydrophilic surface, the drop will rapidly flow from one analyte to the next by gravitational force if the plate is tilted back and forth. For optimal strength and sensitivity, the design of the plate is such that the drop will flow in a circle repeatedly reacting with the bound analyte each time the flow is completed in a full circle.

The developers believe the PreventIt assay combines the strengths of ELISA (the use of a molded device with a standardized surface matrix and repeated interaction of reagents to maximize binding), with that of a lateral flow assay (the use of a visually detectable label or strong fluorescent for digital read-out). Because the device will be simpler and faster than an ELISA assay, it should be suitable for use at the POC in resource-limited settings.

It is expected that the assay will have been developed and evaluated within three years of the start of the project in 2012. Therefore, commercial launch of the product could come as early as 2015.

Combination HIV/Syphilis Assay (MBio Diagnostics®)

MBio Diagnostics® intends to develop a combination HIV/syphilis assay. Each product being developed by MBio Diagnostics is a diagnostic system combining an easy-to-use, software-driven portable reader with single-use disposable cartridges (pictured below). In 2014, MBio Diagnostics will commercialize its first product, the MBio CD4 System. MBio is currently prioritizing the development of an HIV-1/2 antigen/antibody assay over the combination HIV/syphilis assay. Timing for the HIV-1/2 antigen/antibody screening test will be after 2016; the combination assay would follow that. When developed, the HIV/syphilis assay will use the same cartridge design and same blood sample volume (15 µL) as the CD4 assay. While the CD4 assay requires a 20-minute incubation period, none will be required for the HIV/syphilis assay.

Conclusion

Although MTCT of HIV and syphilis can cause significant adverse impacts to infants and young children, including death, these outcomes can be significantly diminished if women are routinely tested for both HIV and syphilis and given appropriate and timely treatment during pregnancy.

While individual rapid tests for HIV and syphilis exist separately, there are currently only two combination rapid tests on the market that simultaneously detect both HIV and syphilis *(3,7)*. However, there are several combination rapid tests in the pipeline that will become available in the next few years. See Annex D for the current pipeline of HIV/syphilis assays.

Given the challenges of diagnostic delivery in resource-limited settings, such combination tests are highly desirable as they will make implementation of both HIV/syphilis testing simpler and, hopefully, more cost effective. However, not all of the tests in the pipeline meet the criteria laid out in the combined HIV/syphilis TPP. Of particular concern are tests with multiple steps, a number of which require precision timing and/or special technique for adding buffer, for example. Another concern is the inflexibility of the read window for some assays, where test results must be read immediately or within a few minutes of the final step in the test process. In some cases, the expected shelf life of reagents is less than 12 months and environmental tolerances of the assays are not within the specifications of the TPP. Cost is also a factor for some of the proposed assays. Current syphilis RDTs range in cost from about US$ 1.00 to US$ 3.00 per test and current HIV RDTs range in cost from about US$ 0.50 to US$ 1.60. Therefore, the cost of a combined HIV/syphilis RDT ideally would be US$ 1.50 or less, but at a minimum should be less than US$ 3.00.

It is hoped that the combined HIV/syphilis assays will continue to be optimized to more closely meet the performance and operational characteristics set forth in the TPP. The development and effective implementation of these tests is an important step towards the goal of elimination of MTCT of HIV and congenital syphilis, which will require sustained efforts on the part of the public and private sectors to accelerate access to these important maternal screening tools.

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Annex A: TPP – combined HIV/syphilis test

Intended use	To detect HIV infection and syphilis infection in pregnant women and key populations[a] for purposes of screening			
Goal of test	Qualitative detection of HIV antibodies and qualitative detection of TP-specific antibodies			
Target patient	Pregnant women and key populations			
Target use setting	Health centres, health posts, PMTCT centres, VCT centres and community outreach			
Results	Clear positive, negative or invalid result with minimal instructions for interpretation			
Equipment	Disposable in vitro diagnostic (IVD) preferred, reader optional (small, portable table-top or hand-held)			
Performance	**HIV infection**		**Syphilis**	
Reference technology	ELISA/EIA		TPPA[b]	
	Minimal	**Optimal**	**Minimal[c]**	**Optimal[c]**
Clinical sensitivity[d]	>98%	>99%	>75%	>90%
Clinical specificity[d]	>98%	>99%	>90%	>95%
Quantitation	None: qualitative test			

Minimal and optimal operational characteristics for combined HIV/syphilis test

	Minimal	**Optimal**
Specimen	Fingerstick capillary blood (maximum 50 µL)	Fingerstick capillary blood (maximum 20 µL)
Specimen preparation	Minimal sample processing; no more than one operator step	Integrated
Steps performed by health-care worker between specimen preparation and result	No more than five operator steps *(only one of which has a timed interval)*, excluding waste disposal	One operator step *(none of which has a timed interval)*, excluding waste disposal
Additional consumables required but not provided within the test kit	None, except for specimen collection	
Cold chain	None required at any point in supply chain or storage	
Test kit	All materials required for test procedure, including devices, reagents or other consumables to diagnose one individual, included in packaged, self-contained kit (either packaged individually as one test per test kit or sufficient to perform the number of tests packaged in the test kit box – e.g. 20, 50 or 100 tests)	
Test kit stability and storage conditions	Stable for 12 months at 2 C–35 C (2 C–40 C preferred), 70% humidity, including transport stress (48 hours with fluctuations up to 50 C and down to 0 C)	Stable for 24 months at 0 C–35 C, 90% humidity, including transport stress (48 hours with fluctuations up to 50 C and down to 0 C)
Environmental tolerance of packaged test kit	Tolerate exposures between 2 C and 45 C at an altitude up to 3000 metres, up to and including condensing humidity	
Operating conditions	Operation between 15 C and 40 C at an altitude up to 2000 metres (between 10 C and 45 C at an altitude up to 3000 metres preferred) Extremely low relative humidity to condensing humidity Result interpretation in low light settings	
Training required	<1 day, health-care worker	<4 hours, health-care worker
Clean water	None	
Time to result[e]	Less than 30 minutes	Less than 15 minutes
Duration of sample stability (time from specimen collection to insertion into test cartridge)	Fingerstick blood must be inserted immediately into cartridge; for whole blood specimen collected in an EDTA tube, at least six hours	Fingerstick blood must be inserted immediately into cartridge; for whole blood collected in an EDTA tube, at least six hours

Stability of valid result (read window)	At least 30 minutes (after which results may be *false or invalid)*	≥1 hour (after which results give *invalid* rather than *false* results)
Safety precautions (biosafety requirements)	Closed, self-contained system; unprocessed sample transfer only; no open handling of biohazardous material	
Waste/disposal requirements	Safe disposal of all waste materials	Small environmental footprint; compostable plastics for test cartridges and other materials
Internal quality control – reagents	Procedural control internalized in cartridge for each individual test run; positive control for internal quality control provided in each box of test kits	
Device control	Indicator of instability or expiration	Indicator of instability, expiration, inadequate sample and incorrect procedure and/or use
Regulatory requirements	ISO 13485:2003 certified; registered for in vitro diagnostic use	
Patient identification capability	Yes; simple, self-contained way to indicate a patient identifier	
Result display; result interpretation	Result can be read with the naked eye with minimal instructions for interpretation required by user, or with an integrated reader with an easy pictorial display: reactive, non-reactive, invalid for each test; no instructions for interpretation required	
Data acquisition and display: reader	If combined with a reader, on-device visual read-out; able to add information (patient ID, operator ID, date, location, etc.); able to store patient results; able to print out results utilizing commoditized paper products (i.e. standard paper specifications and sizes)	
Connectivity: reader	If combined with a reader, reader has integrated GPS module	If combined with a reader, internally integrated GPS/ GPRS module and conformity with HL-7 messaging standards
Data export (for quality assurance): reader	If combined with a reader, full data export over mobile phone network	If combined with a reader, full data export over mobile phone network (data transmission can automatically select between GPRS or more advanced networks and GSM, based on available coverage) GPRS should be able to utilize the internet File Transfer Protocol (FTP) to transmit data: data transfer should be initiated every 6–12 hours automatically by the reader; data can be exported in a format compatible with HL7 standards, where appropriate; instrument tracks and transmits quality assurance data over time (e.g. identify shifts or trends)
Target price for reader	≤US$100 (preferred that cost is amortized over test volumes in price per test)	No cost (any actual cost is amortized over test volumes in price per test)
Target price for disposable test strip/ cartridge	≤US$3.00	≤US$1.50

a Key populations defined as persons most at risk for HIV/syphilis (e.g. MSM and sex workers).
b TPHA also can be used as a reference standard, but there is greater variability in the results than when using TPPA.
c In whole blood specimens against TP-specific reference standard point estimates with 95% CI.
d Based on a sample size sufficient to achieve ±5% CI around a point estimate of sensitivity and specificity.
e As reported by the company in the product insert for the test.

Annex B: Combined HIV/syphilis tests – characteristics of available tests

Test name	**HIV/Syphilis Duo Rapid Test**	**DPP® HIV-Syphilis Assay**	**Multiplo Rapid TP/HIV-Syphilis Antibody Test**
Company	Standard Diagnostics, Inc. (Republic of Korea)	Chembio Diagnostic Systems, Inc. (United States)	MedMira, Inc. (Canada)
Type of technology	Rapid immunochromatographic assay, using lateral flow (RDT)	Rapid immunochromatographic assay (RDT)	Rapid immunofiltration assay (RDT)
Availability	Commercially available	Commercially available	Pipeline (available for research use)
Output	Qualitative detection of HIV-1, including subtype O, and HIV-2 (combined) and/or syphilis TP	Qualitative detection of HIV-1 and HIV-2 (combined) and/or syphilis TP	Qualitative detection of HIV-1, including subtype O, and HIV-2 (combined) and/or syphilis TP
Antigen type (HIV)	Recombinant HIV-1 capture antigen (gp41), recombinant HIV-2 capture antigen (gp36) and recombinant HIV-subtype O antigen	Unspecified mix of HIV-1/2 antigens	Synthetic HIV peptides gp36, gp41, gp120 and HIV group O
Antigen type (syphilis)	Recombinant TP antigens (17kDa)	Unspecified recombinant TP antigen	Unspecified recombinant TP antigen
Sensitivity[a]			
Anti-HIV	100%	98.7%	99.8%
Anti-TP	100%	94.3%	97.9%
Specificity[a]			
Anti-HIV	100%	100%	99.7%
Anti-TP	99.1%	100%	100%
Sample type	Whole blood (fingerstick or venous), serum or plasma	Whole blood (fingerstick or venous), serum or plasma	Whole blood (fingerstick or venous), serum or plasma
Volume of sample required	20 µL of whole blood; 10 µL of serum or plasma	Two drops of fingerstick blood; 10 µL of venous blood, serum or plasma	One drop of whole blood or one drop of serum/plasma
Sample storage	Fingerstick blood must be tested immediately; venous blood may be stored for up to three days at 2 °C–8 °C (36 °F–46 °F); freezing is recommended for storage of whole blood longer than three days If plasma or serum specimens are not tested immediately, they should be refrigerated at 2 °C–8 °C (36 °F–46 °F); freezing is recommended for storage longer than two weeks	Fingerstick blood must be tested immediately; venous blood, serum and plasma may be stored for up to three days at 2 °C–8 °C (36 °F–46 °F); if specimens are not used within three days of collection, serum or plasma specimens should be frozen at -20 °C (-4 °F)	Fingerstick blood must be tested immediately; venous blood may be stored for up to five days at 2 °C–8 °C (36 °F–46 °F); if storage of venipuncture whole blood specimen is required for more than five days, plasma should be separated from the blood and stored at -20 °C (-4 °F or) below
Time to result	~15–20 minutes	~10 minutes	~3-minute test procedure; results must be read immediately

Protocol complexity – steps required	(i) Remove the test device from the foil pouch and place it on a flat, dry surface; (ii) for whole blood specimens using a capillary pipette, add 20 µL of drawn blood specimen with a 20 µL capillary pipette into the sample well of the device (marked S) or if using a micropipette, add 10 µL of plasma or serum or 20 µL of blood into the sample well (S); (iii) add three drops (about 100 µL) of assay diluent into the sample well; (iv) interpret test results in 15–20 minutes	**For fingerstick blood:** (i) remove the DPP® HIV-Syphilis test device from its pouch; (ii) before collecting sample, write sample ID on the sample buffer bottle with the black cap; (iii) remove (unscrew) the white cap, keeping the black cap screwed onto the white part of the cap; (iv) obtain a fingerstick blood sample according to normal laboratory practices; (v) touch the sample loop to the drop of blood allowing the opening of the loop to fill with blood; (vi) insert the sample loop into the sample buffer bottle with the black cap such that the loop is touching the bottom of the bottle; (vii) snap and twist the shaft at the break notch to dislodge the loop into the bottle; (viii) replace the black/white cap assembly onto the bottle and gently shake the bottle for 10 seconds; (ix) remove (unscrew) the black cap keeping the white cap screwed onto the sample buffer bottle; invert the sample buffer bottle containing the collected sample and hold it vertically (not at an angle) over the sample + buffer well 1 on the test kit; (x) slowly add two drops into the sample + buffer well 1; (xi) wait five minutes (by which time the blue and green coloured lines in the rectangular test and control window should have disappeared; if not, discard the test device and repeat the procedures); (xii) add four drops of running buffer (green cap) to buffer well 2 (a reddish colour should begin to flow across the strip within 2–3 minutes); (xiii) read the test result 10–15 minutes after the addition of the running buffer to buffer well 2 **For venous whole blood, serum or plasma:** (i) remove the DPP® HIV-Syphilis test device from its pouch; (ii) obtain a venous blood, serum or plasma sample according to normal laboratory practices; (iii) before adding the sample, write the sample ID on the sample buffer bottle with the black cap; (iv) remove (unscrew) the white cap, keeping the black cap screwed onto the white part of the cap; (v) add 10 µL venous blood, serum or plasma sample using a calibrated pipette into the sample buffer bottle with the black cap such that the pipette tip is touching the bottom of the bottle; (vi) replace the black/white cap assembly onto the bottle and gently shake the bottle for 10 seconds; (vii–xiii) the remaining steps are the same as for the fingerstick blood sample	**For fingerstick whole blood collection and use:** (i) place sample tube in a secured rack on a flat surface; (ii) add five drops from the vial of universal buffer to the sample tube (included); (iii) obtain a fingerstick blood sample according to normal laboratory practices using the sterile lancet provided with the test; (iv) use the auto-fill pipette provided with the test to collect one drop of blood from the fingerstick site by touching the tip of the pipette to the blood sample in a horizontal position (the blood sample is automatically drawn to the black fill line); (v) place the tip of the auto-fill pipette into the universal buffer in the sample tube [prepared in step (ii) above];(vi) squeeze the bulb to empty the blood sample into the tube; (vii) discard the auto-fill pipette; (viii) hold the sample tube and gently tap the side of the tube near the bottom until the mixture becomes a clear reddish colour; (ix) pour the entire contents of the sample tube into the well of the test cartridge; (x) allow the specimen to be absorbed; (xi) place the InstantGold cap on the test cartridge; (xii) dispense the remaining buffer, in drops, from the vial of universal buffer onto the InstantGold cap and allow the solution to be absorbed; (xiii) remove the InstantGold cap, waiting for the solution to be completely absorbed; (xiv) read test results immediately **For venipuncture whole blood collection and use:** (i) use standard venous phlebotomy procedures to collect a whole blood sample; (ii) place the sample tube (provided) in a secured rack on a flat surface; (iii) add five drops from the 30 mL bottle of universal buffer (provided) to the sample tube; (iv) use the transfer pipette provided to collect specimen from the specimen collection tube; (v) add one drop of whole blood into the sample tube prepared in step iii above; (vi) hold the sample tube and gently tap the side of the tube near the bottom until the mixture becomes a clear reddish colour; (vii) pour the entire contents of the sample tube into the well of the test cartridge; (viii) allow specimen to be absorbed; (ix) place the InstantGold cap on the test cartridge; (x) dispense 12 drops from the 30 mL bottle of universal buffer onto the InstantGold cap and allow the solution to be completely absorbed; (xi) remove the InstantGold cap and wait for solution to be completely absorbed; (xii) add three drops of universal buffer to clarify results; (xiii) read test results immediately **For serum/plasma:** (i) apply three drops of universal buffer to the centre of the test cartridge; (ii) allow the buffer to absorb completely; (iii) apply one drop of serum or plasma specimen to the centre of the test membrane; (iv) wait for the specimen to absorb completely before proceeding to the next step; (v) place the InstantGold cap on the test cartridge; (vi) dispense 12 drops of universal buffer onto the InstantGold cap; (vii) allow the solution to be completely absorbed; (viii) remove the InstantGold cap; (ix) wait for the solution to be completely absorbed; (x) add three drops of universal buffer to clarify results; (xi) read test results immediately
Read window	Results should not be read more than 20 minutes after adding assay diluent	15 minutes after running buffer is added to sample	N/A; results should be read immediately after adding universal buffer

Shelf life of test kit	24 months	24 months	18 months
Storage requirements	1 °C–30 °C (test devices and diluent)	2 °C–30 °C (test devices and buffers)	2 °C–30 °C (test devices and buffers)
Test kit components	Two versions: (i) test device individually foil pouched with a dessicant; assay diluent; or (ii) test device individually foil pouched with a dessicant; 20 µL capillary pipettes; lancets; alcohol swabs	DPP® HIV-Syphilis individually pouched test devices; sample loops (10 µL), sample buffer (1 mL); lancets (for fingerstick whole blood samples); band-aids; 1 DPP running buffer bottle (6 mL) – green cap	**Multiplo TP/HIV (POC) – for fingerstick whole blood:** mylar pouches with alcohol swab, each containing: one test cartridge, one InstantGold cap, one auto-fill pipette, one sample tube, one vial universal buffer, one lancet (sterile), one silica gel packet **Multiplo TP/HIV (LAB) – for venipuncture whole blood/ serum/plasma:** mylar pouches, each containing: one test cartridge, one InstantGold cap, one silica gel packet; two bottles universal buffer (30 mL); sample tubes and transfer pipettes **Multiplo TP/HIV (LAB) – for serum/plasma only:** mylar pouches, each containing: one test cartridge, one InstantGold cap, one silica gel package; one bottle universal buffer (30 mL); transfer pipettes
Not included in test kit		Pipettor capable of delivering 10 µL to be used for serum, plasma or venous blood specimens; sterile gauze (for fingerstick samples only), disposable gloves, antiseptic wipes, biohazard disposal containers; Chembio DPP® HIV-Syphilis Reactive and Non-reactive Controls are available separately for use with the assay	
Controls	The device has a self-contained internal control: if the purple colour band is not visible within the result window after performing the test, the result is considered invalid	The device has a self-contained internal control: if the pink/purple line in the control area does not appear within 10 minutes after adding the running buffer to buffer well 2, the test is invalid	The device includes a built-in procedural and reagent control line that demonstrates the validity of the test procedure and reagent function: a vertical red line under the "C" (control region) on the test cartridge indicates that the specimen has been added to the test cartridge and that the test reagents are functioning correctly; the test result is invalid if no red line (or a broken red line) appears under the "C"
Regulatory	Submitted to WHO Prequalification Programme	USAID Waiver List; submitted to WHO Prequalification Programme	Submitted to WHO Prequalification Programme in March 2013
Estimated pricing	US$ 1.20 per test	US$ 2.50–3.00 per test	US$ 3.50 per test

N/A = Not available.
a As reported in the respective package inserts for the tests.

Annex C: Combined HIV/syphilis tests – characteristics of tests in the pipeline

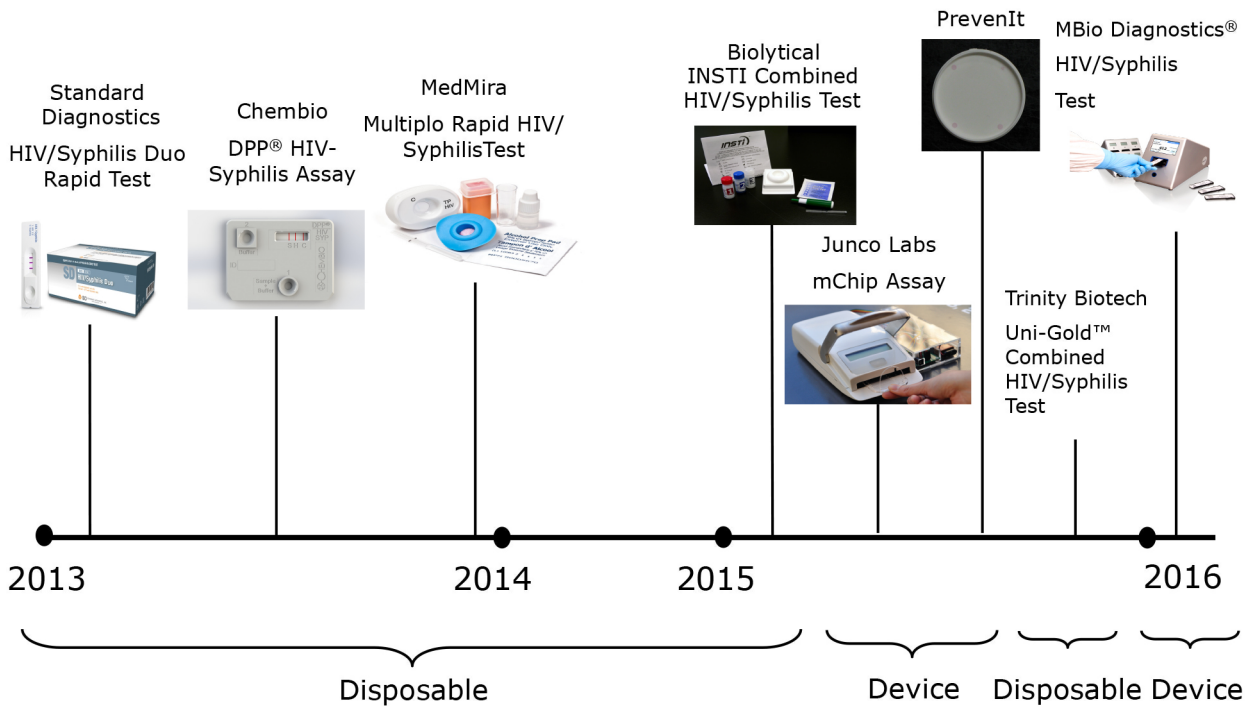
Test name	**INSTI Combined HIV/Syphilis Test**	**mChip Assay**	**Uni-Gold™ Syphilis Test[a]**
Company	BioLytical Laboratories (Canada)	Junco Labs and Columbia University in collaboration with OPKO Health, Inc. (United States)	Trinity Biotech (Ireland)
Type of technology	Immunofiltration (flow through)	Microfluidics	Immunochromatographic assay, using lateral flow (RDT)
Availability	Investigational use only; expected to be commercially available in 2014	Expected to be commercially available in 2015	Combination HIV/syphilis assay could be commercially available in 2015
Output	Qualitative detection of HIV-1 and HIV-2 (combined) and/or syphilis TP	Qualitative detection of HIV-1, including subtype O, and HIV-2 (combined) and/or syphilis TP and non-treponemal) Quantitative detection of anaemia (haemoglobin)	Qualitative detection of syphilis TP
Antigen type (HIV)	Recombinant gp36 (HIV-2) and gp41 (HIV-1)	HIV-1 gp41, O IDR, HIV-2 gp36	N/A
Antigen type (syphilis)	Recombinant p17-p47 fusion protein	TP recombinant antigens r17 (treponemal specific) Cardiolipin (non-treponemal specific)	Unspecified recombinant TP antigens
Sensitivity[b]			
Anti-HIV	100% (136/136 positive)[c]	100% (95% CI, 97–100)	
Anti-TP	96.5% (55/57 positive)	90% (87–93)	99.3% (current Uni-Gold™ Syphilis Test)
Anti-cardiolipin	N/A	95% (92–98)	N/A
Anaemia (haemoglobin)	N/A	0.2 g/dL (0–25 g/dL measurement range)	N/A
Specificity[b]			
Anti-HIV	100% (874/874 negative)	100% (95% CI, 97–100)	
Anti-TP	99.8% (991/993 negative)	90% (87–93)	94.7% (current Uni-Gold™ Syphilis Test)
Anti-cardiolipin	N/A	95% (92–98)	N/A
Anaemia	N/A	N/A	N/A
Sample type	Whole blood (fingerstick or venous), serum or plasma	Whole blood (fingerstick or venous)	Whole blood (venipuncture or fingerstick), serum or plasma
Volume of sample required	50 µL	1 µL	~60 µL
Sample storage	Whole blood collected in EDTA tubes may be stored at 2 °C–8 °C for up to five days; serum or plasma EDTA samples may be stored up to five days at 2 °C–8 °C for up to five days, up to three months at -20 °C and up to one year at -70 °C	Whole blood is stored in a sample holder; once blood is in mChip holder, it should be tested immediately, but can be stored at ambient temperature (15 °C–30 °C) for up to six hours	Whole blood samples should be tested immediately; if assays are not run immediately, a whole blood sample may be stored refrigerated (2 °C–8 °C) and tested within eight hours If testing cannot be carried out within eight hours of blood sample collection, a plasma or serum sample should be generated and stored accordingly If assays are not completed within eight hours, separated serum/plasma should be refrigerated (2 °C–8 °C) up to a maximum of 48 hours; beyond 48 hours, serum/plasma should be frozen at or below -20 °C

Time to result	60 seconds, from addition of sample to sample diluent	15 minutes	~15 minutes
Protocol complexity – steps required	**For fingerstick blood**, (i) obtain a fingerstick blood sample according to normal laboratory practices and instructions in package insert using the sterile lancet provided; (ii) as the blood bubbles up, hold the pipette (provided) horizontally and touch the tip of the pipette to the blood; (iii) transfer the blood held in the pipette to the sample diluent vial (solution 1); (iv) align the tip of the pipette with the sample diluent vial and squeeze the bulb to dispense the sample; (v) tear open the pouch and carefully remove the membrane unit without touching the centre well; (vi) place the membrane unit on a level surface (for sample identification purposes the tab of the membrane unit may be labelled with the patient's name or number); (vii) remix the sample diluent-specimen mixture and pour the entire contents into the centre of the membrane unit well within five minutes after the specimen has been added to the sample diluent vial; (viii) re-suspend the colour developer (solution 2 vial) by slowly inverting to mix the solution thoroughly, continuing this process until careful visual observation confirms that the reagent is evenly suspended; (ix) open the colour developer and add the entire contents to the centre of the membrane unit well (the coloured solution should flow through completely in about 20 seconds); (x) open the clarifying solution (solution 3 vial) and add the entire contents to the centre of the membrane unit well; (xi) immediately read the result while the membrane is still wet **For venous blood, serum or plasma:** (i) obtain a venous blood, serum or plasma sample according to normal laboratory practices; (ii) gather one sealed test pouch containing the membrane unit, and one vial each of the sample diluent (solution 1 vial), colour developer (solution 2 vial), and clarifying solution (solution 3 vial) for each test to be performed; (iii) using a pipette, add 50 µl of whole blood, serum or plasma to the sample diluent vial; (iv) recap the vial and mix by inversion; (v–xi) the remaining steps are the same as for the fingerstick blood sample	**For fingerstick blood**, (i) obtain a fingerstick blood sample according to normal laboratory practices using a sterile lancet; (ii) wick blood into the sample holder capillary tube; (iii) snap sample holder into the microfluidic chip with pre-stored reagents; (iv) insert the microfluidic chip into the dongle (which inserts into a smartphone that is loaded with a dedicated app that provides step-by-step on-screen guidance); (v) read results from smartphone in 15 minutes **For venous blood**, (i) use standard venous phlebotomy procedures to collect a whole blood sample; (ii) use a transfer pipette to collect specimen from a specimen collection tube; steps iii–v are the same as for fingerstick blood	After removing test device and placing it on a flat surface: **For whole blood,** use the capillary tube provided, (i) holding the capillary tube horizontally, touch the tip of the tube to the whole blood sample (making sure not to squeeze the bulb) until sample reaches the indicated line; (ii) touch the end of the capillary tube onto the bottom of the sample port and squeeze out the entire volume; (iii) add three drops of wash buffer to sample port; (iv) keeping test device on level surface, incubate at room temperature; (v) read results immediately after a 15-minute incubation period **For serum or plasma samples**, use the transfer pipette provided, (i) squeeze the transfer pipette midway and place the pipette in the sample to be tested; (ii) draw sample up to the black indication line and remove from sample tube; (iii) touch the end of the pipette onto the bottom of the sample port on the test device and dispense the entire volume; (iv) add three drops of wash buffer to sample port; (v) keeping test device on level surface, incubate at room temperature; (vi) read results immediately after a 15-minute incubation period

Read window	Five minutes, as per package insert; results should not be read if more than five minutes have elapsed following the addition of the clarifying solution	N/A; results are shown on smartphone screen and may be stored or sent to cloud	Test should not be read after a 15-minute incubation period
Shelf life of test kit	15 months	6 months	~12 months
Storage requirements	15 °C–30 °C	15 °C–30 °C	2 °C–30 °C
Test kit components	Blotted membrane units, individually packaged; ready-to-use sample diluent (solution 1 vial); ready-to-use colour solution (solution 2 vial); ready-to-use clarifying solution (solution 3 vial); test kits may be purchased with or without accessories (lancet, pipette, alcohol swab)	Single-use sample holders; individually-pouched, single-use microfluidic chips on which reagents are pre-stored; lancet; package insert	Test device; one disposable pipette per each test device; wash buffer
Not included in test kit	HIV-1, HIV-2, TP and negative controls available	Dongle; smartphone	Venous whole blood collection container; sterile lancet device for fingerstick blood collection; antiseptic wipes; controls
Controls	Test has built-in procedural controls that demonstrate assay validity and adequate sample addition	Internal negative and positive control for each test; external quality control kit is available separately	Built-in procedural control on the test device indicates that the test is functioning correctly; a pink/red band should always appear at the control window
Regulatory	Not yet approved by regulatory bodies; INSTI HIV test is United States Federal Drug Administration approved; Clinical Laboratory Improvement Amendments waived; Health Canada licensed; CE-marked; WHO prequalified		
Pricing	To be determined	US$ 2 per test, US$ 30 mChip device (dongle)	To be determined

N/A = Not available.
a Listed below are the operational characteristics of the current Uni-Gold™ Syphilis Assay. It is expected that the proposed combination HIV/syphilis assay would be functionally similar to this assay, but the company has not yet defined the TPP for the assay.
b As reported by the company from preliminary studies.
c Data from field testing in Bangalore, India, 2012–2013.

Annex D: Available HIV/syphilis products and product pipeline*



*Estimated - timeline and sequence may change