The Point-of-Care Diagnostic Landscape for Sexually Transmitted Infections (STIs)

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Sexually transmitted infections (STIs) continue to be a significant global public health issue, with an estimated 357 million people becoming ill each year with one of 4 STIs: syphilis, *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Trichomonas vaginalis* (TV) *(1)*. In addition, more than 290 million women have a human papillomavirus (HPV), which infection is a necessary cause of high grade cervical intraepithelial neoplasia (grade 2 or higher [CIN2+]) *(1)*.

This report considers the available and pipeline diagnostics for curable STIs, namely syphilis, CT, NG, TV, and HPV. With some exceptions, the existing diagnostics for these STIs are laboratory-based platforms, which typically require strong laboratory infrastructure and well-trained laboratory technicians. In addition, test turnaround time is often long, requiring patients to return for test results on a subsequent clinic visit. This, in turn, leads to significant loss to follow-up. Therefore, while these laboratory-based diagnostics are effective, they may not always be suitable for use in resource-limited settings where diagnostic access and delivery are difficult.

There are now a variety of tests available for use at or near the point of patient care (POC) for STIs *(2)*. These include a wide range of rapid diagnostic tests (RDTs) for human immunodeficiency virus (HIV), hepatitis C virus and syphilis, among others, with which it is possible to detect infection using fingerprick blood, or in some cases, oral fluid.[1] In addition, other types of POC tests, including simple molecular tests for use in primary healthcare settings, have also become available recently. This review focuses on the newest diagnostic platforms for syphilis, including syphilis dual tests, CT, NG, TV and HPV that are designed for use at or near the point of patient care.

Methodology

The Point-of-Care Diagnostic Landscape for Sexually Transmitted Infections (STIs) is compiled by Maurine M. Murtagh with support from the Department of Reproductive Health for Research of the World Health Organization (WHO). The material in this landscape was gathered by the author from publicly available information, published and unpublished reports and prospectuses, and interviews with developers and manufacturers. The prices for diagnostic equipment and reagents cited in this report were obtained directly from manufacturers and are ex works prices, meaning that they are the prices at the manufacturer's factory, and do not include any delivery, distribution, taxes or commission charges. The material is current through 31 May 2018.

Syphilis

The WHO estimates that in 2012, the most recent year for which such statistics are available, there were approximately 6 million new cases of syphilis worldwide *(3)*. The highest disease burden for syphilis is in the African region *(3)*.

[1] Note that RDTs are a subset of POC tests. RDTs are generally lateral fluid or immunofiltration strip or cassette-enclosed tests that are disposable, easy-to-use and have short time to test result. They can be used at all levels of the healthcare system.

Syphilis has particularly profound consequences for pregnant women, considered to be a vulnerable population, and for individuals in certain key populations where its prevalence is high: men who have sex with men (MSM) and sex workers. With respect to pregnant women, maternal syphilis is a significant cause of infant mortality. In 2012, researchers estimated that almost one million cases of syphilis occurred worldwide among pregnant women, many of whom were either untreated or inadequately treated *(4)*. It was estimated that about 350,000 adverse pregnancy outcomes, including 143,000 early fetal deaths and stillbirths, 62,000 neonatal deaths, 44,000 preterm or low weight births, and 102,000 infected infants resulted *(4)*. Although these results are an improvement over the 2008 estimates, 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 *(5)*.

With respect to MSM, the WHO estimates that syphilis infects 5% or more of MSM in at least 42 countries, 10% or more in 20 countries and more than 20% in 8 countries *(6)*. In the United States, the United States Centers for Disease Control and Prevention (CDC) estimates that in 2016, 80.6% of syphilis cases are among MSM and the numbers are increasing *(7)*. The WHO also reports an increasing trend of syphilis in MSM, with 48% of all new syphilis cases in 2012 reported among that population *(8)*. Untreated, syphilis can lead not only to serious complications, but it also increases the risk of acquiring and transmitting HIV.

Finally, per the WHO, syphilis infects more than 5% of sex workers in 37 countries, more than 10% in 23 countries and more than 20% in 7 countries *(9)*. Sex workers include female, male and transgender individuals who receive money/goods in exchange for sexual services, and in many places, they are very vulnerable to HIV and other STIs *(9)*.

Syphilis is usually diagnosed using laboratory-based tests, consisting of both non-*Treponema pallidum* (non-TP) and *Treponema pallidum* (TP) tests. However, given the cost of the tests and the required infrastructure and need for well-trained staff, these tests are generally only available at reference laboratories in resource-limited settings.

In recent years, a range of RDTs for syphilis screening have been developed. These tests are antibody tests that detect TP. Among them are CE-marked rapid tests from AccuBioTech (Accu-Tell® Rapid Syphilis Test), Alere, Inc. (Alere Determine™), Alere/Standard Diagnostics (SD Syphilis 3.0), The Tulip Group/Qualpro (Syphicheck® - WB), Cypress Diagnostics (Syphilis Rapid Test), and Omega Diagnostics (Visitect® Syphilis).[2] These tests are summarized below.

[2] Note that to date the WHO prequalification program for diagnostics has not prequalified syphilis only assays. Therefore, manufacturers have primarily relied on European Union approval through the CE-marking process. However, the WHO will begin accepting applications to prequalify syphilis only RDTs in the 4th quarter of 2018 *(10)*.

Test (Manufacturer)	Specimen	Volume of whole blood or other specimen	Time to result (minutes)	Storage Temperature (°C)	Test life (months)	Test type
Alere Determine™ Syphilis TP (Alere, Inc. USA)	Whole blood (fingerstick), plasma or serum	50 µL	15 minutes (up to 24 hours)	2 - 30°	NA	Lateral flow strip
SD Syphilis 3.0 Alere/SD Bioline (South Korea)	Whole blood (venous or fingerstick), plasma or serum	20 µL (whole blood) 10 µL (plasma or serum)	5 - 20 minutes	2 - 30°	NA	Cassette enclosed test card
Syphicheck® - WB The Tulip Group/Qualpro (India)	Whole blood (venous or fingerstick), plasma or serum	25 µL	15 minutes	4 - 30°	NA	Cassette enclosed test card
Visitect® Syphilis Omega Diagnostics (UK)	Whole blood (venous or fingerstick), plasma or serum	50 µL	30 minutes	4 - 30°	NA	Cassette enclosed test card

NA = Not Available

Table 1. Select RDTs for Detection of Syphilis

Because of the persistence of treponemal antibodies, however, these TP RDTs cannot distinguish between active and past treated infections. But, in resource-limited settings, where many people don't have access to laboratory-based non-TP tests for confirmation of active syphilis, pregnant women who are found to be seropositive with a TP RDT are treated for syphilis in order to prevent transmission of the infection. As indicated by Jafari *et al*: "This is now accepted practice as the risk of over-treatment due to biological false positives which are not syphilis in origin is more acceptable than the risk of non-treatment of syphilis" *(11).*

The other concern about TP RDTs has been performance. However, 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 *(11)*. In this review, adjustments were made for imperfect reference standards using the Bayesian Hierarchical Summary Receiver Operating Characteristic Curve method. The result is point estimates of sensitivity and specificity for each test, using serum and whole blood, around a 95% credible interval (as opposed to a confidence interval), as shown in the table following.

RDT	Sample	Parameters	Assuming Imperfect Reference Standards (95% CrI)
Alere Determine™	Serum	Sensitivity Specificity	90.04% (80.45, 95.21) 94.15% (89.26, 97.66)
	Whole Blood	Sensitivity Specificity	86.32% (77.26, 91.70) 95.85% (92.42, 97.74)
SD Syphilis 3.0	Serum	Sensitivity Specificity	87.06% (75.67, 94.50) 95.85% (89.89, 99.53)
	Whole Blood	Sensitivity Specificity	84.50% (78.81, 92.61) 97.95% (92.54, 99.33)
Syphicheck® - WB	Serum	Sensitivity Specificity	74.48% (56.85, 88.44) 99.14% (96.37, 100.0)
	Whole Blood	Sensitivity Specificity	74.47% (63.94, 82.13) 99.58% (98.91, 99.96)
Visitect® Syphilis	Serum	Sensitivity Specificity	85.13% (72.83, 92.57) 96.45% (91.92, 99.29)
	Whole Blood	Sensitivity Specificity	74.26% (53.62, 83.68) 99.43%, (98.22, 99.98)

Crl = Credible Interval; NA = Not Available.

Table 2. Meta Analysis Data on Performance of Select TP RDTs for Syphilis. Adapted from Jafari *et al (11)*. For further detail, refer to published article.

The conclusions of the meta analysis are that, overall, the four tests (Alere Determine™, SD Syphilis 3.0, Syphicheck® - WB and Visitect® Syphilis) performed well in both sensitivity and specificity when compared to laboratory-based TP-specific tests, including TP haemagglutination assays (TPHAs) and TP particle agglutination assays (TPPAs), which have sensitivities from about 95 – 100% and specificities from about 98 – 100%. Of these, Determine™ had the best sensitivity, and Syphicheck® had the best specificity. In general, therefore, the tests are useful in resource-constrained settings where access to laboratory testing for syphilis is limited *(11)*.[3]

In addition to the four tests that were part of the meta-analysis, additional TP RDTs are available. These include, but are not limited to:

[3] See, also, Tucker JD et al. Accelerating worldwide syphilis screening through rapid testing: a systematic review. Lancet Infect Dis. 2010; 10: 381-86 *(12)*; Mabey D et al. Prospective, multi-centre clinic-based evaluation of four rapid diagnostic tests for syphilis. Sex Transm Infect. 2006; 82:v13-v16. Doi: 10.1136/sti.2006.022467 *(13).*

Manufacturer	Specimen	Volume of whole blood or other specimen	Time to result (minutes)	Storage Temperature (°C)	Test life (months)	Test type
OnSite™ Syphilis Ab Combo Rapid Test CTK Biotech, Inc. (USA)	Whole blood (venous or fingerstick)	1 drop	15 minutes	2 - 30°	NA	Cassette enclosed test card
Syphilis Health Check™ Trinity Biotech (Ireland)	Whole blood (fingerstick), plasma or serum	2 drops	10 minutes	Room temperature	NA	Cassette enclosed test card
Syphilis Rapid Test Cypress Diagnostics	Whole blood, plasma or serum	20 µL (whole blood) 10 µL (plasma or serum)	5 – 20 minutes	8 - 30°	NA	Cassette enclosed test card
Uni-Gold™ Syphilis Treponemal Trinity Biotech (Ireland)	Whole blood (venous or fingerstick), plasma or serum	~60 µL	~15 minutes	2 - 30°	~12 months	Cassette enclosed test card

Table 3. Additional Commercially-Available TP TDTs.

Of these syphilis RDTs, the Syphilis Health Check™ is FDA-approved and CLIA waived in the United States, and the *OnSite*™, Syphilis Rapid Test, and Uni-Gold™ tests are CE-marked.

The *OnSite*™ Syphilis Ab Combo Rapid Test was the subject of a laboratory evaluation in Australia by Causer et al, which found that the test had sensitivity and specificity of 92.5% (95% Confidence Interval [CI] 90.3% - 94.3% in each case) when compared to reference TP assay tests. (*14*) In a laboratory-based clinical evaluation of the Syphilis Health Check™ assay in Uganda, Nakku-Joloba et al found that the test had sensitivity of 89.8% (95%CI, 82.0% - 95.0%) and specificity of 92.3% (95%CI, 85.9% - 96.4%) compared with TPHA (*15*). The sensitivity of the Syphilis Health Check™ against the clinical algorithm of sequential rapid plasma reagin (RPR) and TPHA was 95.3% (95%CI, 88.4% – 98.7%) and the specificity of the test was 98.8% (95%CI, 93.6% – 99.9%) (*15*). In a study in Burkina Faso, the Syphilis Rapid Test from Cypress Diagnostics demonstrated sensitive of 90% (95%CI, 79% - 95%) and specificity of 95% (95%CI, 86% - 98%) *(16)*. No peer reviewed evaluations of the Uni-Gold™ Trepenomal test were found.

Need for Additional Syphilis Tests for Resource-Limited Settings

Because of the generally good performance of syphilis RDTs, there is arguably no need for additional mono tests. However, there are several important needs for new syphilis dual tests, preferably in the form of RDTs, in resource-limited settings. One of these is for a combination TP/non-TP test that can be used to diagnose syphilis at POC where traditional laboratory-based testing may not be available. Another need is for HIV/syphilis dual tests. Both of these are discussed below.

DPP® Syphilis Screen & Confirm Assay (Chembio Diagnostic Systems)

Chembio has developed the first dual non-TP and TP syphilis test for use at the point-of-care.[4] The assay, pictured below, is CE-marked and uses a unique combination of protein A and anti-human IgM antibody, which are conjugated to colloidal gold particles. It also employs a recombinant antigen to TP and synthetic antigens for non-TP, separately bound to the membrane solid phase. The result is an assay that permits the simultaneous, yet separate, detection of both markers.

Figure 1. DPP® Syphilis Screen & Confirm Assay

The DPP® Syphilis Screen & Confirm Assay requires a sample size of only 10 µl of whole blood (fingerstick or venipuncture), and tests can be stored at room temperature (2 to 30°C). The turnaround time of the test is 15 – 20 minutes. The test has been shown to be not only highly sensitive and specific, but also useful for the serological diagnosis of syphilis in primary health care clinics or resource-poor settings.

There are several peer-reviewed publications of the performance of the DPP assay, each of which found that the test performed favorably against laboratory-based reference tests. In a multi-center field study in China, three kinds of specimens (whole blood [WP], fingerprick blood [FP] and blood plasma [BP]) were used to evaluate the sensitivity and specificity of the DPP® platform. The TPPA assay and the toluidine red unheated serum test (TRUST) were used as reference standards. A total of 3,134 specimens (WB 1,323, FB 488, and BP 1,323) from 1,323 individuals were collected. The sensitivities as

[4] Note that Span Diagnostics had also developed TP/Non-TP immunofiltration rapid test, but it is not currently a product that Span is offering commercially. *See* Castro AR, Mody HC, Parab SY, *et al*. An immunofiltration device for the simultaneous detection of non-treponemal and treponemal antibodies in patients with syphilis. *Sex Transm Infect*. 2010; 86: 532-536 *(17).*

compared with TPPA were 96.7% for WB, 96.4% for FB, and 94.6% for BP, the specificities were 99.3%, 99.1%, and 99.6%, respectively. When compared with TRUST, the sensitivities were 87.2% for WB, 85.8% for FB, and 88.4% for BP. The specificities were 94.4%, 96.1%, and 95.0%, respectively. The sensitivity and specificity of the non-TP spot were 96.5% and 97.7%, respectively, when compared to the RPR test. The sensitivity and specificity of the TP test spot were 97.3% and 99.1%, respectively, when compared with the TPPA test *(18)*.

The second study of the DPP assay®, a laboratory-based evaluation, used 1,601 banked serum samples. The TPPA assay and a quantitative RPR test were used as reference standards. Compared to the RPR test, the sensitivity of the DPP® non-TP line was 98.4% when the RPR titers of sera were ≥1:2, and the specificity of the non-TP line was 98.6%. Compared to the TPPA assay, the reactive and nonreactive concordances of the TP line were 96.5% and 95.5%, respectively *(19)*.

Causer et al performed another study in Australia. Of the 1,005 serum samples tested, the DPP TP line sensitivity was 89.8% (95% CI, 87.3% - 91.9%) and specificity was 99.3% (95% CI, 97.0% - 99.9%) (*19*). The DPP non-TP line sensitivity was 94.2% (95% CI, 91.8% - 96.0%) and specificity was 62.2% (95% CI, 57.5% - 66.6%) (*20*). The study compared TP and non-TP lines to corresponding conventional TP and non-TP reference test results: immunoassays and RPR, respectively. The DPP test outcome (considering paired test lines) was concordant with both reference test results for 94.3% of 404 high-titer infections, 90.1% of 121 low-titer infections, 27.5% of 211 past/treated infections, and 78.1% of 242 infections classified as not being syphilis *(20)*. Of the 211 past/treated infections, 49.8% were incorrectly identified as active infection and a further 22.8% as not syphilis *(20)*. The authors conclude that the DPP test would result in identification of more than 93% of active syphilis infections, but note that the sensitivity of the DPP TP line is lower than other TP-only syphilis tests, such as Alere Determine™ *(14,20)*. They further note that while the DPP assay can improve identification of past infections and avoid unnecessary treatment, there may be a trade-off with respect to lower TP sensitivity, which could mean cases that require treatment are missed (*19*). The authors conclude that unless there is a substantial prevalence of past/treated infection in the population at risk, a TP-only POC test may be preferred *(20)*.

Each of the studies concluded, however, that the DPP® Screen & Confirm Assay could be useful for diagnosing syphilis in primary healthcare settings in resource-limited settings.

Other than the Chembio assay, no additional combined TP/non-TP RDTs were identified in the pipeline.

Arguably, however, the greatest need in resource-limited settings now is for a combination test for syphilis and HIV for certain target populations, including MSM, sex workers and pregnant women. Perhaps the most acute of these needs is a dual test to help eliminate mother-to-child transmission (MTCT) of HIV and syphilis, which is a significant cause of death in infants and young children globally each year. Of the approximately 1.7 million new HIV infections among adults and children in 2016, for example, it is estimated that about 160,000 children became newly infected with HIV, most of whom are children who live in sub-Saharan Africa and were infected by HIV-positive mothers during pregnancy, childbirth or breastfeeding *(21)*. 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. WHO estimates that

absent any interventions in pregnancy, transmission from an HIV-positive woman to her child ranges from 15% to 45% *(22)*. It is estimated, however, 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 to 5% *(22)*. It also has been demonstrated that programs that include syphilis testing along with appropriate and timely penicillin treatment for pregnant women who test positive for TP infection can reduce adverse pregnancy outcomes *(23,24,25)*.

WHO has long supported screening of all pregnant women for HIV *(26)*, 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 *(27,28,29,30)*, WHO launched a global initiative for the elimination of congenital syphilis in 2007 (*31*). 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 programs 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. In addition, such tests can be a very important tool in the fight against HIV and syphilis in target populations, including MSM and sex workers, who are typically hard-to-reach populations, making it particularly important to provide a package of testing services to them at a single patient visit.

Combined HIV/syphilis tests currently in the market

This section of the report describes the available combined HIV/syphilis tests designed for use at the point of care, all of which are RDTs. It also describes combined HIV/syphilis tests in the pipeline. The available and pipeline tests are shown in summary form in **Annex A**.

There are currently at least six combination HIV/syphilis (TP) RDTs on the market: the SD Bioline HIV/Syphilis Duo Rapid Test (Alere, Inc.), the DPP® HIV-Syphilis Assay (Chembio Diagnostics Systems, Inc.), the Multiplo Rapid TP/HIV Antibody Test (MedMira, Inc.), the INSTI™ HIV/Syphilis Multiplex Test (biolytical Laboratories Inc.), the *OnSite*™ HIV/Syphilis Ab Combo Rapid Test (CTK Biotech Co), and First Response® HIV 1 +2/ Syphilis Combo Card Test (Premier Medical Corporation).

Of the six tests, three of them – the tests from Alere, Chembio and MedMira – have been the subject of published, independent laboratory evaluations. In the first of these evaluations, the performance of the three tests was compared using sera specimens in the United States. All three RDTs were tested in parallel by trained laboratory technicians. The results of the RDTs for HIV were compared to those via routine testing (EIA and Western blot); while the results of the TP assay were compared to TPPA test results. One hundred and fifty samples were included in the study. The performance of the RDTs was good. Sensitivity for HIV antibody detection by the RDTs ranged from 98 to 99%, and the specificity ranged from 94 to 100%, compared to the Siemens Advia HIV EIA. The authors characterized the performance of the three RDTs as excellent for the detection of TP, ranging from 93 to 95% sensitivity

and 97 to 100% specificity, compared to TPPA. The authors concluded that overall the evaluations "showed performance by the RDTs that was comparable to the reference methods, with excellent sensitivity and specificity" *(32).*

A second simultaneous evaluation of the three dual RDTs from Alere, Chembio and MedMira was conducted at three laboratories in China and Nigeria. A total of 1,514 serum specimens were included in the study. Reference tests varied among the laboratory sites participating in the study. The authors report that all three of the tests had "encouraging" laboratory performance for detection of HIV antibodies, with a combined sensitivity/specificity of 99.6%/97.9% for DPP® HIV-Syphilis Assay (Chembio), 99.5%/98.3% for the Multiplo Rapid TP/HIV Antibody Test (MedMira), and 99.0%/99.0% for SD Bioline (Alere, Inc.) *(32)*. Similarly, the combined sensitivity/specificity of the RDTs for identifying TP antibodies were 97.0%/99.6% for Chembio, 94.2%/97.2% for MedMira, and 96.6%/99.1% for SD Bioline HIV/Syphilis Duo Rapid Test *(28)*. The authors concluded that all three of the HIV/syphilis dual RDTs evaluated have "acceptable sensitivity and specificity to detect HIV or syphilis, although the sensitivity to detect HIV antibodies (99.0% - 99.6%) is generally higher than that for syphilis (94.2% - 97.0%) *(33)*.

The performance of the assays as determined by the studies above are summarized below. Both of the evaluations of the three RDTs for detection of HIV and syphilis were conducted in laboratory settings with trained users using serum specimens.

RDT	Sample	Parameters	Performance (95% CI) HIV Antibody	Performance (95% CI) TP Antibody
SD Bioline HIV/Syphilis Duo Rapid Test (Alere, Inc.)	Sera	Sensitivity Specificity	97.9% (92.0 – 99.6) 99.0% (98.8 – 99.9) 100% (91.5 – 100) 99.0% (98.0 – 99.5)	93.0% (84.8 - 97.1) 99.6% (95.0 – 97.7) 100% (92.9 - 100) 99.1% (98.2 – 99.6)
DPP® HIV-Syphilis Assay (Chembio Diagnostics Systems, Inc.)	Sera	Sensitivity Specificity	98.9%(93.6 – 99.9) 99.6% (98.8 – 99.9) 98.1% (88.6 – 99.9) 97.9% (96.7 – 98.7)	95.3% (87.9 – 98.5) 97.0% (95.5 – 98.0 100% (92.9 – 100) 99.6% (98.9 – 99.9)
Multiplo Rapid TP/HIV Antibody Test (MedMira, Inc.)	Sera	Sensitivity Specificity	97.9% (92.0 – 99.6) 99.5% (99.4 – 99.8) 94.2% (83.1 – 98.5) 98.3% (97.2 – 99.0)	94.1% (86.3- 97.8) 94.2% (92.3 – 95.7) 96.9% (88.2 – 99.5) 99.1% (98.2 – 99.6)

Table 4. Summary of Performance of Three Commercially-available Combined HIV/Syphilis Tests.

Yin *et al* suggest that further research on the above RDTs is needed to evaluate their performance on whole blood samples in primary healthcare settings – i.e., in the hands of less well trained users in target use settings *(33)*.

In addition to the studies described above, Gliddon et al have conducted a systematic review and meta-analysis of studies evaluating the performance and operational characteristics of combined RDTs for HIV and syphilis *(34)*. The overall findings of the authors are that the studies indicate that the dual tests demonstrated high sensitivity with respect to HIV, and somewhat lower, but adequate sensitivity, with respect to syphilis *(34)*. In addition to evaluating the literature with respect to the diagnostic accuracy of the combined HIV/syphilis tests, the authors also evaluated the findings of the studies with respect to cost-effectiveness, feasibility, acceptability and ease of interpretation of the tests. Here the authors found that the studies indicated that combined HIV/syphilis tests are acceptable to clients, feasible for implementation in antenatal care centers, and cost-effective *(34)*.

Each of the six combined HIV/syphilis assays currently in the market is described below. Where available, peer-reviewed, published studies on the individual tests are also cited.

SD Bioline HIV/Syphilis Duo Rapid Test (Alere, Inc.)

One of six HIV/syphilis rapid diagnostic tests currently on the market is the SD Bioline HIV/Syphilis Duo Rapid Test from Alere (pictured below).

Figure 2. SD Bioline HIV/Syphilis Duo Rapid Test

The SD Bioline 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.

There are a number of peer-reviewed, published performance evaluations of the SD Bioline HIV/Syphilis Duo Rapid Test, several of which were done in field settings in Ghana, Mexico, Laos, Togo, Kenya, and Myanmar *(35)*, Uganda *(36)*, Ethiopia *(37)*, Peru *(38)*, Haiti *(39)*, Nepal *(40)*, and the United States *(41)*. All have found good sensitivity and specificity with respect to both components (HIV and TP) of the test, and many authors advocate for wider use of the test; however, Holden et al found that the performance

of the syphilis component of the SD Bioline HIV/Syphilis Duo Rapid Test against TPPA requires further testing and assessment *(41)*.

DPP® HIV-Syphilis Assay (Chembio Diagnostic Systems, Inc.)

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 SampleTainer® specimen collection bottle, which is a safe, closed system for handling potentially infectious blood samples. Turnaround time for the test is about 10 minutes.

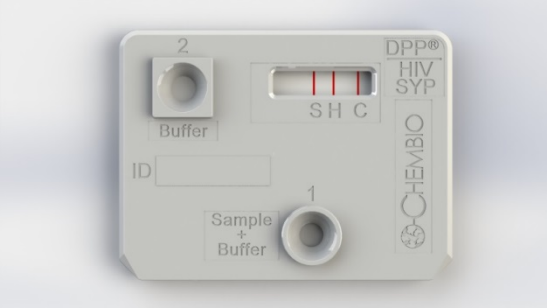


Figure 3. DPP® HIV-Syphilis Assay

In addition to the three simultaneous evaluations of combined HIV/syphilis RDTs described above, two independent, peer-reviewed studies were found with respect to the DPP® HIV-Syphilis Assay. In a laboratory evaluation of the DPP® using 450 previously characterized serum specimens, Leon et al found that the sensitivity of HIV antibody detection was 100% and the specificity was 98.7% (with or without the use of an electronic reader) (*39*). For visual TP antibody detection, the sensitivity of the assay was 94.7% and the specificity was 100.0%; using the electronic reader, the sensitivity of the test was 94.7% and the specificity was 99.7% *(42)*. Similarly, using serum samples, Kalou et al found that the sensitivity and specificity of the DPP® assay were 99.8% and 98.4%, respectively; for syphilis, they were 98.8% and 99.4%, respectively *(43)*. However, the study found that although 344 of 348 co-infected sera were identified accurately by the DPP® assay, 9 HIV specimens and 2 syphilis specimens had false-positive results due to weak reactivity *(43)*.

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

The Multiplo Rapid TP/HIV Antibody Test from MedMira, Inc. (POC format pictured below) is CE-IVD marked, making it available for sale and distribution throughout the European Union, and the company is pursuing product registration in a number of other markets. The combination assay is built on the MedMira Rapid Vertical Flow Technology platform and is sold in in the same packaging formats as the company's rapid HIV antibody test.

Figure 4. Multiplo Rapid TP/HIV Antibody Test

The Multiplo Rapid TP/HIV Antibody test combines qualitative detection of HIV-1 and HIV-2 with qualitative detection of TP in an immunofiltration format. Turnaround time is approximately three minutes.

Two peer-reviewed, published performance evaluations of the Multiplo Rapid TP/HIV Antibody Test were found in the literature. A total of 200 stored serum specimens collected from MSM and transgender women presenting in one of two STI clinics in Lima, Peru were tested in a laboratory setting by Bristow et al (*44*). The estimated sensitivity of the HIV component of the Multiplo test was 100% with a 95% CI of 95.1% - 100%, and the specificity was estimated to be 91.9% (95%CI, 85.7% - 96.1%) (*40*). With respect to the TP antibody component of the test, the sensitivity and specificity estimates were 94.6% (95% CI, 88.5% - 98.0%) and 92.8% (95% CI 84.9% - 97.3%) (*44*). Subsequently, Bristow et al. conducted a field evaluation of the Mutliplo test in Lima, Peru. The sensitivity and specificity of the HIV antibody component of the test were 93.8% (95% CI, 69.8% - 99.8%), and 100% (95% CI, 97.7% - 100%), respectively (*45*). The TP component of the test had a sensitivity of 81% (95% CI, 68.1% - 94.6%) and a specificity of 100% ((95% CI, 97.6% - 100%) (*45*).

INSTI™ HIV/Syphilis Multiplex Test (biolytical Laboratories Inc.)

The INSTI™ HIV/Syphilis Multiplex test, pictured below, 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. Turnaround time is about 60 seconds.

Figure 5. INSTI™ HIV/Syphilis Multiplex Test

The INSTI™ combo test has been introduced onto the market. One peer-reviewed published performance evaluation of the assay was found. De Cortina et al tested 200 stored serum samples from high-risk patients enrolled in a longitudinal study on HIV infection and syphilis in Peruvian MSM and transgender women (*46*). They found that the INSTI™ HIV/Syphilis Multiplex Test detected HIV and TP serum antibodies with sensitivities of 100% (95%CI, 95.9% to 100%) and 87.4% (95% CI, 81.4% to 92.0%), respectively, and specificities of 95.5% (95% CI, 89.9% to 98.5%) and 97.0% (95% CI, 84.2% to 99.9%), respectively (*46*). The authors noted that the sensitivity of the syphilis assay was higher in patients with a RPR titer of ≥1:8 (97.3%) than in those with a titer of ≤1:4 (90%) or a nonreactive titer (66.7%) (*46*).

***OnSite*™ HIV/Syphilis Ab Combo Rapid Test** (CTK Biotech, Inc.)

CTK Biotech has introduced the OnSite™ HIV/Syphilis Ab Combo Rapid Test, pictured below.

Figure 6. *OnSite*™ HIV/Syphilis Ab Combo Rapid Test

The assay is a lateral flow chromatographic immunoassay for the qualitative detection of antibodies to HIV-1, HIV-2 and TP. It is a three-line test that can be used with whole blood, serum or, plasma to detect IgG, IgM and IgA to HIV-1 and HIV-2, and TP. Results are available within 15 minutes.

No published, peer-reviewed studies on the performance of the *OnSite*™ HIV/syphilis dual test were found in the literature.

First Response® HIV 1+2/Syphilis Combo Card Test (Premier Medical Corporation)

Premier Medical Corporation has developed the First Response® HIV 1+2/Syphilis Combo Card Test, which is based on the principle of immunochromatography for the qualitative detection of antibodies (IgG and IgM) specific for HIV 1+2 and/or syphilis. It is a three-line test that can be used with whole blood, serum, or plasma with results within 15 minutes.

No published, peer reviewed studies on the performance of the First Response® HIV 1+2/Syphilis Combo Card Test were found in the literature.

The detailed performance (sensitivity and specificity), as reported by the companies in product inserts, and operational characteristics of the six assays described above are detailed in **Annex B**. Both the SD Bioline and DPP dual assays have been evaluated by the CDC and are on the USAID Waiver List (https://www.usaid.gov/sites/default/files/documents/1864/Rapid-Test-Kit-List-February-1-2017-508_0.pdf). The SD Bioline and INSTI assays are CE-IVD marked. All but the CTK assay have been submitted to WHO for prequalification, but to date, only the SD Bioline assay has been prequalified, and there are currently no products actively under review for prequalification.

Combined HIV/syphilis tests in the pipeline

In addition to the assays described above, another combined HIV/syphilis test for use at POC is in the pipeline - an assay from Junco Labs and Columbia University in collaboration with OPKO Health, Inc. (See **Annex C** for operational characteristics of the assay.) .

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

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-TP syphilis and the quantitative detection of anemia (hemoglobin) in a device-based format that utilizes a reusable microfluidic mChip and a smart phone (pictured below) for read-out of results. The technology has been evaluated in Rwanda with good results *(47)*. No commercial launch date for the HIV/syphilis TP assay has been set.

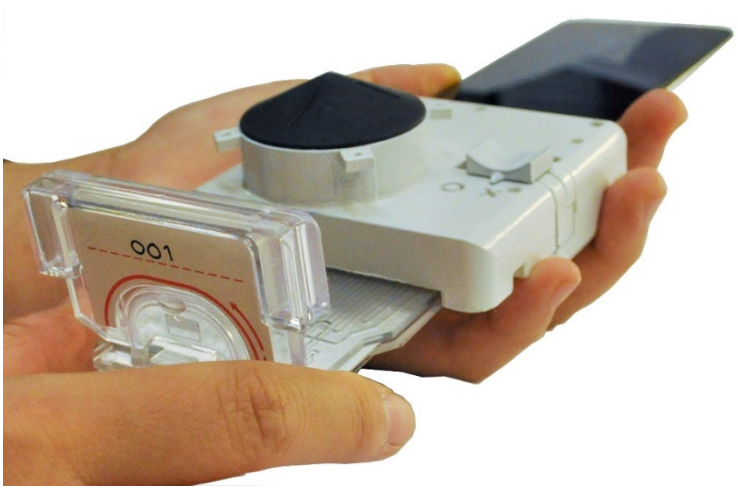


Figure 7. mChip Platform with Smart Phone

In conclusion, with respect to combined HIV/syphilis assays, given the challenges of diagnostic delivery in resource-limited settings, such tests are highly desirable as they will make implementation of both tests simpler, and hopefully, more cost-effective. But, not all of the available tests or those in the pipeline meet the desired criteria for such an assay. 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 do not achieve desired specifications. Cost is also a factor for some of the proposed assays. Therefore, continued optimization of an HIV/syphilis dual test in line with the existing target product profile (TPP) is still required.[5]

Chlamydia trachomatis, Neisseria gonorrhoeae

Both CT and NG are significant global health problems. The WHO estimates that in 2012 approximately 131 million and 78 million new cases of CT and NG, respectively, were diagnosed worldwide *(3).* Traditional CT and NG testing utilizes culture or serological techniques; however, nucleic acid

[5] The TPP for HIV/syphilis dual test is available on the website of the International Diagnostics Centre: http://www.idc-dx.org/resources/target-product-profile-combined-hivsyphilis-test.

amplification tests (NAATs) are considered the gold standard assays for detection of both CT and NG (as well as HPV), and a number of such assays are already available from Abbott Laboratories, BD Biosciences (BD), Hologic, Roche Diagnostics, and others. In numerous studies, the performance of the laboratory-based tests for both CT and NG has been shown to be good (*48*).

However, these platforms require significant infrastructure, including continuous power, clean running water and climate control. In order to reach patients in resource-limited settings, patient samples must be transported from urban, peri-urban and rural settings to the laboratory for processing. This is done using sample transport networks in-country. But, frequently, these services are not well developed, leading to long delays in returning sample results to patients and loss to follow-up. The conclusion is that what is needed in resource-limited settings is more sensitive, easier-to-use and cheaper tests for both CT and NG the results of which can be delivered in a single patient visit (*49,50*).

There are already antibody tests for CT available in lateral flow/RDT format, which are easy to use and relatively inexpensive. These include, but are not limited to: ACON Chlamydia (ACON Laboratories, USA), aQcare Chlamydia TRF kit (Medisensor, Republic of Korea), BioRapid Chlamydia Ag Test (Biokit S.A., Spain), Chlamydia Rapid Test SAS (Diagnostics for the Real World, UK), Clearview Chlamydia (Alere, Inc., USA), and Chlamydia test card (Ultimed Products, GmbH, Germany), HandiLab-C (HandiLab, USA), and QuickVue (Quidel, USA). In a systematic review of the listed assays , Kelly et al found that although CT antigen detection rapid POC tests exhibited high specificity across all specimen types (range 97% - 100%), pooled sensitivity was much lower, as shown in Table 5 below (*51*).

Pooled Performance of POC Antigen Detection Assays for CT			
Specimen type	Number of studies; N	Sensitivity (95% CI)	Specificity (95% CI)
Cervical swab	8; 4,588	53.1% (34.7 to 70.8)	98.9% (98.0 to 99.4)
Vaginal swab	10: 6,252	36.6% (22.9 to 52.9)	96.9% (94.0 to 98.4)
Male urine	5; 2,568	62.5% (43.2 to 78.5)	98.0% (95.1 to 99.0)

Table 5. Pooled Performance of POC Antigen Detection Assays for CT. Adapted from Kelly et al (*51*).

Of the tests included in these studies, the aCare Chlamydia TRF kit had the best performance, demonstrating overall sensitivity in cervical swabs of 93.8% (95% CI 88.6 to 97.0), and specificity of 96.8% (95% CI 94.8 to 98.1). Overall sensitivity and specificity in urine were 88.2% (95% CI 67.4 to 97.7) and 94.7% (95% CI 90.1 to 96.9), respectively (*52*).

With respect to NG, there are a limited number of immunoassays designed for use at POC available, four of which have been evaluated: the ACON Duo (*53*), NG ACON Plate (ACON Laboratories, USA) (*53*), BioStar Optical ImmunoAssay-Gonorrhea (BioStar, Inc., USA) (*54*), and the One Step Gonorrhea RapiCard InstaTest (Cortez Diagnostics, USA) (*55*). The performance results are summarized below:

Performance Evaluation of Four NG Assays					
Assay	**Specimen Type**	**Reference Test**	**Participants (N)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
ACON CT/NG Duo Test	Endocervical swab	COBAS AMPLICOR Analyzer CT/NG assay (Roche, USA)	491 sexually active females age 14-49, asymptomatic	12.5% (0 to 41.7)	99.8% (99.3 to 100)
ACON NG individual test	Endocervical swab	COBAS AMPLICOR Analyzer CT/NG assay (Roche, USA)	773 sexually active females age 14-49, asymptomatic	Not quantifiable (no true positives)	97.2% (96-98.5)
BioStar Optical ImmunoAssay	Urine	Aptima Combo 2 assay (Hologic, USA)	57 men, age 18+, attending sexual health clinic	100% (57 to 100)	98% 98 – 100)
BioStar Optical ImmunoAssay	Urine	Microscopy	33 men, age 18+, attending sexual health clinic	100% (51 to 100)	93% (78 to 98)
BioStar Optical ImmunoAssay	Urine	Culture	32 men, age 18+, attending sexual health clinic	100% (51 to 100)	93% (77 to 98)
One Step Gonorrhea RapiCard InstaTest	Women: endocervical swab Men: urethral swab	BD ProbeTec SDA Culture	138 overall (86 women, 52 men)	SDA: 33.3% (20.4 to 49.4) Culture: 32.4% (18.9–49.7)	SDA: 97.9% (91.9 to 99.5) Culture: 96% (89.8–98.5)

Table 6. Adapted from de Cortina SH et al (*48*) and Abbai et al (*55*).

As illustrated above, the BioStar assay was considerably more sensitive than the ACON or One Step Gonorrhea RapiCard InstaTest assays, but it should be noted that the sample size for the BioStar study is small. Similar to the CT studies discussed above, the ACON Duo and ACON NG tests had sensitivities <25% (*53*). While the One Step Gonorrhea RapiCard InstaTest had overall sensitivity of just over 30%, its sensitivity in women was considerably lower, at 7.1% versus SDA (*55*).

It has been concluded by many researchers that although current lateral flow/RDT diagnostic tests for CT and NG often have specificities >90%, sensitivities are often <50% or lower, and as such, they do not perform adequately to be used as screening tests; improved assays are required *(48,51,55,56,57)*. The need is particularly acute with respect to women, where the syndromic approach to managing STIs is inadequate *(58)*. In particular, NAAT-based platforms for use at or near POC are needed.

There are currently three NAAT-based platforms available for near-patient diagnosis of CT, NG, CT/NG combined, and TV (discussed more fully in the next section)– the GeneXpert® system from Cepheid, the the Truelab Real Time micro PCR system, and the STI Array/Vivalytic Analyzer (Randox Biosciences, UK)/Bosch Healthcare Solutions, Germany). Additional CT, NG and combination CT/NG tests are in the

pipeline. These are discussed below. (*See* **Annex D** for existing and pipeline tests for CT, NG and CT/NG for use at or near the point of care, including those for TV and HPV.)

GeneXpert® System (*Cepheid*)

The Cepheid® GeneXpert® System (pictured below) is a fully-automated and integrated system for PCR-based NAAT, currently has 21 FDA-cleared and 27 CE-IVD marked assays, including a CT assay (Xpert® CT) a combined CT and NG assay for simultaneous detection (Xpert® CT/NG), an HPV assay (Xpert® HPV), and a test for TV (Xpert® TV), which was also FDA cleared for symptomatic and asymptomatic men in the US.

Figure 8. GeneXpert® Platform (left) and Cartridge (right)

The Xpert® CT/NG assay, performed on the GeneXpert® system is a qualitative *in vitro* real-time PCR test for automated detection and differentiation of genomic DNA from CT and/or NG. It is CE-IVD marked and FDA cleared. The assay may be used on the following specimens from both asymptomatic and symptomatic patients: female and male urine, endocervical swab, and patient-collected vaginal swab (collected in a clinical setting). The test process is straightforward, with total hands-on time estimated to be less than one minute. The operator (i) obtains either urine or swab samples, which are previously collected and stored in the Cepheid Transport Reagent; (ii) transfers the sample to the Xpert® cartridge; and (iii) inserts the cartridge into the Xpert® system and starts the assay. Time to result is approximately 90 minutes. The performance of the Xpert® CT/NG assay has been evaluated and found to be very good relative to established laboratory based assays (*59,60.61*).

The Xpert® TV assay was CE-IVD marked in September 2014 and FDA cleared for female specimens in 2015 and for male urine specimens in 2016. It is the first molecular TV test that is cleared to detect TV from both male and female specimens. The time to result of the test is approximately 1 hour. A quick laboratory assessment report on the performance of the TV assay is available *(62)*.

The Xpert® HPV assay was CE-IVD marked as of early 2014. Xpert® HPV is a <60 minute test for cervical cancer-related human papillomaviruses. It is a multiplexed test that targets the E6 and E7 oncogenes of

14 high risk HPV types and independently calls out genotype 16- the most common type associated with invasive cervical cancer worldwide (*63*); a combined call out for genotypes 18 and 45 -types also closely associated with invasive cervical cancer (*64*); and 11 other high risk genotypes detected in combined channels. Xpert® HPV uses samples from endocervical cells collected in Preservcyt® Solution (Hologic Corp.) with either a broom-like device or an endocervical brush/spatula combination. The clinical performance of the Xpert® HPV assay was assessed in screening (*65,66*) and colposcopy referral populations (*67,68*) and was found to be highly concordant with other clinically validated laboratory-based assays. In a more recent study, Xpert® HPV was evaluated according to the 2009 International standards set by Meijer and was shown to have acceptable inter- and intra- laboratory reproducibility for women aged 30 or above and has since been added to the list of tests validated for primary cervical cancer screening (*69*). With the flexibility of the GeneXpert System, Xpert® HPV offers rapid and accurate results within the laboratory as well as at POX, thereby increasing accessibility and screening uptake while offering a faster definitive result for risk-based triage of women based on high-risk HPV status. Early detection of HPV would allow for better disease management, thereby reducing unnecessary treatment costs and the overall burden on healthcare. More recently, Xpert® HPV was validated with self- collected vaginal samples against clinician- collected cervical samples and performance was found to be comparable (*70*). The use of the assay at point-of-care was also assessed which proves to be a suitable test and treat solution for developing countries in absence of specialist infrastructure.

All of Cepheid's sexually-transmitted infection tests benefit from a unique Sample Adequacy Control (SAC). Each self-contained cartridge includes a SAC, which detects the presence of a single copy human gene and monitors whether the sample contains human DNA for enhanced results integrity (*71*).

The GeneXpert® System integrates and automates sample preparation, amplification, and detection in a single-use, self-contained cartridge, pictured above right. Most liquids and dry reagents along with enzymes are prefilled so that pre-analytical steps are minimized, greatly reducing opportunities for sample mix-ups and operational errors. GeneXpert® cartridges can handle a variety of sample volumes (micro- to milliliter volume range) within macro fluidic chambers and then concentrate the target material down to microfluidic volumes, which can increase the sensitivity of the assays, if needed.

Further, the GeneXpert® System is modular. Individual modules contain solid state circuitry that controls temperature, pressure, rotation of the valve that moves the liquid between reservoirs in the cartridge, and the detection software. These individual modules are packaged in cabinets that can hold up to 1, 2, 4, 16, 48, or 80 modules. The latter two systems (Infinity-48 and Infinity-80) are fully automated, walk-away robotic systems, developed for high-throughput laboratory applications. Additionally, the modules can be removed and replaced individually so that the entire system is not incapacitated if one module fails.

The GeneXpert® System is sufficiently simple that training can usually be completed within half a day or less. Further, although the system was designed to use AC power, its low wattage requirements allow it to be powered by a 12VDC/120VAC voltage converter in mobile laboratories. It has also been installed in remote clinic sites powered by solar panels. The GeneXpert® software comes pre-installed on a desktop

or laptop computer and results can be displayed for each module in real time or uploaded via an Internet connection to a central database or institutional LMIS system. Cepheid C360 allows for systems monitoring to observe instrument performance data, and disease surveillance to aggregate and monitor disease state testing data. Countries in sub-Saharan Africa, EU and North America are currently scaling up their GeneXpert program with Cepheid C360.

The price of the Xpert STI assays for resource-limited settings are: $16.20 per test for Xpert® CT/NG, $16.70 for Xpert® HPV, and $19.00 for Xpert® TV. Cepheid's high burden developing countries (HBDC) terms apply. The price of the GeneXpert® 4-module systems under the same HBDC program is $17,000.

Arguably, the GeneXpert® platform is best used at district hospitals and above in the tiered laboratory system in-country. It is not as well suited to use at health centers and below for reasons including cost, the need for stable electricity and remote calibration requirements. However, the GeneXpert® has been used quite successfully at remote Aboriginal healthcare facilities in Australia and many other decentralized settings (*72,73,74*).

Truelab™ Real Time micro PCR System (*Molbio Diagnostics Pvt. Ltd.*)

Molbio Diagnostics has developed a comprehensive, rapid, near-patient RT PCR platform, called the Truelab™ Real Time micro PCR System. The system is portable and includes all instrumentation, reagents and essential accessories that are required for the operator to conduct a real time, quantitative PCR assay, from sample preparation through to final result reporting, all within one hour. A Truelab™ micro PCR printer is also available. The system works on ready-to-use Truenat™ disease-specific assays that are stable at room temperature. Assays for MTB, HBV, dengue fever, Chikungunya, H1N1, salmonella, malaria (both *P. falciparum and P. vivax*), CT, NG, TV and HPV (16/18/31/45) are currently available, and assays for HCV and HIV viral load, among others, are in development.

The testing process begins with sample collection (blood, serum or plasma) followed by extraction, which uses the Trueprep™ Auto Sample Prep Device and Trueprep™ Auto sample prep kits. The completely automated extraction process takes about 20 minutes per sample. From there, 6 µL of the extracted nucleic acid is dispensed into the reaction well of the disease-specific Truenat™ micro PCR chip. The chip, which contains all of the chemistry required to complete an assay, is then inserted into the Truelab™ Uno Dx Real Time micro PCR Analyser, pictured below. Thermal cycling takes place automatically within the analyser. The Truelab™ Duo and Truelab™ Quattro Realtime microPCR analyser systems that allow a higher throughput and random access are also commercially available.

During amplification, the Truenat™ micro PCR chip exponentially releases flurophores. These signals are captured by sensors and are displayed as an amplification curve on the Truelab™ screen. Test results are compared to lot-specific standard values preset into the Truena™t chip, which enables quantitative estimation of the test analyte and display as RT PCR results in approximately 30 minutes. An internal control is provided from the extraction stage for a complete validation of the test results.

Figure 9. Truelab™ Real Time micro PCR System

Test results are automatically stored in the analyser memory (up to 20,000 results), can be printed, and transported wirelessly to any server/compatible device by Wi-Fi, GPRS, Bluetooth or even SMS.

Peer-reviewed, published evaluations of the CT, NG, TV and HPV assays were not found in a literature search.

Platforms/Assays in the Pipeline

STI Multiplex Array/Vivalytic Analyzer (Randox Laboratories, UK/Bosch Healthcare Solutions, Germany)

Bosch Healthcare Solutions has developed the Vivalytic Analyzer, a universal, cartridge-based platform for sample to answer molecular diagnostics (pictured below), with the first tests available being the Randox STI Multiples Array and the Respiratory Multiplex Assay.

Figure 10. Bosch Vivalytic Analyzer.

The Vivalytic platform can accommodate a wide variety of samples and allows for different methods of analysis to run in a fully-automated way in a short timeframe, with results from 30 minutes. Single or multiple pathogens can be detected simultaneously in the patient sample. In addition, the Vivalytic platform is an open system that can process molecular diagnostic tests from various assay manufacturers.

The Vivalytic Analyzer is a small footprint, fully automated device with no peripherals, capable of quantitative and qualitative PCR procedures with three stable isothermal zones, where rapid microfluidic transfer between these zones achieves fast heating and cooling cycles. This ensures high test quality and reproducibility. The analyzer has a universal optical evaluation unit, which enables microarrays, qualitative or quantitative PCR, as well as melting curve analyses to be read out in one system. Four standard color channels can be evaluated per PCR strand. This corresponds to a degree of multiplexing of up to eight for qualitative or quantitative PCR, or up to sixteen in multi-channel melting curve analysis. Via geometrical multiplexing with the help of microarrays, a much higher number can be achieved. Up to 100 properties can be examined here.

The Vivalytic system has built-in connectivity and can be easily integrated with popular standard IT systems. Further, an analyzer device can be networked and combined with many other devices, so that several series of tests can be carried out at the same time.

Randox Laboratories Ltd (UK) has developed a number of CE-IVD marked infection arrays that have been adapted for use with the Vivalytic Analyzer. The first two of these, a respiratory tract infection array and an STI Multiplex Array (pictured below) will be available on the release of the Vivalytic Analyzer in Q3 2018.

Figure 11 Randox Respiratory Tract Infection and STI Panels

The STI array detects 10 of the most important bacterial, viral and protozoan sexually transmitted infections, providing a comprehensive infection profile from a single swab sample. The test panel includes: CT, NG, and TV, as well as Mycoplasma genitalium (MG), UU, Haemophilus ducreyi (HD), Mycoplasma hominis (MH), TP, and HSV-1 & HSV 2.

All reagents required for a test are stored on the STI array cartridge and all are stable at room temperature; no cold storage or special shipping conditions are required. The cartridge also employs the Randox Biochip Array, a 9x9mm solid state unit that facilitates multiple target testing from a single patient sample. Each STI Biochip has 25 discrete test regions (DTRs), and each DTR holds an individual test. A single sample is added to one cartridge, which then provid multiple test results.

The cartridge contains internal controls that indicate successful extraction, amplification, hybridization and detection; all of these must pass acceptance criteria in order for the Vivalytic Analyzer to return patient results. Further, test results do not require interpretation; positive or negative results are indicated for each target without ambiguity.

There is currently no peer reviewed, published performance data on the STI Multiplex Array, although data is expected to be published later in 2018.

Atlas Genetics io® Platform (*Atlas Genetics*)

Atlas Genetics has developed a multi-assay instrument and disposable test-specific cartridge, the Atlas Genetics **io®** (pictured below), which is designed to bring the sensitivity and accuracy of laboratory-based platforms to the point of patient care. The **io®** platform uses an electrochemical DNA detection technology based on differential pulse voltammetry. Bacteria or viruses from a clinical specimen are lysed; nucleic acids are extracted and purified in preparation for amplification. PCR amplification of a specific target sequence produces a DNA amplicon to which a target-specific electrochemically labeled probe is hybridized. A double-strand specific exonuclease recognizes and digests the complex releasing a free electrochemical label that is detected using a carbon electrode. The system is fully automated and capable of providing nucleic acid testing in under 30 minutes. All steps, including sample processing, occur on the cartridge.

Figure 12. Atlas Genetics io® Platform and Cartridge

The company indicates that the **io®** instrument, which has a small footprint, is very robust, easy-to-use and maintain because it contains no fragile optical sensors or reagents. Further, after addition of a raw sample to the disposable **io®** Cartridge and loading the Cartridge onto the instrument, no further operator intervention is required. The sample is processed using pneumatically-controlled fluidic movement and all reagents required to perform the test are located on the Cartridge in an ambient-stable format. A single Cartridge can detect up to 24 genetic targets in a single patient sample.

Atlas Genetics considers tests for STIs to be one of its core focus areas. In addition to a CE-marked test for CT, the company has several other STI assays in development, including combination CT/NG, combination CT/NG/TV, and *Mycoplasma genitalium* (MG). Each of the assays has undergone, or is undergoing, preliminary clinical evaluations (primarily in the United States through the Johns Hopkins STD POC Centre). The company is also developing a Cipro-sensitive NG resistance test in collaboration with St. George's Hospital London. Currently, a research stage clinical study on the assay is ongoing, and dependent on results, Atlas expects to move this test into its development pipeline. The company indicates that early data are encouraging. Atlas envisages that this 30-minute NG resistance assay will be used in countries where Cipro sensitivity is still at a significant level; the test can be used to help a clinician decide if this alternative to ceftriaxone can be used safely.

Atlas expects to launch a combined CT/NG assay and the **io®** system, both of which will be CE-IVD marked, in Europe in 2018. A clinical trial for the CT/NG assay is expected to be conducted in the United States in 2018, with FDA approval and product launch to follow. Evaluations of the CT assay have demonstrated clinical sensitivity of 94.4% to 96.1% and specificity of 97.7% to 98.2% (*75,76*).

Atlas Genetics has developed manufacturing facilities that will enable the company to produce the **io®** Reader and Cartridge at commercial scale.

No price information is available on either the instrument or the individual assays.

GeneXpert® Omni (*Cepheid*)

In July 2015, Cepheid announced the development of the GeneXpert® Omni system (pictured below). The system leverages existing Xpert® cartridge technology (described earlier in this report). However, the GeneXpert® Omni is highly portable, measuring just 9 inches tall (about 23 cm) and weighing 2.2 pounds (about 1 kg). The system is battery-operated (with up to 4 hours of operation and a supplemental rechargeable battery with an additional 12 hours of battery life), and is wireless and connectivity-enabled. Advanced microfluidics regulate all aspects of the testing process within the test cartridge – from sample preparation and nucleic acid extraction to amplification and detection. Additionally, the platform has solid state digital electronic architecture, which means it is durable.

Figure 13. GeneXpert® Omni Platform

The GeneXpert Omni® platform will use a dedicated mobile device to control each test module. The platform will also use a secure, hosted platform that collects and aggregates real-time information. A single system can store more than 20,000 test results.

The initial assays planned for availability on the system will be the Xpert® MTB/RIF, Xpert® MTB/RIF Ultra, Xpert® HIV-1 Qual, Xpert® HIV-1 Viral Load, and Xpert® HCV Viral Load. Xpert® CT/NG and Xpert® HPV. Over time, it is Cepheid's intent to have the majority of the Xpert menu available on the GeneXpert® Omni.

RT CPA CT Test (*Ustar Biotechnologies*)

Ustar Biotechnologies has developed Cross Priming Amplification (CPA), a novel isothermal NAAT with multiple iterative designs that can address a wide variety of key obstacles to traditional amplification technologies such as PCR. By using multiple crossing primers and probes, target DNA sequences can be

rapidly and precisely amplified at a uniform temperature (typically 63°C) in an easy-to-use protocol with high sensitivity and specificity. By utilizing its CPA technology on its dedicated platform (pictured below), Ustar is now developing assays for *Mycoplasma pneumoniae* (MP), *Chlamydia pneumoniae* (CP), CT[6], NG, *Ureaplasma urealyticum* (UU) and Herpes Simplex Virus.

Figure 14. RT CPA-CT Platform (left) and Cartridge (right)

Ustar's goal is to develop a qualitative RT CPA CT cartridge to be used in conjunction with a robust and user-friendly portable instrument. For this purpose, Ustar has developed a high quality, fully integrated and automated (sample-in, answer-out) molecular diagnostic system (qualitative or quantitative).

The final Ustar diagnostic test kit is comprised of a reagent-containing cartridge and a portable device for sample preparation, amplification and detection. Reagents will consist of glassified enzymes for ambient temperature transport and storage, a reconstitution buffer, and sample preparation buffers, all of which are pre-loaded and housed in the cartridge.

The cost of the Ustar platform, which is being designed for health center laboratory facilities, is expected to be less than $5,000. The cost per test is not yet determined. Ustar is currently focused on CE authentication of its TB assay, which is expected to be launched by Q3 of 2018. The company expects to turn to its CT assay subsequent to that. As a result, the CT assay will not be available until after 2018.

Alere™-i Platform *(Alere, Inc.)*

The Alere™-i platform (pictured below) from Alere is an instrument-based, molecular diagnostic test utilizing isothermal nucleic acid amplification technology (iNAAT) for the qualitative detection of

[6] Note that Ustar currently manufactures a CT Isothermal Amplification Diagnostic Kit that can be used with separate equipment: Micropipette and disposable tips, heating block, water bath or any isothermal devices; centrifuge; vortex, timer; 1.5 mL centrifuge tubes, with safe-lock feature; and normal saline. It is a highly manual process that is not designed for use at the point of care. The narrative above describes a test system optimized for use on portable instrument, which is being designed for use at or near the point of patient care.

infectious disease targets. Molecular testing involves the extraction and analysis of DNA or RNA strands to detect sequences associated with viral and bacterial causes of infections. The proprietary technology embodied in the Alere™-i platform utilizes iNAAT, which, unlike polymerase chain reaction (PCR) testing, does not require temperature cycling and can therefore deliver results more quickly and to a broader range of settings. Alere has acquired several companies with iNAAT technologies, including RPA, a nucleic amplification system that uses prokaryotic enzymes (recombinases) to guide synthetic oligonucleotide primers to target sites in sample nucleic acids, and NEAR, which uses DNA polymerase and a nicking endonuclease. Assays developed for the Alere™-i platform may use various iNAAT technologies.

Figure 15. Alere™-i Platform

Alere™-i Influenza A & B was the first molecular test to be granted CLIA waiver in the U.S and delivers actionable, lab-accurate results in less than 15 minutes on a user-friendly platform. Alere™-i Strep A was launched in 2015, and with test results in 8 minutes or less, is the fastest CLIA-waived molecular Strep A test. Alere™-i RSV (respiratory syncytial virus) was launched in October 2016, and is the first molecular test that can be used at the point-of-care to detect RSV in 13 minutes or less. Alere has received 510k clearance and CLIA waiver of Alere™ i Strep A 2 and Alere™ i Influenza A & B 2 with planned launches in Q3 2018. Alere™ i Influenza A & B 2 provides a result in 13 minutes or less with the added benefit of returning a positive result in as little as 5 minutes with the early call out feature. A test for CT/NG is currently in the pipeline.

Microwave-Accelerated Metal-Enhanced Fluorescence Test (MAMEF)

Scientists at the University of Maryland Baltimore County and Johns Hopkins, led by Chris D. Geddes, have developed a MAMEF test for CT and NG. The test combines lower-power microwave acceleration

to hasten biological reactions, reducing assay run times over 1000-fold, with metal-enhanced fluorescence, whereby the close proximity of silver nanoparticles amplifies the fluorescence of labels in the near field (*77*). Per the developers, it is this combination of enhanced fluorescence emission coupled with a significantly reduced assay turnaround time that makes MAMEF a powerful technology for POC testing.

CT MAMEF-based detection

Two MAMEF assays have been evaluated for the detection of CT DNA from vaginal swabs compared to those of NAATs. The overall rates of agreement with NAAT results for the assays were 89.3% (16S rRNA assay) and 91.0% (cryptic plasmid assay), and the authors of the study concluded that the "sensitivity, specificity, and rapid detection of the plasmid-based MAMEF assay appear to be suited for clinical POC testing" (*78*).

The image below shows a flow chart of the current sequence of steps and timeline for a MAMEF-based CT test:

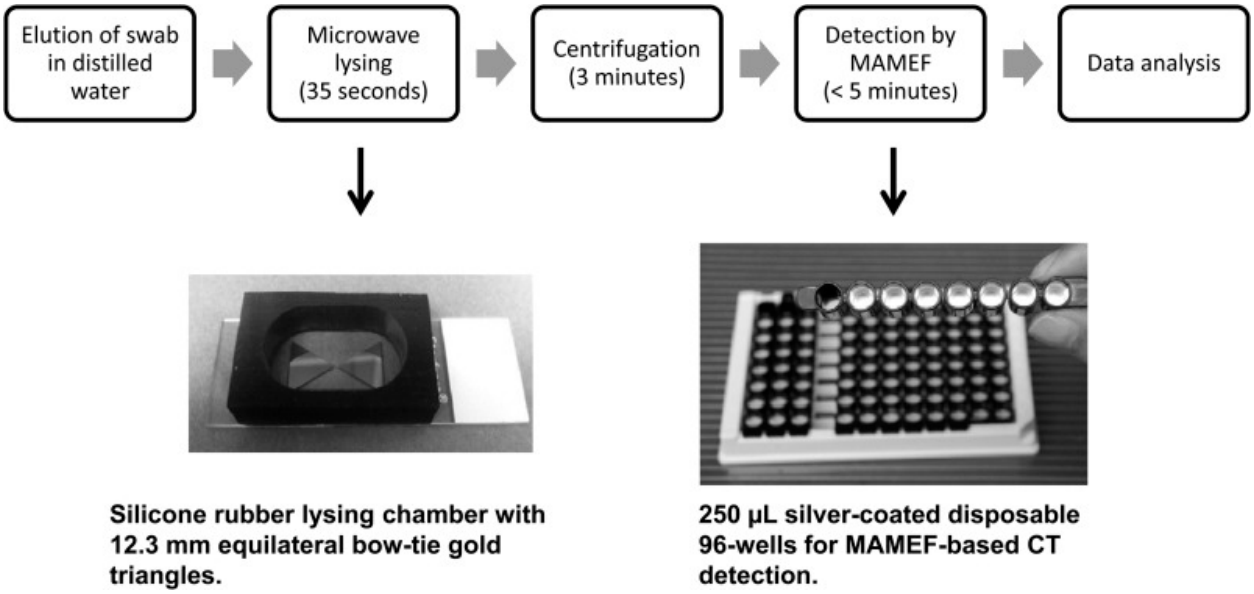


Figure 16. Flow Chart of Sequence of Steps and Timeline for a MAMEF-based CT Test

The process is quite fast; it involves four steps: (i) elution of the sample from the swab; (ii) microwave-based cell lysis and DNA fragmentation; (iii) separation of DNA and cellular debris by centrifugation; and (iv) MAMEF-based DNA detection.[7] The cost is quite low, with the developers estimating that each assay would cost about $1.00 plus an additional $1.00 per lysing procedure.

While the MAMEF-based test is clearly fast and inexpensive, in its present configuration, it would likely not be appropriate for use at POC in resource-limited settings. In general, one of the advantages of NAAT-based approaches in those settings is that many such assays have been evaluated and are well-

[7] Images and description from Melendez et al, pp 2914-15. (*78*)

validated; the assays are available in quality-assured kits; and clinicians are comfortable interpreting the results. In order for the MAMEF-based assays to be suitable for use in resource-limited settings, they would likely need to be further optimized, simplified and standardized for use in laboratories that are very basic. These tasks are currently being undertaken by Professor Geddes' laboratory; commercial launch of the STI assays is expected within 12 – 24 months.

One technology that can significantly aid STI identification has recently been commercialized by Professor Geddes and his team, namely Lyse-It®. Lyse-It®'s commercialization has enabled the lysing component of the technology to be significantly simplified and readily available as a tool for researchers and clinicians alike. Lyse-it® starter lysing kits, including the microwave hardware, cost about US$700.[8] Disposable lysing slides are subsequently available to lyse samples, as a consumable when the hardware is in place. Per the company, this enables the rapid and efficient lysing of samples, at significantly lower cost, more easily and more quickly than any other lysing technology available today, and the technology could also be used with other PCR tests for STI detection, significantly simplifying sample preparation and potentially lowering the cost of healthcare.

Gonorrhea MAMEF-based detection

A MAMEF-based assay, similar to the CT assay, has now been developed for detection of NG. The assay targets the *PorA* gene of *N. gonorrhoeae* and has shown excellent specificity against pathogens commonly found in genital specimens. Preliminary studies with dry vaginal swabs suggest that the sensitivity of the assay is approximately 83% and specificity is 93% (*79*). A blinded comparison evaluation of the NG MAMEF-based assay with vaginal and urethral swabs is currently underway.

In addition to testing for STIs, Dr. Geddes and his team have also developed MAMEF DNA-based assays for Bacillus anthracis, various *Salmonella* bacteria, C. *difficile*, Listeria monocytogenes as well as for a variety of antibody-based assays. The assay component of the MAMEF technology is expected to be commercialized as a multi-use platform technology in 2018.

MobiNAAT (*Johns Hopkins University BioMEMS lab*)

Researchers at Johns Hopkins University BioMEMS laboratory have created a new smartphone DNA test capable of diagnosing CT. Early results in a small study on the platform in the U.S., in which the assay correctly identified two positives among 30 patients, are very promising (*80*). Additional validation testing at Johns Hopkins is the next step for this device followed by field testing.

The device, called MobiNAAT, is a low-cost NAAT platform that integrates sample preparation, DNA amplification and data processing into one instrument the size of a coffee mug (pictured below). The device is battery-powered and uses a microfluidics cartridge to identify CT DNA in genital swab samples. Results are analyzed via a smartphone that allows the user to control the testing and process data with an app. Because the smartphone diagnostic is automated, it does not require a lab technician to process results.

[8] For additional information, see www.lyse-it.com.

Figure 17. MobiNAAT Platform

In its current form, the NAAT uses touchscreen user interface and Bluetooth communication along with a phone camera for data collection, and was designed with an iPhone/Android in mind. Modifications would be required to generalize the platform for any other mobile devices.

The cost of each microfluidics cartridge is less than $2.00.

There is currently no expected launch date for the CT assay on the MobiNAAT platform, which is at the prototype stage of development.

Trichomonas Vaginalis

Like CT, NG and syphilis, TV is a significant health problem globally. The WHO estimates that there were about 143 million new cases of TV worldwide in 2012 *(3)*. Diagnosis of TV infection has traditionally been performed by microscopy of vaginal secretions, but this technique requires immediate evaluation of a wet preparation and is only about 50% sensitive when compared with culture or NAAT *(81,82)*. Diagnosis of TV in men is typically from wet mount with microscopic visualization of the parasites on slide preparations from urethral secretions *(83)*. Modern nucleic acid-based testing for TV is convenient, accurate, and more sensitive than traditional methods. Like testing for CT and NG, there are a number of reliable laboratory-based molecular systems for TV testing. These include the APTIMA *Trichomonas vaginalis* Assay (Hologic), the BD ProbeTec *Trichomonas Vaginalis* Qx Amplified DNA Assay (BD), and the Affirm VPIII Microbial Identification Test (BD), which also detects *Gardnerella vaginalis* and *Candida albicans*.

In addition to the above molecular tests, there is at least one rapid diagnostic test for detection of TV: the OSOM® Trichomonas Test (Sekisui Diagnostics), which studies have shown performed reasonably well when compared to wet mount and culture *(84,85)*.

Of the companies developing assays for molecular platforms discussed earlier in this report in connection with tests for CT and NG, GeneXpert® (Cepheid), TrueLab™ (Molbio) and STI Array

(Randox/Bosch) have commercialized assays for TV (discussed earlier in this report). In addition, Atlas io™ has a combined CT/NG/TV assay in its development pipeline. Finally, Quidel Corporation (Quidel) has launched a near-POC TV assay for its Solana® platform, which is described below.

Solana® (*Quidel Corporation*)

Figure 18. Solana® Platform from Quidel

Quidel is developing assays based on the company's isothermal molecular diagnostic platform (Solana®); one of these is a qualitative assay for TV. Solana® features Quidel's proprietary helicase-dependent amplification (HDA) technology which uses a helicase enzyme to unwind double-stranded DNA into single strands, eliminating the need for a thermocycler. The company emphasizes that unlike other isothermal amplification methods, HDA uses a probe-based detection method, thereby resulting in greater specificity. In addition, because HDA only detects amplicons, rather than turbidity caused by amplification as with Loop Mediated Amplification (LAMP), it provides assurance that amplification of only the intended target will be identified as positive. HDA can also multiplex in a single tube.

Solana® is a compact benchtop instrument measuring 9.4" x 9.4"x 5.9" that allows for rapid detection of TV from vaginal swabs and female urine specimens obtained from symptomatic and asymptomatic females. Turnaround time for the test is 35 minutes. In addition, the platform permits operators to batch up to 12 samples in a single run, allowing for testing scale-up as needed.

The workflow for the Solana® is detailed below:

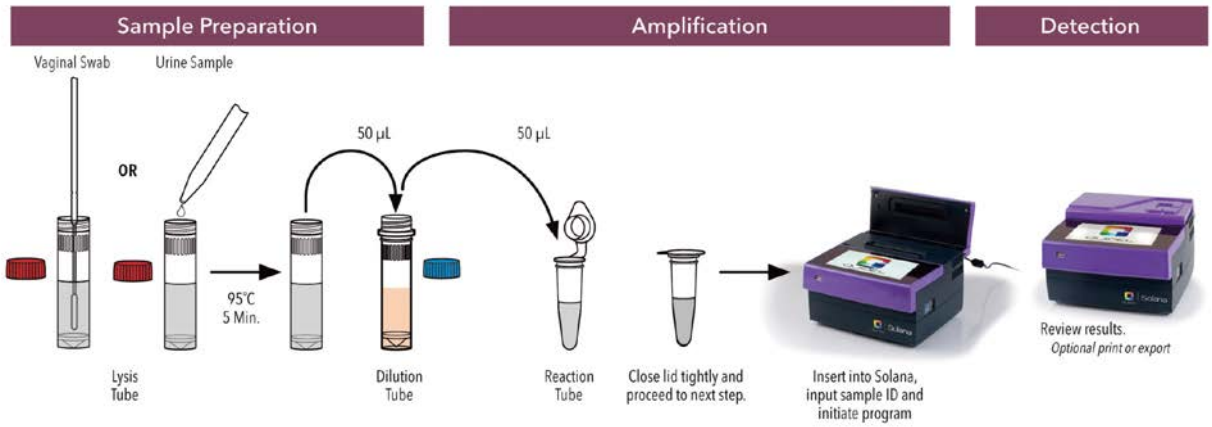


Figure 19. Typical Workflow on Solana® Platform

The Solana® platform is designated as moderately complex by the US FDA. The company believes the platform is ideal for small to medium-sized microbiology labs where the low total cost of the instrument and disposables enable molecular testing at the volumes seen in these settings. In resource-limited settings, this would likely translate into use at district hospitals and above.

Additional assays that can be run on the Solana® platform include Influenza A+B, Group A Strep, Group B Strep, and HSV 1+2/VZV, all of which are FDA approved. Quidel is also developing additional assays for the Solana® platform.

The Solana® TV assay has been independently evaluated and performed well compared to the reference assay (*79*). Gaydos et al collected vaginal swabs and urine specimens from 501 asymptomatic and 543 symptomatic women (*79*). Prevalence of TV was 11.5%. For swabs, Solana® demonstrated high sensitivity and specificity from asymptomatic and symptomatic women, 100%/98.9% and 98.6%/98.5%, respectively, compared to the reference method. For urine specimens, results were also good, with sensitivity and specificity from asymptomatic women of 98.0% and 98.4%, respectively, and from symptomatic women of 92.9% and 97.9%, respectively (*86*).

Human Papilloma Virus

In the United States, the Papanicolaou (Pap) test has been the gold standard for detecting cervical cancer in women over 30 years of age, most of which is caused by HPV. However, the U.S. Food and Drug Administration (FDA) recently recommended that the cobas® HPV Test (Roche Diagnostics) should be the first line of screening in the United States.[9] However, HPV screening using either of these methods or using visual inspection with acetic acid (VIA), is difficult in resource-limited settings. For example, the cobas® HPV Test must be done in centralized laboratory facilities using sophisticated instrumentation. The same is true for other HPV screening using molecular-based testing – e.g., the

[9] Currently in the U.S., use of the Pap test and HPV test in tandem (i.e., co-testing) is the preferred method of screening in women over 30.

RealTi*m*e High Risk HPV test from Abbott, the Qiagen *digene* HC2 HPV Test or the Onclarity™ HPV assay on the BD Viper™ LT System.

However, there are several POC or near-POC platform/assay options currently available for use in resource-limited settings. Each of GeneXpert® (Cepheid) and the Truelab Real Time micro PCR system (Molbio) has a commercially-available HPV assay; these are discussed earlier in this report. In addition, there is the Onco***E6***™ assay from Arbor Vita Corporation. Qiagen has also introduced an HPV assay, the *care*HPV™ Test, a molecular diagnostic test for HPV that is designed for use in low resource settings. Each of these platforms is described below.

Onco*E6*™ Assay (Arbor Vita Corporation, USA)

The Onco***E6™*** test from Arbor Vita Corporation, pictured below, is a lateral flow immunoassay that detects the E6 oncoprotein from two high risk (HR) HPV types (HPV16, HPV18), which cause approximately 75% of invasive cervical cancer (ICC). The Onco***E6***™ test is in a dipstick-like format and is simple, quick, non-invasive, and requires no refrigeration. The test is compatible with specimens collected for either a regular Pap smear or liquid Thinprep© from Arbor Vita.

The Onco***E6***™ does not require complex equipment for processing. The equipment costs around US$2,000 and can process 45 specimens per operator per day, a volume that can be processed in a clinic within 2-2.5 hours.

Figure 20. Onco*E6*™ Assay

The performance of the Onco***E6***™ assay has been evaluated in at least two studies. In a study of the performance of the assay for the detection of CIN2+ among 7,621 women and CIN3 among 7,421 women in China, Zhao et al found sensitivity and specificity of 42.4% and 99.1%, respectively, for the detection of CIN2+, and 53.5% and 98.9%, respectively, for the detection of CIN3+ in HIV-negative women (*87*). In another study, Chibwesha et al evaluated the performance of the assay in 200 women living with HIV in Zambia. The reported sensitivity and specificity of the Onco***E6***™ assay were 31.3%

(95%CI from 16 to 50) and 99.4% (95%CI from 97 to 100), respectively, for CIN2+ detection (*88*). Kelly et al concluded that the low sensitivity, but higher specificity, of the Onco***E6***™ assay for CIN2+ detection suggests that it might be "useful as a 'screen-and-treat' or triage test," but further studies are needed (*89*).

_care_HPV™ System (Qiagen, Germany)

The *care*HPV™ Test (Qiagen, Germany) provides primary, stand-alone screening for high-risk HPV in women 30 years and older. The test, which is a nucleic acid hybridization assay with signal amplification using microplate chemiluminescent, detects 14 HR HPV types in about 2.5 hours, which permits same-day follow-up. The company reports clinical sensitivity of 88% and clinical specificity of 85% for CIN2+, although researchers have noted that sensitivity and specificity may be lower in women living with HIV/AIDS (*89*).

The *care*HPV System includes the *care*HPV Test Controller, *care*HPV Test Shaker, *care*HPV Test Luminometer, and the *care*HPV Test Magnetic Plate Holder, pictured below. The automated components are designed for a universal power supply and operate on mains electricity or a lead acid battery.

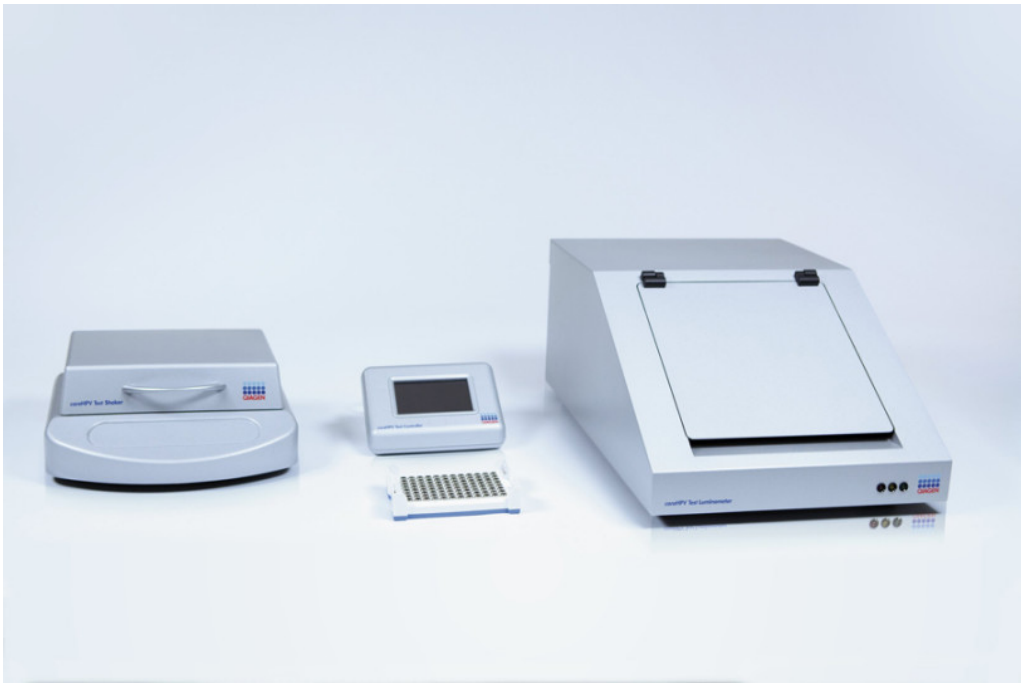


Figure 21. _care_HPV Test System Components

Qiagen also provides simple sample collection materials with multiple day stability in the form of the *care*HPV Sample Collection Device, which consists of the *care*Brush and *care*HPV Collection Medium. These permit both healthcare-provider sampling and self-sampling, which has the possibility to increase uptake and screening.

A recent meta-analysis and systematic review found that, using clinician-collected swabs, the *care*HPV assay performed well, with sensitivity and specificity for the detection of CIN2+ of 88.1% and 83.7%, respectively, and with sensitivity and specificity of 90.3% and 85.3%, respectively for detection of CIN3+ (*89*). The sensitivity of the test was slightly lower when using self-collected vaginal swabs, with sensitivity of 73.6% for detection of CIN2+ and 75.2% for detection of CIN3+; specificity remained high (89).

Performance of the *care*HPV assay has been good relative to testing using either the Pap test or VIA *(89,90,91,92)*. The assay has also performed better than cervical ctytology (*91*). Kelly et al conclude that the *care*HPV test performs as well as visual inspection and cervical cytology (*92,93*), and has the advantage of allowing for self-sampling (*89*).

Additional Technologies in the Pipeline for STIs

NEDxA Platform (GENOMICA S.A.U., Spain)

GENOMICA, which has a diagnostics product line for the detection and genotyping of different types of HPV by means of multiplex PCR followed by visualization in low-density arrays based on CLART® Technology, has developed an HPV genotyping assay for its NEDxA platform. The system detects and identifies 14 HR HPV genotypes. The NEDxA platform, pictured below, is a lightweight, compact, desktop device with a folding screen.

Figure 22. NEDxA Platform

The NEDxA platform accepts dry endocervical swabs from females; no DNA extraction is required. Commending in June, the system will also accept ThinPrep and SurePath™ liquid samples. The NEDxA cartridge, pictured below, is based on microfluidic technology.

Figure 23. NEDxA Cartridge

NEDxA is a closed system; all reagents are included in the cartridge, which can be shipped and stored at room temperature (from 4°C to 25°C). The sample is inserted directly into the cassette; no pipetting is required. The sample is then moved to the PCR chamber where the DNA amplification takes place. Following the PCR process, the amplified product moves to the detection chamber for hybridization of targets with specific probes. Reagents are then pumped into the detection chamber for incubation of the solution. Finally, an electric potential is applied through 64 Au electrodes to obtain an instant electrochemical detection. Turnaround time for the HPV test is approximately 75 minutes.

The NEDxA system is easy to use. The operator selects the analysis to be done, places the cartridge on a tray, and starts the test run. The running test screen displays the test status and test time remaining. Results are reported automatically. The NEDxA platform provides LAN connection and a USB port; the system is compatible with LIMS systems.

The company indicates that its aim is to provide a complete CE-marked IVD solution to address women's health issues, focusing on clinical sensitivity to improve patient management. The NEDxA system is expected to be launched in 2019.

GENOMICA reports >95% sensitivity and 100% specificity for the HPV assay. Currently, no peer reviewed, published evaluations are available.

cobas® Liat® System (Roche Molecular Diagnostics)

The **cobas®** Liat® System, pictured below, is a compact, real-time PCR platform designed for on-demand STAT testing at the point of care or in the laboratory to support time-sensitive diagnoses and treatment decisions. All nucleic acid testing processes are fully automated, including sample preparation, amplification and real-time detection for qualitative and quantitative results. Each **cobas®** Liat® assay tube contains all assay reagents for a single test.

The System currently has assays clinically validated, CE-IVD marked and FDA cleared for the detection of Influenza A/B, Strep A, & Influenza A/B and RSV. All three assays have received CLIA Waiver from the

FDA. A CLIA Waiver determines that there is little risk of error due to the simple use of the test, and that no special training is required. Additional assays are under development, including for HIV.

Figure 24. cobas® Liat® System. *The **cobas®** Liat® Analyzer compresses the assay tube to move the sample and selectively release reagents from tube segments, under temperature controlled conditions.*

To aid the operator and provide reliable results, the **cobas®** Liat® System incorporates a variety of intelligent and advanced features. The system self-checks at power on and has an error diagnostic system with comprehensive real-time monitoring, continuous self-calibrations and error message display. Sample and quality control features include barcode data entry that avoids errors in sample or assay coding and on-screen prompts provide easy-to-follow directions to guide the operator through sample loading and tube insertion. Volume sensing ensures the appropriate amount of sample is used for the test, or delivers a warning if the sample volume is insufficient. A comprehensive set of sensors further monitors system operations in real time. Internal Controls are pre-packed and processed through every step, and quality control reagents are used with each new assay tube lot.

As illustrated below, the test procedure is straightforward, with no sample manipulation or reagent loading steps, other than inputting the sample directly into the **cobas®** Liat® assay tube. The **cobas®** Liat® System is a closed system, thus minimizing cross-contamination and biohazard risks, and allowing testing to be performed in non-laboratory or near patient facilities. The **cobas®** Liat® System is small and portable, weighing 3.76 kg. It executes all required assay steps and reports a test result in 20 minutes for Influenza A/B and RSV and in 15 minutes for Strep A.

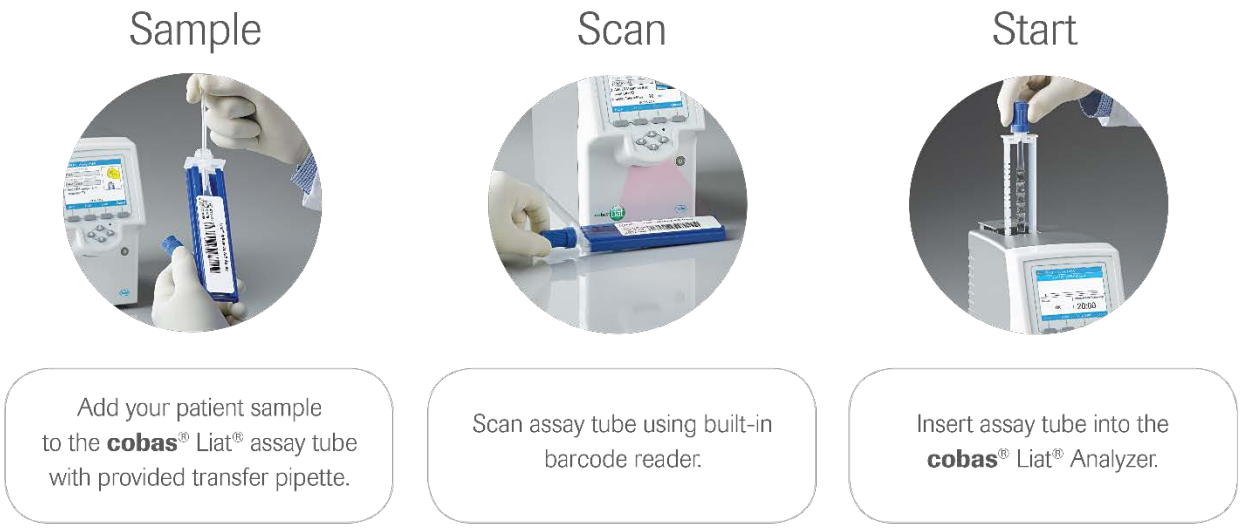


Figure 25. cobas® Liat® Test Procedure

The **cobas®** Liat® System has an internal optical system that provides independent optical detection channels for real-time monitoring and quantification, allowing for the detection of multiple targets in each test and providing future expandability for detection of multiple diseases. It can be powered by AC mains or by battery, allowing mobile use.

No price information is available on either the instrument or the individual assays for resource-limited settings. Roche launched the system in the US at the end of 2014 and now has expanded globally. STI assays are currently under development for the **cobas®** Liat® System, but the target profile and stability profiles for STI and future assays are also not available at this time.

PanNAT® Platform – *(Micronics, Inc.)*

Micronics, Inc., a subsidiary of Sony Corporation of America, has developed the PanNAT® system (pictured below), which is a small, portable microfluidic platform for near-patient use in *in vitro* molecular diagnosis of infectious diseases in resource-limited settings. It is a fluorescent-based reader capable of processing individual, disposable, assay-specific test cartridges, each of which is designed to perform a single and/or multiplexed nucleic acid assay. The cartridge includes all necessary reagents on board. The system is lightweight, mains-powered, can store up to 1,000 test results before prompting the user to download or delete results, and can provide results in approximately 1 hour, depending upon assay parameters. Battery-operation and WiFi-enabled options will be available with results output to USB, local network, LIS/HIS and printer.

Figure 26. PanNAT® Platform and Cartridge

The PanNAT® test cartridge incorporates all required reagents and controls for purification, amplification and detection, and because it is a closed cartridge system, there is no PCR product cross-contamination. The cartridge design permits storage at ambient temperatures for prolonged periods. All waste is captured in the cartridge for safe disposal.

Micronics has a number of tests in development, including certain STIs, respiratory and healthcare-associated infections (the specifics of which are confidential), an assay for Shiga toxin-producing E-coli, as well as other infectious disease diagnostics. Commercial launch of a first test and system is targeted for 2018.

A brief summary of the STI diagnostic products available and in the pipeline for CT, NG, CT/NG, TV and HPV is attached as **Annex E**.

Next-Generation Technologies

In addition to the platforms described above that use conventional lateral flow and molecular techniques, some diagnostic platforms use what might be described as "next-generation" technologies. These include the mChip assay for simultaneous detection of HIV and syphilis, the Vivalytic Analyzer, and the PanNAT® platform, all of which use microfluidic techniques, the MAMEF test for CT, which uses microwave acceleration and metal-enhanced fluorescence, the Solana® platform from Quidel, which has unique HDA technology, and the IDAlert, which uses electrochemical immunoassay technology with an immune-electrode detector. Additional diagnostic platforms/tests are being developed using a variety of next generation technologies that may make it possible to further enhance diagnostic capabilities at or near POC in resource-limited settings. The development of techniques that permit microscale fabrication and processing methods using silicon and the advances in plastics engineering can facilitate mass-produced, low cost, ultra-portable instrumentation with sophisticated sample and information processing capabilities that can be used effectively in diagnostics for use at the point of care *(94)*.[10]

Diagnostics involve two key processes: sample preparation and target detection. Sample preparation has proven to be a quite challenging problem. Specimens, including blood and body fluids, generally contain a significant amount of cells (e.g., proteins, DNA, etc.) other than the target analyte. These cells/debris need to be removed prior to target detection. But simplifying and miniaturizing sample preparation protocols have proven to be difficult.

Once the target biomarker has been washed and purified (and amplified in the case of nucleic acids), target detection is required. A number of techniques have been developed to detect biological signals at the micro- and nanoscale. These include optical sensing methods (e.g., from using color changes visible to the human eye to single-molecule fluorescence sensors), as well as electrochemical, electromagnetic and mass sensors. New technologies for sample preparation and target detection are generally characterized as microfluidics and nanotechnology, a few of which are described below.

Microfluidic Sample Preparation

There are a variety of microfluidic sample preparation approaches. These include mechanical, magnetic, electrokinetic, immunoaffinity, and chemical techniques. The approach used for any particular diagnostic will depend on both the sample type (e.g., whole blood, sputum) and the target analyte. As an example, immunoaffinity techniques can be used in microfluidic sample processing when an antibody with specificity for the target is available. Micro- or nanoparticles, particularly magnetic particles, functionalized with antibodies can be mixed with the sample to bind the analyte and then separated downstream. For example, one piece of recent research has demonstrated that magnetic particle binding can detect HIV capsid protein p24 as low as 0.1 pg/ml as part of a bio-barcode detection system *(95).* Other techniques, like hydrodynamics models using channel designs to induce turbulent flow and electrokinetic methods to enrich or concentrate a target in a biological sample are also being examined. However, to date, most of these methods have drawbacks and they have generally not been commercialized.

[10] This section of the report draws significantly from the publication of Damhorst et al. *(94)*.

Micro- and Nanoscale Detection Technologies

Similarly, micro- and nano-technologies offer a number of potential solutions for disease detection, but each technology has advantages and disadvantages for use at the point-of-care. Non-optical detection methods, including electrical impedance sensing, are attractive for their simplicity; while optical sensing methods have often proven to be too costly and cumbersome, requiring large lasers, photodetectors and cameras, many of which are not robust. However, the latest advances in camera technologies put increasingly sophisticated imaging ability into smartphones, which are already being used for diagnostic applications. For example, researchers at the University of California Berkeley have developed a mobile phone-based microscope by pairing a smartphone with a 3D-printed plastic base. Called the video CellScope, the device, pictured below, uses video to detect and quantify infection by parasitic worms in blood. Turnaround time is approximately 2 minutes *(96)*.

Figure 27. Video CellScope Device

Some of the most common optical detection methods are fluorescence, absorbance and chemiluminescence *(97)*. Fluorescence is the most common of these used in diagnostics, including in microscopy, flow cytometry and PCR. Micro-scale approaches often use fluorescence detection, frequently incorporating a laser or light emitting diode (LED) for excitation of the tag. For example, fluorescence microscopy has been a standard method of detecting mycobacterium bacteria (TB) in sputum samples. More recently, LED-based microscopy has increased access to microscopy in resource-limited settings.

Fluorescence is also commonly used as an indicator in NAAT-based testing, either as a DNA intercalating dye or as part of a fluorophore-quencher system conjugated to probe DNA. One example is the digital PCR device from SlipChip, which has been shown to be capable of detecting 37 copies/mL of viral RNA with HIV and HCV samples *(98,99)*. Currently the device is used only in research in clinical labs and has not been commercialized. However, many NAAT-based platforms use isothermal amplification (e.g., Ustar's CPA platform and the Alere™-i platform), and these have been commercialized. Although none of the commercialized platforms is yet an ultra-portable, handheld device, a Harvard-led team is working to develop such a device, for which proof of principle has already been established (*100*).

In addition to fluorescence, colorimetry and chemiluminescence techniques are also being used in diagnostics for use at the point of care. Colorimetry has the advantage of providing a signal that is visible to the naked eye, which can eliminate the need for cameras in tests. Drawbacks include that instrument-based analysis of colorimetric signals is not as precise as other methods. Chemiluminescence, on the other hand, has the advantage of not requiring an external light source, but has the drawback that there are limited reagents available to produce such a signal. Nonetheless, there are at least two enhanced chemiluminescence immunoassays for screening of hepatitis C: VITROS Anti-HCV assay (Ortho-Clinical Diagnostics) and ARCHITECT Anti-HCV test (Abbott). These assays have been reported to have slightly higher sensitivity than traditional enzyme immunoassays *(101)*.

Additional optimal detection technologies include a lens-less shadow imaging technique, plasmon resonance, and shadow imaging, which has been used for whole cell detection in microfluidic devices including for point-of-care CD4 testing *(102,103)*. These methods are generally not yet commercialized, however.

Nonoptical Methods of Detection

In addition to optical methods of detection, there are also non-optical methods, which have their own advantages and disadvantages. Although electrical sensing techniques are frequently simpler and less expensive than optical methods, the downside is that they typically rely heavily on sample processing steps to remove background noise.

One electrical sensing method that has shown promise is impedance spectroscopy, which generally using microfabricated electrodes, measures electrical impedance of an aqueous solution as a function of AC frequency. Several applications in CD4 cell counting have been developed *(104,105)*, and impedance-based cell counting approaches have also been used in the context of malaria diagnosis *(106,107)*. Some commercial applications are already emerging.

Other promising technologies include electrochemical approaches, although they are limited to enzymes and reagents that are capable of producing an electrochemical signal. In addition, other approaches may detect mass or mechanical forces. The potential downside is that mechanical sensors may not be robust enough for hand-held diagnostic test platforms. In addition, thanks to improved microfabrication techniques, innovative approaches are being made possible by increasingly miniaturized measurement techniques that have the potential to be used in diagnostics. For example, mass spectrometry has already been miniaturized and coupled with microfluidic devices *(108).*

In summary, some microfluidics and nanotechnologies appear to have potentially promising applications for diagnostics at the point-of-care, but to date few of them have been commercialized. In addition, there is a long and arduous road from demonstrating the use of these technologies either for enrichment of a biological sample or the sensitive detection of an analyte, on the one hand, to a combined sample-in, result-out diagnostic platform, on the other hand. The integration of these techniques is a big challenge in diagnostic development. But only when all components of a test have been combined into a self-contained device that can be used at the point of patient care can new technologies realize their full promise for improving global health.

Described below are a number of integrated, next-generation, POC diagnostic technologies that have STIs in their medium-term pipeline of tests. This is not an exhaustive group of technologies; additional developers/companies with potential STI tests in the future include: ChipCare (Canada), Click Diagnostics (USA), HiberGene Diagnostics (Republic of Ireland), and Diagnostics for All (USA), among others.

Blusense Platform (Blusense Diagnostics ApS, Denmark)

The Blusense Platform (pictured below) uses microfluidics and next generation latex immune-turbidimetry in the form of an Immuno-Magnetic Assay (IMA) methodology, a patented opto-magnetic nanoparticle-based readout technology, which can be used to detect antigens, antibodies, small molecules, RNA, DNA, and micro-RNA.

TECHNICAL DATA

Screen	7" Color touchscreen, 800 x 480
Memory	1GB RAM, 16GB SD (up to 128GB)
Data transfer	USB, LIS*
External device support	Barcode reader, printer*
Power adapter	DC 19 V /4.73 A
Dimensions (WxDxH)	210 x 200 x 170 mm
Weight	3.2 kg

OPERATIONS CONDITIONS

Temperature range	15 - 35 C
Relative humidity	10 - 80% (non condensing)

Figure 28. Blusense BluBox® Platform

The core idea of IMA consists of measuring the presence of a target molecule by optically measuring the change in dynamic rotation of magnetic nanoparticles (NPs) upon specific cluster formation due to the presence of the target analyte. An AC magnetic field is used to force nano-cluster rotation, which causes a temporal scattering, cross-sectional variation that is optically measured using a Blu-ray laser unit and a photodetector, based on mass-produced electronics components. The phase difference between the applied field and the modulated transmitted light through the nanoparticles precisely correlates with the amount of target analyte (e.g., specific antigen or antibodies). The readout system is implemented on a polymer microfluidic cartridge based on centrifugal microfluidics, which allows fast blood plasma separation, metering, mixing and resuspension of dry nanoparticles without the need for any user sample preparation. For example, for the company's dengue (DENV) assay, commercial superparamagnetic nanoparticles are coated with coupled anti-DENV NS-1 antibodies (for NS-1 detection) or DENV envelope proteins (for IgM/IgG detection) capable of forming sandwich agglutination in the presence of the target analyte.

The Blusense solution consists of an easy-to-use reader (the BluBox) and a single-use test cartridge (the VIRO-Track), specifically designed to detect and quantitate various viruses. The platform is rugged, has high sensitivity and specificity, is easy to use (with 20 seconds hands-on operations), and provides

sample to result in 10 minutes. The platform can take whole blood, plasma, serum or capillary blood and requires 10 -30 µ of sample volume. The platform contains embedded connectivity as well.

Figure 29. Blusense Solution Workflow

Products currently available from BlueSense are its ViroTrack Acute Dengue NS1 Ag (pictured below), the ViroTrack Duo Dengue IgG/IgM, and the ViroTrack Combo Dengue NS1/IgG/IgM assays. Additional assays for ZIKV and DENV/ZIKV/CHIKV differential are currently in development. Blusense is also planning to add assays for certain STIs to its platform. The timeframe for these would be 2020 and beyond.

Figure 30. Blusense Viro-Track® Acute Dengue Cartridge line

Diassess Platform (Diassess Inc., USA)

Diassess Inc. is an *in vitro* diagnostics manufacturer focused on rapid molecular infectious disease tests for physician offices and healthcare settings with limited resources. Diassess has developed an instrument-free, deployable, disposable, and simple-to-use molecular test with a usability profile equivalent to current rapid immunoassay diagnostic tests. The test does not require instrumentation, external power, cold chain supply, or operator training. Results are obtained in 20 minutes or less with performance equivalent to laboratory molecular testing. Upon market entry, the Diassess platform will establish a new category of rapid molecular tests: disposable molecular testing. As shown in Figure 31, the test consists of two parts: a Sample Prep Tube (SPT) and a Detection Module (DM). To use the product, the user inserts an unprocessed patient sample into a proprietary elution buffer contained within the SPT. The buffer elutes and lyses cells from the sample. The SPT is then engaged with the DM, after which point the test is automated and the results are displayed on the on-board LCD screen.

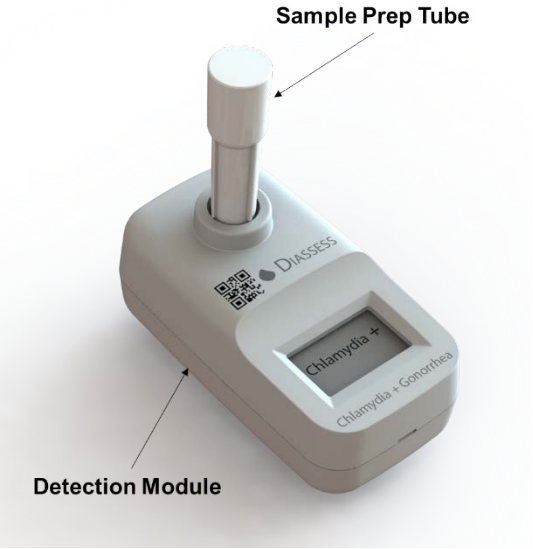


Figure 31. Diassess Platform

The Diassess platform utilizes an isothermal amplification scheme to amplify pathogenic genetic targets in the patient sample that can be indicative of infectious diseases such as CT or NG. These reactions occur in independent chambers within an automated and pump-free fluidic module. The proprietary colorimetric chemistry leads to a color change when DNA is amplified and facilitates the real-time monitoring of amplification within each chamber enabling semi-quantitative results. This color change is detected using inexpensive and disposable electronic elements and the diagnostic result is displayed on the test. The platform is powered by removable alkaline batteries enabling molecular testing free from the traditional bounds of instrumentation, operator training, and external power requirements. The platform is designed to tolerate extreme operating conditions and be performed by minimally trained health-care workers (Level I settings) in decentralized clinical settings. The system is self-contained to ensure biosafety and shelf-life stable for extend periods of time (stability studies are pending) with internal positive, negative, and sample controls ensuring test validity and quality. An unlimited number of test can be run in parallel during times of high patient volume.

Diassess has been awarded multiple NIH Phase I and II grants for the development of the CT and NG product lines. Prior evaluation of the CT assay, using remnant and fresh vaginal swab samples, has demonstrated Positive and Negative Percent Agreement of >95% when compared to the Cepheid Xpert[11]. Both the CT and NG assays are undergoing pilot clinical evaluations through collaboration with the UCSF Benioff Children's Hospital Oakland, California, USA.

No launch date for the Diassess platform has been set by the company.

[11] Unpublished data from an evaluation by Diassess incollaboration with USCF Benioff Children's Hospital, Oakland, California, USA.

IDAlert (*Aalto Bio Reagents*)

The IDAlert platform is the first lab-on-a-chip technology that uses an electrochemical immunoassay technology with an immune-electrode detector to produce a sample to answer result in less than 15 minutes on a patient sample. The technology utilizes a self-contained, portable electrochemical enzyme linked immunoassay (EEIA) system composed of a handheld battery operated electronic reader and sample assay chip card (both of which are shown below). The sample is applied to the chip card via a sampling strip that contains reagents required for a specific ELISA procedure. The card is inserted into the reader and the test begins.

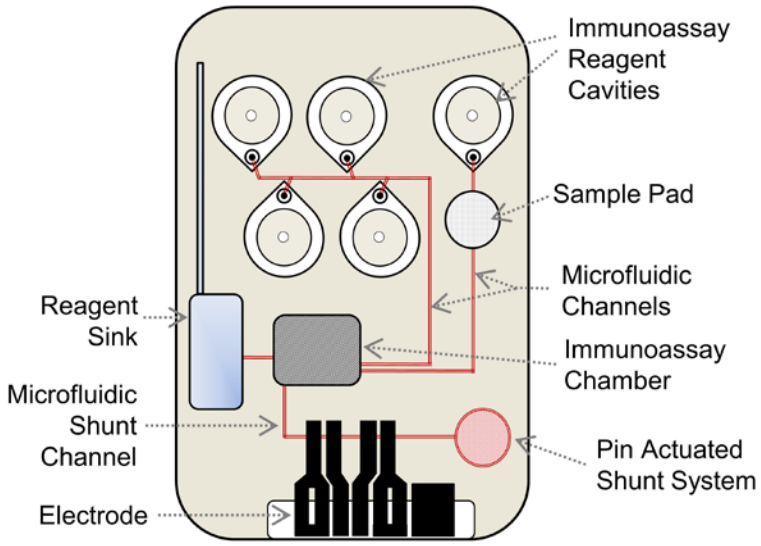


Figure 32. IDAlert Chip Card

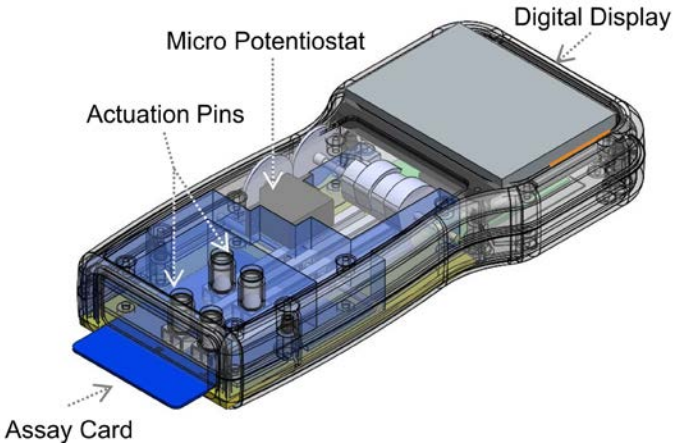


Figure 33. IDAlert Assay Reader

The chip detection methodology is based on charge measurement or coulometry for the detection and sensitive quantitation of peroxidase labels in EIAs. The detector uses a series of electrodes coated with

antigen specific for the target antibody. The chip also houses pressure-sensitive cavities and the reagents are moved throughout the card via a series of microchannels to the detector through pin actuation. Electrochemical activity is measured by the on-board potentiostat and results are given on the reader's digital display panel.

The technology has been developed over a number of years particularly to focus on the unmet need for diagnosing emerging or re-emerging diseases like Ebola, Marburg, MERS and existing STIs - CT, NG, HIV, HPV, Herpes Simplex Virus (HSV), syphilis, and TB. With diabetes in mind, it is envisaged that the technology can also be rolled out to help with chronic disease management.

A feasibility study of the IDAlert system was recently performed using anti-HSV-2 blood antibody as the diagnostic target.[12] The diagnostic performance of the HSV-2 biochip tested was determined by testing a panel of serum samples (n = 60) and comparing results to data generated on clinically validated HSV-2 serological assays (DiaSorin LIAISON® HSV-2 and Focus HerpeSelect® 2 IgG ELISA). The sensitivity and specificity of the IDAlert HSV-2 biochip test was 100% compared to the LIAISON® test. The sensitivity and specificity of the system were 96.7% and 100%, respectively, compared to the HerpeSelect® 2 assay.

The company is currently focused on developing a triplex test to detect Zika, Dengue and Chikungunya virus infections. An STI panel is expected to follow.

oncgnostics GmbH/BLINK AG (Germany)

Oncgnostics GmbH specializes in the development of *in vitro* diagnostics for all areas of cancer detection. The tests are based on epigenetic markers, disease specific DNA methylation patterns, which are characteristic for cancer cells. In this regard, the company has developed its CE-IVD marked GynTect® assay, which detects epigenetic markers for cervical cancer. The assay was developed as a triage tool for detecting severe cervical lesions (CIN3) and cervical cancer in HPV positive women.

[12] The study results were presented by Aalto Bio Reagents in a poster at the Lab-on-a-Chip Microfluidics and Microarrays World Congress held from 26 – 28 September 2016 in San Diego, California and shared with the author in personal correspondence.

Figure 34. GynTect® Assay

The GynTect® assay is performed using a cervical smear transferred to a standard specimen transport medium (STM) (e.g. from Qiagen, BD). The specimen is lysed and a bisulfite treatment is performed. The bisulfite conversion enables the specific detection of methylated DNA by methylation-specific PCR. Following detection of the methylated DNA markers, data analysis is done. Currently, the GynTect® assay must be performed on an Applied Biosystems 7300 or 7500 Real-time PCR System (ThermoFisher, USA).

The GynTect® assay performed well in a recent study of women referred to a hospital colposcopy unit in Germany for a diagnostic work-up; in other words, the population did not represent a primary screening population, and the performance measures reported are not applicable to primary screening. Schmitz et al (*109*) found the overall sensitivity of the assay for the detection of CIN3 or cervical cancer (CIN3+) was 67.7% (95% CI 57.3 - 77.1) (*109*). All cancer cases were detected by GynTect®. The overall false positive rate for women with no CIN was 17.4% (95% CI 12.5 – 23.1), with a higher proportion among HPV-positive women (24.0%, 95% CI 16.0 – 33.6) (*109*). The authors concluded that GynTect® "is a robust and highly reproducible assay for the triage of HPV-positive women" (*109*).

In partnership with BLINK AG, oncgnostics is now developing its GynTect® assay for use at POC on BLINK's open IVD platform – thus bringing cervical cancer detection nearer to the point of patient care. BLINK is developing a multiplex, multi-analyte platform called the BLINK Box (pictured below).

Figure 35. The BLINK Box.

The BLINK technology supports a variety of workflows for the detection of different analytes, such as cells, nucleic acids and proteins. Moreover the company is developing a novel assay format that provides for the digital detection and quantification of individual analyte molecules in a sample and ensures a substantial dynamic quantification range. The system is designed for processing sample sizes from a few microliters (µl) up to large samples > 10mL and to be compatible with all common sample matrices.

Further, the underlying architecture of the platform supports the flexible setup of different workflows required by a given assay. The BLINK platform will also permit random and continuous access testing, with samples being subjected to multiple tests in a random-access fashion. Individual test panels can be selected from a menu of test cassettes onboard the instrument; any sample can be loaded on the instrument at any time. In short, the platform has a novel, disposable format that bridges laboratory and POC applications.

BLINK will also work with assay developers, like oncgnostics, with test development facilitated through an “open access” development interface.

Q-POC™ (QuantuMDx Group, UK)

QuantuMDx Group is developing a small handheld diagnostic device, Q-POC™, and test cassette (pictured below) that can deliver patient results in less than 20 minutes.

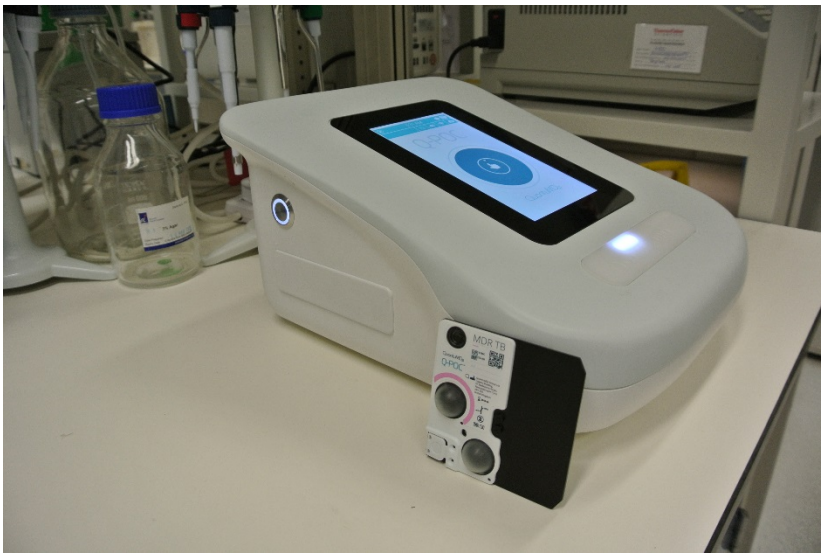


Figure 36. Q-POC™ Cassette and Device

The Q-POC™ device is a portable and simple to use, sample-to-answer molecular platform that runs end-point PCR chemistries, qPCR chemistries and includes a microarray after the amplification step. It is the first MDx POC platform that combines the ability to quantitate pathogens through its 6-channel qPCR and additionally perform multiplex detection of ~50 markers with its integrated microarray. The device can run raw sample to answer assays between 7 to 20 minutes, depending on the complexity of the assay. It does this thanks to its rapid microfluidic thermal cycler that performs a 35-cycle end-point PCR in as fast a couple of minutes.

The first assay being developed on the Q-POC™ platform is an HPV genotyping assay that provides individual genotypes for 13 high-risk HPV subtypes in under 20 mins, direct from a swab sample. The assay is presently in clinical field studies to demonstrate the clinical utility of screen and treat programs in LMICs. QuantuMDx's second assay is a CT/NG/TV triplex assay, also run direct from swab samples in under 15 minutes. Exploiting Q-POC™'s low cost, rapid turn-around time and ability to detect >50 markers in a single test, the Company is developing an antimicrobial resistance NG assay to complement its triplex STI detection assay.

The Limits of Diagnostic Technology

Despite the increasing sophistication of novel diagnostic technologies, the impact of such technologies will be limited unless they can successfully accommodate the weaknesses in healthcare systems in resource-constrained settings, which often affect the successful delivery of diagnostics in-country. These include: shortages of human resources and lack of training for staff; supply chain challenges; lack of diagnostic equipment and equipment breakdowns; and a lack of robust quality assurance and quality control systems.

These weaknesses suggest not only the in-country need for training of test operators and service and maintenance contracts for diagnostics, but also suggest that the following operational specifications for 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 healthcare worker in a day's training or less, including its methods of sample collection and preparation.

High tolerance to difficult environmental conditions. Test kits must be stable at high temperature 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 (either internal or external), 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/GPRM 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. Further, as an optimal standard, compostable plastics for test kits and other materials would be preferred.

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

Cost. The cost of platforms and 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 (TAT) that will fit all settings. Rather, these specifications will depend on the volume of testing and TAT for each assay at the target use setting (e.g., district hospital, health

center). 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 center settings is greatly abbreviated (6 hours or less), and the TAT 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.

These factors, along with required technical performance, must be considered and prioritized by developers of diagnostics intended for use at or near the point of patient care in resource-limited settings.

Conclusions

With respect to STIs, with the exception of screening for syphilis and combined HIV/syphilis and of testing for TV, it is generally the case that RDTs do not perform sufficiently well relative to laboratory-based platforms, in particular NAAT-based platforms. However, given their cost, sophistication and infrastructure requirements, such platforms are generally available only at central reference laboratories (or the equivalent) in resource-limited settings. This severely limits access to STI testing, particularly for CT, NG and HPV. Testing platforms for these infections that can be used at or near the point of patient care are needed.

There is a reasonably robust platform for molecular platforms for which assays for CT, NG, CT/NG, TV and HPV are currently being developed. One platform, the GeneXpert® already provides assays for CT, CT/NG, TV and HPV. Additional platforms designed for use at or near the point of patient care will soon have similar capabilities. However, a number of these platforms, including the Xpert®, are most appropriate for use at the district hospital or above (Level II setting) in resource-limited settings. This gives some degree of test decentralization and should help to increase access to testing, but in order to truly expand access and reach the most patients, it would be necessary to locate test platforms at the level of the health center (Level I setting) where laboratories are quite basic.

Therefore, it is useful to consider what assay/platform characteristics are recommended for STI testing to effectively reach the point of patient care – meaning that test results can be provided to patients and patients can be linked to clinical care in a single visit. One way of doing this is to develop TPPs for each of the desired tests. A TPP for a dual HIV/syphilis test has already been developed and published. Similarly, TPPs for CT, NG, combined CT/NG, TV and HPV tests/platforms have also been developed and are published on the WHO/RHR website.[13] Each TPP sets out not only the performance requirements for each test relative to the appropriate gold standard technology, but also the operational characteristics for the assays/platforms for the desired target use setting in-country. It is only when the required technical specifications and preferred operational specifications are married in a single platform/platforms that new tests for STIs will be well positioned to achieve the desired level of uptake and impact in global health.

[13] Available at: http://www.who.int/reproductivehealth/topics/rtis/POCTs-target-product-profiles.pdf.

REFERENCES

1. Sexually transmitted infections (Fact Sheet 110). Geneva: WHO; 2016 (http://www.who.int/mediacentre/factsheets/fs110/en/, accessed 21 November 2016).

2. Peeling RW. Applying new technologies for diagnosing sexually transmitted infections in resource-poor settings. Sex Transm Infect. 2011; 87:ii28-ii30.

3. Newman LR et al. Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. PLoS ONE. 2015;10(12):e0143304. Doi:10.1371/journal.pone.0143304.

4. Wijesooriya NS et al. Global burden of maternal and congenital syphilis in 2008 and 2012: a health systems modelling study. Lancet Glob Health. 2016; 4:e525-33.

5. Gomez GB et al. Untreated maternal syphilis and adverse outcomes of pregnancy: a systematic review and meta-analysis. Bull World Health Organ. 2013;91:217–26. doi:10.2471/BLT.12.107623.

6. Men who have sex with men and syphilis. Geneva: Global Health Observatory (GHO) data – WHO; 2018 (http://www.who.int/gho/sti/sex_msm/text/en, accessed 3 April 2018).

7. Syphilis – CDC Fact Sheet (Detailed). Atlanta: Centers for Disease Control and Prevention; 2018 (http://www.cdc.gov/std/syphilis/stdfact-syphilis-detailed.htm, accessed 3 April 2018).

8. Global incidence and prevalence of selected curable sexually transmitted infections. Geneva: WHO;2015; unpublished data.

9. Sex workers with active syphilis. Geneva: Global Health Observatory (GHO) data – WHO; 2018 (http://www.who.int/gho/sti/sex_workers/text/en, accessed 3 April 2018.

10. Prequalification of in vitro diagnostics scope to be to syphilis RDTs. Geneva: WHO: 2017 (http://www.who.int/diagnostics_laboratory/pq-syphilis-rdts/en/, accessed 3 April 2018.

11. Jafari Y et al. Are treponema pallidum specific rapid and point-of-care tests for syphilis accurate enough for screening in resource limited settings? Evidence from a meta-analysis. PLoS One. 2013; 8(2):e54695. Doi:101371/journal.pone.0054695.

12. Tucker JD et al. Accelerating worldwide syphilis screening through rapid testing: a systematic review. Lancet Infect Dis. 2010; 10: 381-86.

13. Mabey D et al. Prospective, multi-centre clinic-based evaluation of four rapid diagnostic tests for syphilis. Sex Transm Infect. 2006; 82:v13-v16. doi: 10.1136/sti.2006.022467.

14. Causer LM et al. A laboratory-based evaluation of four rapid point-of-care tests for syphilis. PLoS One. 2014;9(3):e91504; doi:10.1271/journal.pone.0091504.

15. Nakku-Joloba E et al. Clinical evaluation of 2 point-of-care lateral flow tests for the diagnosis of syphilis. Sex Transm Dis. 2016; 43(10):623-625.

16. Bocoum, FY et al. Evaluation of the diagnostic performance and operational characteristics of four rapid immunochromatographic syphilis tests in Burkina Faso. African Health Sciences. 2015; 13(2): 360-367.

17. Castro AR et al. An immunofiltration device for the simultaneous detection of non-treponemal and treponemal antibodies in patients with syphilis. Sex Transm Infect. 2010; 86: 532-536.

18. Yin YP et al. A dual point-of-care test shows good performance in simultaneously detecting nontreponemal and treponemal antibodies in patients with syphilis: a multisite evaluation study in China. Clin Infect Dis. 2013; 56: 659-65.

19. Castro AR et al. Novel point-of-care test for simultaneous detection of nontreponemal and treponemal antibodies in patients with syphilis. J Clin Microbiol. 2010; 48: 4615-4619.

20. Causer LM et al. An evaluation of a novel dual Treponemal/Nontreponemal point-of-care test for syphilis as a tool to distinguish active from past treated infection. Clin Infect Dis. 2015; 61:184-191.

21. Global Statistics. The Global HIV/AIDS Epidemic. Geneva: UNAIDS; 2018 https://www.hiv.gov/hiv-basics/overview/data-and-trends/global-statistics, accessed 6 April 2018).

22. Mother-to-Child Transmission of HIV. Geneva: WHO; 2018 (http://www.who.int/hiv/topics/mtct/en/, accessed 6 April 2018).

23. Berman SM. Maternal syphilis: pathophysiology and treatment. Bull World Health Organ. 2004; 82: 433–8.

24. Blencowe H et al. Lives Saved Tool supplement detection and treatment of syphilis in pregnancy to reduce syphilis related stillbirths and neonatal mortality. BMC Public Health. 2011;11:S9. doi:10.1186/1471-2458-11-S3-S9.

25. Hawkes S et al. Effectiveness of interventions to improve screening for syphilis in pregnancy: a systematic review and meta-analysis. Lancet Infect Dis. 2011; 11: 684–91.

26. PMTCT strategic vision 2010–2015. Geneva: WHO; 2010 (http://www.who.int/hiv/pub/mtct/strategic_vision/en/index.html, accessed 5 July 2015).

27. Terris-Prestholt F et al. Is antenatal syphilis screening still cost effective in sub-Saharan Africa? Sex Transm Infect. 2003; 79: 375–81.

28. Hira SK et al. Syphilis intervention in pregnancy: Zambian demonstration project. Genitourin Med. 1990; 66: 159–64

29. Jenniskens F et al. Syphilis control in pregnancy: decentralization of screening facilities to primary care level, a demonstration project in Nairobi, Kenya. Int J Gynaecol Obstet. 1995; 48: S121–8.

30. Fonck K et al. Syphilis control during pregnancy: effectiveness and sustainability of a decentralized program. Am J Public Health. 2001; 91: 705–7.

31. The global elimination of congenital syphilis: rationale and strategy for action. Geneva: WHO; 2007 (http://www.who.int/reproductivehealth/publications/rtis/9789241595858/en/, accessed 5 July 2015.)

32. Humphries RM et al. Laboratory evaluation of three rapid diagnostic tests for dual detection of HIV and treponema pallidum antibodies. J Clin Microbiol. 2014; 52: 4394-4397.

33. Yin YP et al. Laboratory evaluation of three dual rapid diagnostic tests for HIV and syphilis in China and Nigeria. Intl Jrl Gyn and Obst. 2015; 130: S22-S26.

34. Gliddon HD et al. A systematic review and meta-analysis of studies evaluating the performance and operational characteristics of dual point-of-care tests for HIV and syphilis. Sex Transm Infect, 2017;93:S3-S515.

35. Bristow CC et al. Multi-site laboratory evaluation of a dual HIV/syphilis point-of-care rapid test for simultaneous detection of HIV and syphilis. Open Forum Infect. Dis. 1:ofu15, http://dx.doi.org/10.1093/ofid/0fu015.

36. Omoding D et al. Evaluation of the SD Bioline HIV/syphilis Duo assay at a rural health center in Southwestern Uganda. *BMC Res Notes*. 2014; 7:746. doi: 10.1186/1756-0500-7-746.

37. Shimelis T and Tadesse E. The diagnostic performance evaluation of the SD Bioline HIV/syphilis Duo rapid test in southern Ethiopia: a cross-sectional study. BMJ Open. 2015; 5: e007371, doi: 10.1136/bmjopen-2014-007371.

38. Bristow CC et al. Field evaluation of a dual rapid diagnostic test for HIV infection and syphilis in Lima, Peru. Sex Transm Infect. 2016; 92(3):182-185.

39. Bristow CC et al. Dual rapid lateral flow immunoassay fingerstick wholeblood testing for syphilis and HIV infections is acceptable and accurate, Port-au-Prince, Haiti. BMC Infect Dis. 2016;16:302.doi: 10.1186/s 12879-016-1574-3.

40. Shakya G et al. Evaluation of SD Bioline HIV/syphilis duo rapid test kits in Nepal. BMC Infect Dis. 2016;16:450. Doi: 10.1186/s 12879-016-1694-9.

41. Holden J et al. An evaluation of the SD Bioline HIV/syphilis duo test. Int J STD AIDS. 2018; 29(1):57-62.

42. Leon SR et al. Laboratory evaluation of a dual-path platform assay for rapid point-of-care HIV and syphilis testing. J Clin Microbiol. 2016;54:492-494.

43. Kalou MB et al. Laboratory evaluation of the Chembio Dual Path Platform HIV-syphilis assay. Afr J Lab Med. 2016;5(1):433.

44. Bristow CC et al. Laboratory evaluation of a dual rapid immunodiagnostic test for HIV and syphilis infection. JCM. 2015; 53: 311-313.

45. Bristow CC et al. Field evaluation of a dual rapid immunodiagnostic test for HIV and syphilis infection in Peru. Sex Transm Dis. 2016;43:57-60.

46. De Cortina SH et al. Laboratory evaluation of a point-of-care downward-flow assay for simultaneous detection of antibodies to *Treponema pallidum* and human immunodeficiency virus. J Clin Microbiol 2016;54:1922-1924.

47. Chin CD et al. Mobile device for disease diagnosis and data tracking in resource-limited settings. Clin Chem. 2013; 59: 629-640.

48. De Cortina SH et al. A systematic review of point of care testing for Chlamydia trachmomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis. Inf Dis Ob and Gyn. 2016; Article ID 4386127; available at: http//dx.doi.org/10.1155/2016/4386127.

49. Vickerman P et al. Sensitivity requirements for the point of care diagnosis of chlamydia trachomatis and neisseria gonorrhoeae. Sex Transm Infect. 2003; 79: 363-7.

50. Vickerman P et al. Detection of gonococcal infection: pros and cons of a rapid test. Mol Diagn. 2005; 9: 175-9.

51. Kelly et al. Systematic reviews of point-of-care tests for the diagnosis of urogenital Chlamydia trachomatis infections. Sex Transm Infect. 2017;93:522-30.

52. Ham et al. Highly sensitive and novel point-of-care system, aQcare Chlamydia TRF Kit for detecting Chlamydia trachomatis by using Europium (Eu) (III) chelated nanoparticles. Ann Lab Med. 2015;35:50-56.

53. Nuñez-Forero L et al. Diagnostic accuracy of rapid tests for sexually transmitted infections in symptomatic women. Sex Transm Infect. 2016;92:24-28.

54. Samarawickrama A et al. Pilot study of use of the BioStar Optical ImmunoAssay GC point-of-care for diagnosing gonorrhea in men attending a genitourinary medicine clinic. J Med Microbiol. 2014;63:1111-1112.

55. Abbai NS et al. Clinical evaluation of the OneStep Gonorrhea RapiCard Instatest for detection of neisseria gonorrhoeae in symptomatic patients from KwaZulu-Natal, South Africa. J Clin Microbiol. 2015;53:1348-1350.

56. Gaydos C and Hardick J. Point of care diagnostics for sexually transmitted infections: perspectives and advances. Expert Rev. Anti infect Ther. 2014; Early online, 1-16.

57. Huppert J et al. What is the point? How point-of-care sexually transmitted infection tests can impact infected patients. Point of Care. 2010; 9: 36-46.

58. Peeling RW et al. Rapid tests for sexually transmitted infections (STIs): the way forward. Sex Transm Infect. 2006; 82: v1-v6.

59. Xpert® CT NG White Paper. Available at: http://www.cepheid.com/en/cepheid-solutions-uk/clinical-ivd-tests/sexual-health/xpert-ct-ng, accessed 21 December 2016.

60. Gaydos CA et al. Performance of the Cepheid CT/NG Xpert rapid PCR test for the detection of chlamydia trachomatics and neisseria gonorrhoeae. J Clin Microbiol. 2013; doi: 10.1128/JCM.03461-12.

61. Tabrizi SN et al. Analytical evaluation of GeneXpert CT/NG, the first genetic point-of-care assay for simultaneous detection of neisseria gonorrhoeae and chlamydia trachomatis. J Clin Microbiol. 2013; 51: 1945-1947.

62. Badman SG et al. Rapid laboratory assessment of a new [Gx] GeneXpert molecular point-of-care test for detection of Trichomonas vaginalis," in Proceedings of the Australasian Sexual Health Conference, Sydney, Australia, 2014; available at: https://www.researchgate.net/publication/266319343_Rapid_laboratory_assessment_of_a_new_GeneXpert_molecular_point-of-care_test_for_detection_of_Trichomonas_vaginalis_Aust_Sex_Hlth_Conference_10_Oct_2014.

63. Clifford et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet. 2005; 366: 991- 998.

64. Li et al. Human papillomavirus type distribution in 20, 848 invasive cervical cancers worldwide: variation by geographical region, histological type and year of publications. Int J Cancer. 2011; 128: 927- 935.

65. Cuzick J et al. Performance of the Xpert HPV assay in women attending for cervical screening. Papilloma Res 2015; 1:32-37.

66. Arbyn et al. Which high-risk HPV assays fulfill criteria for use in primary cervical cancer screening? Clin Microbiol Infect. 2015;21: 817- 826.

67. Castle PE et al. Reliability of the Xpert HPV assay to detect high-risk human papillomavirus DNA in a colposcopy referral population. Am J Clin Pathol. 2015;143:126-33.

68. Einstein MH et al. Clinical evaluation of the cartridge-based GeneXpert human papillomavirus assay in women referred for colposcopy. J Clin Microbiol. 2014; 52: 20-2095.

69. Cuschieri K et al. Performance of a cartridge based assay for the detection of clinically significant HPV infection – lessons from VALGENT (Validation of HPV Genotyping Tests) 2016; J Clin Microbiol; 54:2337–2342.

70. Toliman et al. Field evaluation of the Xpert HPV Point of care test for the detection of human papillomavirus infection using self-collected vaginal and clinician- collected cervical specimens. J. Clin. Microbiol. 2016;7:1734-1737.

71. Bristow et al. Characteristics of the Sample Adequacy Control (SAC) in the Cepheid Xpert® CT/NG assay in female urine specimens. Microbiol Exp. 2014;1:00026. http://dx.doi.org/10.15406/jmen.2014.01.0026.

72. Causer LM et al. A field evaluation of a new molecular-based point-of-care test for chlamydia and gonorrhoea in remote aboriginal health services in Australia. Sex Health. 2014; doi: 10.1071/SH14158.

73. Natoli L et al. “I do feel like a scientist”; a qualitative study of acceptability of POC CT-NG to primary care in remote settings. PLoS 2015; http://dx.doi.org/10.1371/journal.pone.0145993.

74. Natoli L et al. Public health implications of molecular point-of-care testing for chlamydia and gonorrhea in remote primary care services in Australia: a qualitative study. BMJ Open-2015; 5:e006922 doi:10.1136/bmjopen-2014-006922.

75. Harding-Esch EM et al. A 30-minute nucleic acid amplification point-of-care test for genital Chlamydia trachomatis infection in women: a prospective, multi-center study of diagnostic accuracy. EBioMedicine. 2018;28:120-127.

76. Pearce DM et al. Clinical performance evaluation of a new, rapid point-of-care system for detecting Chlamydia trachomatis. ISSTDR Poster Presentation 2015; available at: https://www.eiseverywhere.com/file_uploads/1f5c0493f2ff843b049992ec0c24f7e9_DavidPearce.pdf.

77. Zhang Y et al. Development of a microwave-accelerated metal-enhanced fluorescence 40 second, < 100 dfu/mL point of care assay for the detection of chlamydia trachomatis. IEEE Trans Biomed Eng. 2011; 58: 781-784. doi:10.1109/TBME.2010.2066275.

78. Melendez JH et al. Blind evaluation of the microwave-accelerated metal-enhanced fluorescence ultrarapid and sensitive *Chlamydia trachomatis* test by use of clinical samples. J. Clin. Microbiol. 2013; 51: 2913-20.

79. Melendez JH et al. Detection of Chlamydia and Gonorrhea by Microwave-Accelerated Metal-Enhanced Fluorescence. CFTCC Annual Symposium Poster Session 2014; available at: http://www.slideshare.net/lenais/boston-214; accessed 28 November 2016.

80. Shin et al. Mobile nucleic acid amplification testing (mobiNAAT) for Chlamydia trachomatis screening in hospital emergency department settings. Scientific Reports. 2017;7:4495-4504.

81. Madico G et al. Diagnosis of trichomonas vaginalis infection by PCR using vaginal swab samples. J Clin Microbiol. 1998; 36: 3205-10.

82. Wendel KA et al. Trichomonas vaginalis polymerase chain reaction compared with standard diagnostic and therapeutic protocols for detection and treatment of vaginal trichomoniasis. Clin Infect Dis. 2002; 35: 576-80.

83. Laboratory detection of trichomonas. Silver Spring: APHL. 2013 (http://www.aphl.org/AboutAPHL/publications/Documents/ID_2013August_Advances-in-Laboratory-Detection-of-Trichomonas-vaginalis.pdf, accessed 5 November 2015).

84. Huppert JS et al. Rapid antigen testing compares favorably with transcription-mediated amplification assay for the detection of trichomonas vaginalis in young women. Clin Infect Dis. 2007; 45: 194-98.

85. Gaydos CA et al. Rapid and point-of-care tests for the diagnosis of *Trichomonas vaginalis* in women and men. Sex Transm Infect. 2017;93:S31–S35.

86. Gaydos CA et al. Clinical performance of the Solana® Point-of-Care Trichomonas Assay from clinican-collected vaginal swabs and urine specimens from symptomatic and asymptomatic women. Expert Rev Mol Diagn. 2017;17:303-306.

87. Zhao FH et al. An evaluation of novel, lower-cost molecular screening tests for human papillomavirus in rural China. Cancer Prev Res. 2013;9:929-936.

88. Chibwesha CJ et al. Clinical performance validation of 4 point-of-care cervical cancer screening tests in HIV-infected women in Zambia. J Low Genit Tract Dis. 2017;20:218-223.

89. Kelly H et al. A systematic review and meta-analysis of studies evaluating the performance of point-of-care tests for human papillomavirus screening. Sex Transm Infect. 2017;93:S36-S45.

90. Jeronimo J et al. A multicountry evaluation of careHPV testing, visual inspection with acetic acid, and papanicolaou testing for the detection of cervical cancer. Int J Gynecol Cancer. 2014; 24: 576-85.

91. Labani S et al. CareHPV cervical cancer screening demonstration in a rural population of north India. Eur J Obstet Gynecol Reprod Biol. 2014; 14: 138-39.

92. Fokom-Domgue J et al. Performance of alternative strategies for primary cervical cancer screening in sub-Saharan Africa: systematic review and meta analysis of diagnostic test accuracy studies. BMJ. 2015;351:h33084.

93. Arbyn M et al. Pooled analysis of the accuracy of five cervical cancer screening tests assessed in eleven studies in Africa and India. Int J Cancer. 2008;123:153-160.

94. Damhorst GL et al. Microfluidics and nanotechnology for detection of global infectious diseases. Proceedings of the IEEE. 2015; 103:150-162.

95. Kim E et al. Detection of HIV-1 p24 Gag in plasma by a nanoparticle-based bio-barcode-amplification method. Nanomedicine. 2008; 3: 293. doi:10.2217/17435889.3.3.293.

96. Yang S. Smart phone video microscope automates detection of parasites in blood. Berkeley News. May 2015 (http://news.berkeley.edu/2015/05/06/video-cellscope-automates-detection-of-parasites; accessed 8/7/2015).

97. Kuswandi B et al. Optical sensing systems for microfluidic devices: a review. Anal. Chim. Acta. 2007; 601: 141-155.

98. Shen F et al. Digital PCR on a slipchip. Lab Chip. 2010; 10: 2666-2672.

99. Selck DA et al. Increased robustness of single-molecule counting with microfluidics, digital isothermal amplification, and a mobile phone versus real-time kinetic measurements. Anal Chem. 2013; 85: 11129-11136.

100. Tsaloglou M-N et al. Handheld isothermal amplification and electrochemical detection of DNA in resource-limited settings. Anal Biochem. 2018; 543:116-121.

101. Ornopia GL and Kuramoto K. Detection of anti-hepatitis C virus using chemiluminescence. J Viral Hepat. 1995: 2: 215–19.

102. Gorocs Z and Ozcan A. On-chip biomedical imaging. IEE Rev. Biomed. Eng. 2013: 29-46.

103. Moon S et al. Integrating microfluidics and lensless imaging for point-of-care testing. Biosens Bioelectron: 2009; 24: 3208-3214.

104. Watkins NN et al. Microfluidic CD4+ and CD8+ T lymphocyte counters for point-of-care HIV diagnostics using whole blood. Sci. Transl. Med. 2013; 5: 214.

105. Cheng X et al. Cell detection and counting through cell lysate impedance spectroscopy in microfluidic devices. Lab Chip. 2007; 7: 746-755.

106. Du E et al. Electric impedance microflow cytometry for characterization of cell disease states. Lab Chip. 2013; 13: 3903-3909.

107. Ha S et al. Microfluidic electric impedance spectroscopy for malaria diagnosis. Miniaturized Syst. Chem. Life Sci. 2012; 1960-1962.

108. Gao D et al. Recent advances in microfluidics combined with mass spectrometry: technologies and applications. Lab Chip. 2013; 13: 3309-3322.

109. Schmitz et al. Performance of a methylation specific real-time PCR assay as a triage test for HPV-positive women. Clin Epigen. 2017; 9: 118-125.

ANNEX A

Combined HIV/Syphilis tests – available and pipeline

POC HIV/Syphilis Tests – Available and Pipeline*

2013

Standard Diagnostics

HIV/Syphilis Duo Rapid Test

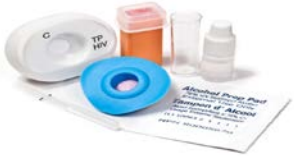
Chembio

DPP® HIV-Syphilis Assay

2014

MedMira

Multiplo Rapid TP/HIV AntibodyTest



CTK Biotech

OnSite™ HIV/Syphilis

2015

Biolytical INSTI Combined HIV/Syphilis Test

2016 - 2018

Premier Medical First Response® HIV 1+2/syphilis

2020

Junco Labs mChip Assay*

Disposable

Device

*Estimated as of May 2018 - timeline may change

---· No market launch date set by company.

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 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, using immunofiltration (RDT)	Rapid Vertical Flow (RVF)
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 *Treponema pallidum* (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	Recombinant TP antigens 15kDa, 17kDa, 47kDa
Sensitivity[1]			
Anti-HIV	100%	98.7%	99.6%
Anti-TP	100%	94.3%	95.8%

[1] As reported by the company in product insert.

Specificity[1]			
Anti-HIV	100%	100%	98.2%
Anti-TP	99.1%	100%	98.0%
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. Serum/Plasma: For optimal results, it is recommended to use fresh specimens. Fresh specimens may be tested immediately upon receipt or stored at 2-8°C for up to 5 days prior to testing. If storage is necessary for than 5 days, serum/plasma specimens should be stored at -20°C 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	**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.

		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 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.
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) Cat. No. 815311005021 – for fingerstick whole blood:** box of 20 pouches each containing: one test cartridge, one InstantGold cap, one auto-fill pipette, one sample tube, one vial Universal Buffer, one lancet (sterile), one alcohol swab, one package insert, and one silica gel packet **Multiplo TP/HIV (LAB+) Cat. No. 815311005138 – for venipuncture whole blood/serum/plasma**: box of 50 pouches

			each containing: one test cartridge, one InstantGold cap, one silica gel packet; and two bottles Universal Buffer (30 mL); 50 sample tubes, 50 transfer pipettes, and one package insert **Multiplo TP/HIV (LAB S/P) Cat. No 815311005145 – for serum/plasma only**: box of 50 pouches each containing: one test cartridge, one InstantGold cap, one silica gel package; and two bottles Universal Buffer (30 mL); 50 transfer pipettes, and one package insert
Not included in test kit			
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 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"	Built-in Control: 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". External Test Controls are available as an

			accessory Cat. No. 815311006074
Regulatory	WHO Prequalified USAID Waiver List CE-IVD Marked	USAID Waiver List CE-IVD Marked	
Estimated pricing	US$ 1.30 – 1.50 per test	US$ 3.50 per test	US$ 2.20 to $4.50 per test. This range is dependent on the packaging format and available volume discount.

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

Test name	**INSTI HIV/Syphilis Multiplex Test**	***OnSite*™ HIV/Syphilis Ab Combo Rapid Test**	**First Response® HIV 1+2/Syphilis Combo Card Test**
Company	BioLytical Laboratories (Canada)	CTK Biotech, Inc. (United States)	Premier Medical Corporation (India)
Type of technology	Immunofiltration (flow through)	Lateral flow chromatographic immunoassay	Lateral flow chromatographic immunoassay
Availability	Commercially available	Commercially available	Commercially available
Output	Qualitative detection of HIV-1 and HIV-2 (combined) and/or syphilis TP	Qualitative detection of HIV-1 and HIV-2 (combined) and/or syphilis TP	Qualitative detection of antibodies (IgG and IgM) specific for HIV 1&2 and/or syphilis
Antigen type (HIV)	Recombinant gp36 (HIV-2) and gp41 (HIV-1)	Unspecified antigens to HIV 1 & 2	Recombinant for HIV 1 (gp41) and HIV 2 (gp36)
Antigen type (syphilis)	Recombinant p17-p47 fusion protein	Unspecified recombinant TP antigens	TP antigen (p24, p45, p17, p15)
Sensitivity[1]			
Anti-HIV	100% (136/136 positive)[c]	99%	100%
Anti-TP	96.5% (55/57 positive)	98.1%	100%
Specificity[1]			
Anti-HIV	100% (874/874 negative)	99.2%	100%
Anti-TP	99.8% (991/993 negative)	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	50 µL	20 µL	20 µL

required			
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 specimens should be stored in refrigeration (2 °C–8 °C) if not tested immediately. The specimens must be tested within 24 hours of collection. Serum or plasma samples may be stored for up to 5 days at 2 °C–8 °C; the specimens should be frozen at -20 °C for longer storage.	Whole blood specimen may be used for testing immediately or may be stored at 2 °C–8 °C for up to 3 days. If serum or plasma specimens are not immediately tested, they should be refrigerated at 2 °C–8 °C. For storage periods greater than 3 days, freezing at -20 °C is recommended up to 4 months.
Time to result	60 seconds, from addition of sample to sample diluent	15 minutes from addition of sample diluent	15 minutes from addition of assay buffer
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	For whole blood, (i) collect specimen (either venous or fingerstick blood) into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®); (ii) when ready to test, open the pouch at the notch and remove the device and place the test device on a clean, flat surface; (iii) label the device with the specimen's ID number; (iv) fill the capillary tube with specimen (about 20 µL) not to exceed the specimen line on the tube; for better precision, transfer specimen using a pipette capable of delivering a 20 µL volume; (v) holding the capillary tube vertically, dispense the entire specimen into the center of the sample well making sure that there are no	For **fingerstick blood**, (i) obtain a fingerstick blood sample according to instructions in package insert using the sterile lancet provided; For **whole blood,** (i) collect the specimen by venipuncture in collection tubes containing anticoagulants like EDTA, Heparin, or Sodium citrate; For **plasma**, (i) collect the whole blood by venipuncture in collection tubes containing anticoagulants like EDTA, Heparin, or Sodium citrate and centrifuge at 3000 rpm for 10 - 15 minutes to obtain plasma; For serum, (i) collect whole blood by

	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	air bubbles; (vi) immediately add 2 drops (60 – 80 µL) of sample diluent to the sample well with the bottle positioned vertically; (vii) read result in 15 minutes. **For plasma**, (i) collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer® by venipuncture; (ii) separate the plasma by centrifugation; (iii) carefully withdraw the plasma into a new pre-labeled tube. When ready to test, continue with step (ii) under whole blood above. **For serum**, (i) collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by venipuncture; (ii) allow the blood to clot; (iii) separate the serum by centrifugation; (iv) carefully withdraw the serum into a new pre-labeled tube. When ready to test, continue with step (ii) under whole blood above.	venipuncture in collection tubes without having any anticoagulants; keep it in a standing position for 30 minutes and centrifuge it at 3000 rpm for 10 – 15 minutes to obtain serum. Following specimen collection,(ii) add one drop (20µl) of capillary/whole blood, serum or plasma to the specimen well using the specimen transfer device; (iii) add 2 drops of the assay buffer vertically to the specimen well; (iv) observe for development of colored lines in the results window; and (v) interpret test results 15 minutes after adding assay buffer

	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		
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	Fifteen minutes; results should not be read after 20 minutes.	Fifteen minutes; should not be read after 25 minutes
Shelf life of test kit	15 months	N/A	N/A
Storage requirements	15 °C–30 °C	2 °C–30 °C (unopened pouches)	4 °C–30 °C for assay buffer (opened and unopened) and for unopened test devices
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)	Individually sealed foil pouches containing: (a) one cassette device and (b) one dessicant; capillary tubes (20 µL); sample diluent (5 mL/bottle); One package insert (instructions for use).	Test device pouch containing 1 test device and 1 dessicant; specimen transfer device; assay buffer bottle; sterile lancets; alcohol swabs; instructions for use.
Not included in test kit	HIV-1, HIV-2, TP and negative controls available	HIV Ab positive control; HIV Ab negative control; Syphilis Ab positive control; Syphilis Ab negative control.	Venipuncture blood collection kit (if whole blood is collected by venipuncture)

Controls	Test has built-in procedural controls that demonstrate assay validity and adequate sample addition	Test has a built-in procedural control.	Test has a built-in procedural control
Regulatory	CE-IVD marked		
Pricing	To be determined	N/A	N/A

N/A = Not available.

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

Test name	**mChip Assay**
Company	Junco Labs and Columbia University in collaboration with OPKO Health, Inc. (United States)
Type of technology	Microfluidics
Availability	Expected to be commercially available in 2018
Output	Qualitative detection of HIV-1, including subtype O, and HIV-2 (combined) and/or syphilis TP and non-treponemal) Quantitative detection of anaemia (haemoglobin)
Antigen type (HIV)	HIV-1 gp41, O IDR, HIV-2 gp36
Antigen type (syphilis)	TP recombinant antigens r17 (treponemal specific) Cardiolipin (non-treponemal specific)
Sensitivity[2]	
Anti-HIV	100% (95% CI, 97–100)
Anti-TP	90% (87–93)

[2] As reported by the company from preliminary studies.

Anti-cardiolipin	95% (92–98)
Anaemia (haemoglobin)	0.2 g/dL (0–25 g/dL measurement range)
Specificity[b]	
Anti-HIV	100% (95% CI, 97–100)
Anti-TP	90% (87–93)
Anti-cardiolipin	95% (92–98)
Anaemia	N/A
Sample type	Whole blood (fingerstick or venous)
Volume of sample required	1 µL
Sample storage	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
Time to result	15 minutes
Protocol complexity – steps required	**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
Read window	N/A; results are shown on smartphone screen and may be stored or sent to cloud
Shelf life of test kit	6 months
Storage requirements	15 °C–30 °C
Test kit components	Single-use sample holders; individually-pouched, single-use microfluidic chips on which reagents are pre-stored; lancet; package insert
Not included in test kit	Dongle; smartphone
Controls	Internal negative and positive control for each test; external quality control kit is available separately

Regulatory	
Pricing	US$ 2 per test, US$ 30 mChip device (dongle)

N/A = Not available.

b As reported by the company from preliminary studies.
c Data from field testing in Bangalore, India, 2012–2013.

ANNEX D
POC STI Tests: available and pipeline

POC STI Tests: Available and Pipeline*

Onco*E6*™
Arbor Vita Corp.
HPV

careHPV™
Qiagen
HPV

GeneXpert®
Cepheid
CT/NG, TV, HPV

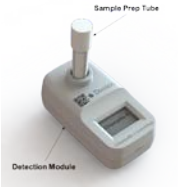
Solana®
Quidel
TV

Truelab™ PCR
Molbio/bigTec
CT, NG, TV, HPV

Vivalytic
Randox/Bosch
CT/NG/TV/panel

Alere™-i
Alere
CT/NG

Diassess Platform
Diassess
CT/NG



GynTect®
Oncgnostics/BLINK
HPV

XenoStrip-Tv™,

OSOM®
RDTs for TV

Atlas io™
Atlas Genetics
CT/NG – 2018
CT/NG/TV - 2019

NEDxA®
GENOMICA
HPV - 2019

GeneXpert®
Omni
CT/NG, HPV

RT CPA
Ustar
CT

Prior to 2015 | 2015 | 2016 | 2017 | 2018 and beyond

*Estimated as of May 2018 - timeline and sequence may change.

---No market launch date set by company.

ANNEX E

POC STI Diagnostics Products Available and in the Pipeline – Summary Tables

STI POC DIAGNOSTICS AVAILABLE AND IN THE PIPELINE
SUMMARY CHART
PLATFORMS FOR CT, CT/NG, TV and HPV

PLATFORM	SYSTEM LEVEL	TECHNOLOGY	CT	NG	CT/NG	TV	HPV
GeneXpert® Cepheid	Multiplex Level 2	PCR-based NAAT	**√** CE-IVD FDA	N/A	**√** CE-IVD FDA	**√** CE-IVD FDA	**√** CE-IVD (FDA, 2018)
Solana® Quidel Corporation	Multiplex Level 2	iNAAT-HDA	N/A	N/A	N/A	**√** CE-IVD FDA	N/A
Onco*E6*™ Assay Arbor Vita Corporation	Singleplex	Lateral flow immunoassay	N/A	N/A	N/A	N/A	**√** CE-IVD
***care*HPV™ System** Qiagen Corporation	Singleplex	NAAT	N/A	N/A	N/A	N/A	**√** CE-IVD
Truelab™ RT micro PCR Molbio	Multiplex Level 2	RT-PCR	**√**	**√**	N/A	**√**	**√**

STI POC DIAGNOSTICS AVAILABLE AND IN THE PIPELINE
SUMMARY CHART
PLATFORMS FOR CT, CT/NG, TV and HPV

PLATFORM	SYSTEM LEVEL	TECHNOLOGY	CT	NG	CT/NG	TV	HPV
Atlas io™ Atlas Genetics	Multiplex Level 1 (possible)	NAAT, immunoassay and small molecule chemistry	N/A	N/A	**2018**	Pipeline	N/A
Vivalytic Randox/Bosch	Multiplex Panels (CT/NG/TV plus 7 others) Level 2	iNAAT	**2018**	**2018**		**2018**	
RT-CPA CT Test Ustar	Multiplex Level 2	iNAAT – CPA	**2019**	N/A	N/A	N/A	N/A
Alere™ i **Platform** Alere Inc.	Multiplex Level 2, Level 1 (possible)	iNAAT – RPA or NEAR	N/A	N/A	N/A	N/A	N/A

STI POC DIAGNOSTICS AVAILABLE AND IN THE PIPELINE
SUMMARY CHART
PLATFORMS FOR CT, CT/NG, TV and HPV

PLATFORM	SYSTEM LEVEL	TECHNOLOGY	CT	NG	CT/NG	TV	HPV
Diassess Platform Diassess	Multiplex Level 2, Level 1 (possible)	iNAAT - instrumentless	Pipeline	Pipeline	N/A	N/A	N/A
Q-POC QuantuMDx Group	Multiplex Level 2	Continuous flow PCR	Pipeline	Pipeline	N/A	Pipeline	Pipeline
GynTect™ ocgnostics/BLINK	Multiplex Level 2, Level 1 (possible)		N/A	N/A	N/A	N/A	**√**
cobas™ Liat Roche	Multiplex Level 1 (possible), Level 2	PCR-NAAT	Unknown which of these assays will be developed by the company				
PanNAT® Micronics, Inc.	Multiplex Level 2	NAAT	Unknown which of these assays will be developed by the company				
IDAlert Alto Bio Reagents	Multiplex Level 1	EEIA	Unknown which of these assays will be developed by the company				

Level 1 – primary healthcare centre; Level 2 – district hospital; N/A = Not applicable

STI POC DIAGNOSTICS AVAILABLE AND IN THE PIPELINE
SUMMARY CHART
PLATFORMS FOR CT and CT/NG

Company	**Cepheid**	**Atlas Genetics**	**Alere**
Assay Name	**GeneXpert®** **CT, CT/NG**	**Atlas io™** **CT/NG (pipeline)**	**Alere™ i** **CT/NG (pipeline)**
Use Setting	Table-top, not portable Level 2	Table-top; portable Level 1	Table-top; portable Level 1
Specimen	Female and male urine, endocervical swab/ patient-collected vaginal swab	Self-collected and clinician-collected vaginal swabs from symptomatic and asymptomatic females, and urine from males	Female and male urine, endocervical swab/ patient-collected vaginal swab
Steps	~4; sample prep automated	~4; automated sample prep on instrument	~6 simple steps; raw sample added to device
Time to result	~90 minutes	30 minutes	
Cold Chain; Reagent stability	No; TBD	Cartridges with reagents stable at 2 – 25C	No; >12 months
Power	Mains power required; solar power possible	Mains power required	AC mains and DC from external AC/DC supplied plug pack
Training	Less than ½ day	Less than one hour; no formal training required; self-explanatory user guide and screens on instrument	Less than ½ day

STI POC DIAGNOSTICS AVAILABLE AND IN THE PIPELINE
SUMMARY CHART
PLATFORMS FOR CT and CT/NG

Connectivity	Yes; computer/Internet required; remote calibration	Yes, via middleware	Yes; USB and Ethernet outlets
Equipment Cost ($US); Per test cost	~$17,000 (with 4 modules), but could be higher; $16.20 (CT/NG)	TBD	TBD

Level 1 – primary healthcare centre; Level 2 – district hospital; N/A – Not available; TBD = To be determined

STI POC DIAGNOSTICS AVAILABLE AND IN THE PIPELINE
SUMMARY CHART
PLATFORMS FOR CT and CT/NG

Company	**Molbio/bigTec**	**Ustar**
Assay Name	**Truelab™ PCR** **CT, NG**	**RT CPA HIV-1 Viral Load** **CT (pipeline)**
Use Setting	Level 2; 2 instruments; not portable	Level 2
Specimen	TBD	TBD
Steps	Multiple pipetting steps	~3 – 5 steps from sample to result
Time to Result		
Cold Chain; Reagent Stability	No; 3 months at temperatures to 40C	
Power	Rechargeable Lithium ion battery	Mains power or rechargeable battery
Training	Less than ½ day	Approximately ½ day
Connectivity	Yes; wireless connectivity	Will be used with Genie® device; TBD
Equipment Cost ($US); Per test	~$8,000; TBD	<$5,000; TBD

Level 1 – primary healthcare centre; Level 2 – district hospital; N/A – Not available; TBD = To be determined

STI POC DIAGNOSTICS AVAILABLE AND IN THE PIPELINE
SUMMARY CHART
PLATFORMS FOR TV

Company	**Cepheid**	**Quidel**	**Atlas Genetics**
Assay Name	**GeneXpert®**	**Solana®**	**Atlas io™**
Use Setting	Table-top, not portable Level 2	Table Top; not portable Level 2	Table Top; portable Level 1
Specimen	Female and male urine, endocervical swab/, patient-collected vaginal swab	Vaginal swabs and female urine specimens obtained from symptomatic and asymptomatic females	Self-collected and clinician-collected vaginal swabs from symptomatic and asymptomatic females, and urine from males
Steps	~4; sample prep automated	Moderately complex; 13 steps	~4; automated sample prep on instrument
Time to result	~60 minutes	35 minutes Batch up to 12 samples in a single run	30 minutes
Cold Chain; reagent stability	Kit storage: 2 – 28C	Kit storage: 2 to 8C	Cartridges with reagents stable at 2 to 25C
Power	Mains power required; can use solar	Mains power required for heat block and Solana instrument	Mains power required
Training	Less than ½ day	Less than ½ day	Less than one hour; no formal training required; self-explanatory user guide and screens on instrument

STI POC DIAGNOSTICS AVAILABLE AND IN THE PIPELINE
SUMMARY CHART
PLATFORMS FOR TV

Connectivity	Yes; computer/Internet required; remote calibration	Yes; bi-directional	Yes, via middleware
Equipment Cost ($US); per test	~$17,000 (with 4 modules, but could be higher; $19.00	TBD	TBD

Level 1 – primary healthcare centre; Level 2 – district hospital; N/A – Not available; TBD = To be determined

STI POC DIAGNOSTICS AVAILABLE AND IN THE PIPELINE
SUMMARY CHART
PLATFORMS FOR HPV

Company	**Cepheid**	**GENOMICA S.A.U.**
Assay Name	**GeneXpert®**	**NEDxA** **(Pipeline)**
Use Setting	Table-top, not portable Level 2	Table-top Level 2
Specimen	Female endocervical swab	Female endocervical swab. From June, ThinPrep and SurePath™ liquid samples.
Steps	~4; sample prep automated	~4; no sample prep
Time to result	~60 minutes	~75 minutes
Cold Chain; reagent stability	Kit storage: 2 – 28C	Between 4 and 25C
Power	Mains power required; can use solar	Mains power required. Low power consumption.
Training	Less than ½ day	No training needed
Connectivity	Yes; computer/Internet required; remote calibration	Yes; compatible with LIMS systems
Equipment Cost ($US); per test	~$17,000 (with 4 modules), but could be higher; $16.70	7.500€ per instrument. 16-20€ per cartridge (per sample including 14 targets);

		pricing is volume-based.

Level 2 – district hospital