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

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Introduction

Sexually transmitted infections (STIs) continue to be a significant global public health issue, with an estimated 378 million people becoming ill in 2016 with one of 4 STIs: syphilis, *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Trichomonas vaginalis* (TV) *(1)*. In addition, more than 291 million women have a human papillomavirus (HPV) infection, which 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 infrastructure and well-trained laboratory technicians. In addition, test turnaround time (TAT) 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 (HCV) 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 The material in this landscape was gathered by the author from publicly available information, published and unpublished reports and prospectuses, and interviews with developers and manufacturers.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 30 June 2019.

[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

WHO estimates that in 2016, the most recent year for which such statistics are available, there were approximately 6 million new cases of syphilis worldwide *(1)*. The highest disease burden for syphilis is in the Africa region *(3,4)*.

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 2016, researchers estimated that approximately 661,000 cases of syphilis occurred worldwide among pregnant women, many of whom were either untreated or inadequately treated *(5)*. It was estimated that about 355,000 adverse pregnancy outcomes, including 143,000 early fetal deaths and stillbirths, 61,000 neonatal deaths, 41,000 preterm or low weight births, and 109,000 infected infants resulted *(5)*. Although these results are an improvement over the 2012 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 *(6)*.

With respect to MSM, in 2017, WHO estimated that syphilis infects 5% or more of MSM in 22 of 34 reporting countries, 10% or more in 12 countries, and more than 20% in 3 countries *(7)*. In the United States, the United States Centers for Disease Control and Prevention (CDC) estimated that in 2017, 79.6% of syphilis cases were among MSM and the numbers are increasing *(8)*. Untreated, syphilis can lead not only to serious complications, but it also increases the risk of acquiring and transmitting HIV.

Finally, per WHO, syphilis infected more than 5% of sex workers in 18 of 31 countries and more than 10% in 12 countries in 2017 *(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 Abbott Laboratories, Inc. (Alere Determine™), Standard Diagnostics (SD Syphilis 3.0), The Tulip Group/Qualpro (Syphicheck® - WB), Cypress Diagnostics (Syphilis Rapid Test), and Omega Diagnostics (Visitect® Syphilis), all of which have been the subject of peer-reviewed, published evaluations.[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, recently, the WHO began accepting applications to prequalify syphilis only RDTs *(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 (Abbott Laboratories, 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 SD Bioline (Abbott)	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	90.04% (80.45, 95.21)
		Specificity	94.15% (89.26, 97.66)
	Whole Blood	Sensitivity	86.32% (77.26, 91.70)
		Specificity	95.85% (92.42, 97.74)
SD Syphilis 3.0	Serum	Sensitivity	87.06% (75.67, 94.50)
		Specificity	95.85% (89.89, 99.53)
	Whole Blood	Sensitivity	84.50% (78.81, 92.61)
		Specificity	97.95% (92.54, 99.33)
Syphicheck® - WB	Serum	Sensitivity	74.48% (56.85, 88.44)
		Specificity	99.14% (96.37, 100.0)
	Whole Blood	Sensitivity	74.47% (63.94, 82.13)
		Specificity	99.58% (98.91, 99.96)
Visitect® Syphilis	Serum	Sensitivity	85.13% (72.83, 92.57)
		Specificity	96.45% (91.92, 99.29)
	Whole Blood	Sensitivity	74.26% (53.62, 83.68)
		Specificity	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. 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^{o}C). TAT 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 with respect to the performance of the DPP assay, including a metaanalysis by Marks et al (*17*). Each of the studies found that the test performed favorably against laboratory-based reference tests. In a multi-center field study in China by Yin et al, 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 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 by Castro et al, 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 that they 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 *(32)*.

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 seven combination HIV/syphilis (TP) RDTs on the market: SD Bioline HIV/Syphilis Duo Rapid Test (Abbott), DPP® HIV-Syphilis Assay (Chembio Diagnostics Systems, Inc., USA), Multiplo Rapid TP/HIV Antibody Test (MedMira, Inc., Canada), INSTI™ HIV/Syphilis Multiplex Test (biolytical Laboratories Inc., Canada), *OnSite*™ HIV/Syphilis Ab Combo Rapid Test (CTK Biotech Co, USA), First Response® HIV 1 +2/ Syphilis Combo Card Test (Premier Medical Corporation Private Limited, India), and STANDARD Q HIV/Syphilis Combo (BD Biosensor, Republic of South Korea).

Of the seven tests, five of them – the SD Bioline, Chembio, MedMira, biolytical, and SD Biosensor assays– have been the subject of published, independent laboratory evaluations. In the first of these evaluations, Humphries et al evaluated the comparative performance of three tests (SD Bioline, Chembio and Multiplo) in the United States using sera specimens (*33*). 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 ADVIA HIV EIA (Siemens, USA). 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" *(33).*

Yin et al conducted a second simultaneous evaluation of the same three dual RDTs at three laboratories in China and Nigeria (*34*). 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 (Abbott) *(34)*. 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%) *(34)*.

Finally, as part of the WHO pre-qualification of diagnostics assessment program, Van Den Heuvel et al evaluated the performance of the SD Bioline, Chembio, MedMira and biolytical dual HIV/syphilis assays at the Institute of Tropical Medicine (Antwerp, Belgium) (*35*). Each of the four assays was evaluated using the same characterized evaluation panel of 400 sera or plasma specimens from a variety of geographies. The authors found excellent sensitivities and specificities for HIV, ranging from 99.5 to 100% and from 93.5 to 99.5%, respectively. Results for the TP antibodies were not as good. Although specificities ranged from 99.0 to 100%, sensitivities ranged from 73.5% to 87.0%. Nonetheless, the authors concluded that the tests could be introduced into screening programs to increase the accessibility of HIV/syphilis diagnosis and treatment for hard to reach populations.

The performance of the assays as determined by the studies above are summarized below. Each of the evaluations of the RDTs was conducted in a laboratory setting with trained users.

RDT	Sample	Parameters	Performance (95% CI) HIV Antibody	Performance (95% CI) TP Antibody
SD Bioline HIV/Syphilis Duo Rapid Test (Abbott)	Sera/Plasma	Sensitivity Specificity	97.9% (92.0 – 99.6) 99.0% (98.8 – 99.9) 100% (98.2-100) 100% (91.5 – 100) 99.0% (98.0 – 99.5) 99.5% (97.2 – 100)	93.0% (84.8 - 97.1) 99.6% (95.0 – 97.7) 87.0% (81.5 – 91.3) 100% (92.9 - 100) 99.1% (98.2 – 99.6) 99.5% (97.2-100)
DPP® HIV-Syphilis Assay (Chembio Diagnostics Systems, Inc.)	Sera/Plasma	Sensitivity Specificity	98.9%(93.6 – 99.9) 99.6% (98.8 – 99.9) 100% (98.2 – 100) 98.1% (88.6 – 99.9) 97.9% (96.7 – 98.7) 97.5% ((94.3 - 99.2)	95.3% (87.9 – 98.5) 97.0% (95.5 – 98.0 86.5% (81 – 90.9)) 100% (92.9 - 100) 99.6% (98.9 – 99.9) 100% (98.2 – 100)
Multiplo Rapid TP/HIV Antibody Test (MedMira, Inc.)	Sera/Plasma	Sensitivity Specificity	97.9% (92.0 – 99.6) 99.5% (99.4 – 99.8) 99.5% (97.2 – 100) 94.2% (83.1 – 98.5) 98.3% (97.2 – 99.0) 99.5% (97.2 – 100)	94.1% (86.3- 97.8) 94.2% (92.3 – 95.7) 73.5% (66.8 – 79.5) 96.9% (88.2 – 99.5) 99.1% (98.2 – 99.6) 99.5% (97.2 – 100)
INSTI™ Multiplex HIV-1/HIV-2/Syphilis Antibody Test (biolytical Laboratories, Inc.)	Sera/Plasma	Sensitivity Specificity	N/A N/A 99.5% (97.2 – 100) N/A N/A 93.5% (89.1 – 96.5)	N/A N/A 81.0% (74.9 – 86.2) N/A N/A 99.0% (96.4 – 99.9)

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

In each case, the first performance line shows results from a laboratory evaluation in the US (Humphries *et al* [2014]); the second performance line shows results from a laboratory evaluation at 3 sites, 2 in Nigeria and 1 in China (Yin *et al* [2015]); and the third line shows results for a laboratory evaluation at the Institute of Tropical Medicine in Antwerp, Belgium (Van Den Heuvel *et al* [2019]). The *OnSite*™, First Response and STANDARD Q assay were not evaluated in any of these comparative studies.

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 *(34)*.

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 *(36)*. 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 *(36)*. 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 *(36)*.

Each of the seven 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 (Abbott, Inc.)

One of seven HIV/syphilis rapid diagnostic tests currently on the market is the SD Bioline HIV/Syphilis Duo Rapid Test from Abbott (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 *(37)*, Uganda *(38)*, Ethiopia *(39)*, Peru *(40)*, Haiti *(41)*, Nepal *(42)*, and the United States *(42)*. 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 *(43)*.

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. TAT 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) (*44*). 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% *(44)*. 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 *(45)*. 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 *(45)*.

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

The Multiplo Rapid TP/HIV Antibody Test from MedMira, Inc. (POC format pictured below) is a 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. 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. TAT is approximately three minutes.

Figure 4. Multiplo Rapid TP/HIV Antibody Test

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 (*46*). The estimated sensitivity of the HIV component of the Multiplo test was 100% with a 95% CI of 95.1% - 100%, and specificity was estimated to be 91.9% (95%CI, 85.7% - 96.1%) (*46*). 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%) (*64*). 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 (*47*). 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%) *(47*).

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. TAT is about 60 seconds.

Figure 5. INSTI™ HIV/Syphilis Multiplex Test

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 *(48)*. 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 *(48)*. 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%) *(48)*.

STANDARD Q HIV/Syphilis Combo (SD Biosensor)

The STANDARD Q HIV/Syphilis Combo test, pictured below, is a qualitative, lateral flow assay to detect antibodies specific to HIV-1, HIV-2, and syphilis (TP) in serum, plasma and whole blood using immunochromatography. TAT is approximately 15 minutes.

Figure 6. STANDARD Q HIV/Syphilis Combo Test

Recently, Bristow et al conducted an evaluation of the STANDARD Q HIV/Syphilis Combo Test in a laboratory setting in Peru using 400 stored sera specimens *(49)*. The sensitivity and specificity of the assay for HIV antibody detection was 100.0% (95%CI: 98.2 – 100) and 99.5% (95%CI: 97.2- 100.0), respectively. The sensitivity and specificity for TP antibody detection was 97.5% (95%CI: 94.3 – 99.2) and 100.0% (95%IC: 98.2 – 100), respectively.

***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 7. *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 Private Limited)

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 seven assays described above are detailed in **Annex B**. The SD Bioline, INSTI, DPP®, and Multiplo, assays are CE-IVD marked. Standard Q HIV/Syphilis Combo Test currently has an active application for WHO prequalification, but to date, only the SD Bioline and First Response® HIV 1 +2/ Syphilis Combo Card Test assays have been prequalified.

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 *(50)*. No commercial launch date for the HIV/syphilis TP assay has been set.

Figure 8. 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.[4]

Chlamydia trachomatis*, *Neisseria gonorrhoeae

Both CT and NG are significant global health problems. The WHO estimates that in 2016 approximately 127 million and 87 million new cases of CT and NG, respectively, were diagnosed worldwide *(1).* In addition, antimicrobial resistance (AMR) in NG is particularly problematic. With resistance to both cephalosporins, including third generation extended spectrum cephalosporins, as well as fluoroquinolones, NG is a multidrug-resistant pathogen. Resistance appears to be outpacing new antibiotics for NG. As a result, WHO considers NG to be a priority organism for AMR monitoring in the Global Antimicrobial Surveillance System (GLASS) and for drug development in the context of AMR *(51,52)*.

Traditional CT and NG testing utilizes culture or serological techniques; however, nucleic acid 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) (USA), Hologic (USA), Roche Diagnostics (USA), and others. In numerous studies, the performance of the laboratory-based tests for both CT and NG has been shown to be good (*53*). It should be noted, however, that currently available assays for laboratory-based molecular testing platforms for NG do not detect antimicrobial resistance.

The laboratory-based NAAT 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, including genotypic or phenotypic antimicrobial susceptibility testing (AST) for NG, the results of which can be delivered in a single patient visit *(54,55)*.

[4] The TPP for an 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.

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 (Abbott, USA), 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 (*56*).

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 (*56*).

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 *(57)*.

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 (*58*) and NG ACON Plate (ACON Laboratories, USA) (*58*), BioStar Optical ImmunoAssay-Gonorrhea (BioStar, Inc., USA) (*59*), and the One Step Gonorrhea RapiCard InstaTest (Cortez Diagnostics, USA) (60). 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 (*53*) and Abbai et al (*60*).

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% (*58*). 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 (*60*).

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 *(53,56,60,61,62)*. The need is particularly acute with respect to women, where the syndromic approach to managing STIs is inadequate *(63)*. In particular, NAAT-based platforms, including platforms capable of AMR testing, 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 (Cepheid, a subsidiary of Danaher Corporation, USA), the the Truelab Real Time micro PCR system (Molbio Diagnostics Pvt. Ltd., India*)*, 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, as well as 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 9. 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, patient-collected vaginal swab (collected in a clinical setting), and rectal and pharyngeal swab specimens. 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 (*64,65,66,67*).

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 *(68)*.

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 (*69*); a combined call out for genotypes 18 and 45 -types also closely associated with invasive cervical cancer (*70*); 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 (*71,72*) and colposcopy referral populations (*73,74*) 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 *(75)*. With the flexibility of the GeneXpert System, Xpert® HPV offers rapid and accurate results within the laboratory as well as near-patient settings, 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 (*76*). 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 (*77*).

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 LIMS system. In addition, Cepheid's C360 platform provides epidemiology and systems monitoring functionality that allows users 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.

Arguably, the GeneXpert® platform is best used at district hospitals and above in the tiered laboratory system in-country. While the GeneXpert platform can be used at tiers such as health centers and below,

it is not as well suited there for reasons including the need for stable electricity, temperature conditions and calibration requirements. However, the GeneXpert® has been used quite successfully at remote Aboriginal healthcare facilities in Australia and many other decentralized settings (*78,79,80*).

Additionally, GeneXpert® Edge, launched in 2018, is Cepheid's new offering based on the existing GeneXpert instrument family to move molecular testing beyond the laboratory. By including an easy-to-use touch-screen workflow, external battery pack and dust filter, GeneXpert® Edge enables testing in challenging environments.

Truelab™ Real Time micro PCR System

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 10. 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

Bosch Healthcare Solutions (Germany) 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. In mid-2019, it was announced that aprimeo diagnostics, a R-Biopharm company, will execute all activities regarding the commercialization of the Vivalytic platform in several European countries.

Figure 11. 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 through aprimeo diagnostics in Germany, Austria, Switzerland, BeNeLux, France and all Nordic countries beginning in the 3rd quarter of 2019.

Figure 12 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), *Ureaplasma urealyticum* (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 9x9 mm 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 provides 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.

binx io® system (*binx health, inc., formerly Atlas Genetics, UK*)

The binx io® platform is a rapid, multiplex, molecular diagnostic system that can deliver laboratory quality results in about 30 minutes. The system consists of a small instrument and disposable cartridge, pictured below, that contains all reagents necessary to run a test, is designed to be easy to use, and is fully automated. The operation of the instrument is designed to be simple and intuitive; the user interacts with the instrument through a touchscreen interface which then guides the user through the io® system test process. Once the raw sample has been added to the cartridge and loaded into the instrument, no further interaction is required. The instrument fits easily on a bench-top and is fully integrated enabling the movement of a sample and reagents within the cartridge.

The cartridge has three main assay steps: sample preparation to isolate and purify target DNA, ultra-rapid PCR, which amplifies specific regions of DNA from the target organisms, and a proprietary electrochemical detection to identify the presence of amplified DNA. Once the test is completed, a qualitative 'Detected/Not Detected' result is available with no clinical or laboratorian interpretation needed.

Figure 13. binx io® instrument (left) and cartridge (right)

binx health's core focus on STIs leads with their first application to diagnose two of the most tested for STIs globally: CT and NG. The binx test is designed to provide a result directly from an unpurified patient sample in about 30 minutes with equivalent accuracy and performance as current standard of care platforms run in central laboratories (which can take seven or more days). The goal is to help provide earlier diagnosis and accurate detection and ultimately more timely treatment in order to aid in the prevention of onward transmission and the serious consequences that go with undiagnosed and prolonged infection. The company believes bringing this platform to patients will help to create a new model in caring for infections that are of epidemic proportions globally.

The dual target CT/NG assay received CE marking for use within Europe in April 2019 and received FDA 501(k) clearance in August 2019. No published, peer-reviewed data on performance of the CT/NG assay were found.

binx continues to develop additional targets to add to its test menu, including an expansion of its current CT/NG multiplex test to include two other STIs with rapidly increasing prevalence: TV and MG. In addition, the company is also developing a NG resistance assay to detect Ciprofloxacin-sensitive strains. This work, which is funded by the NIHR and in collaboration with St. Georges Hospital in London, will allow greater antibiotic stewardship and open the breadth of treatments available to address this major public health crisis.

The io® cartridge is manufactured in the UK by Bespak (part of the Consort Medical Group) and the instrument by LRE Medical (Germany).

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 8 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 14. GeneXpert® Omni Platform

The GeneXpert Omni® platform will use a dedicated mobile device to control each test module. The provided mobile device can control up to 3 Omni instruments, thus providing scalability and flexibility. The platform will also use a secure, hosted platform that collects and aggregates real-time test and test telemetry 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 Ultra, Xpert® MTB/RIF, Xpert® HIV-1 Qual, Xpert® HIV-1 Viral Load, Xpert® HCV Viral Load 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, China*)

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[5], NG, UU, and Herpes Simplex Virus (I & II).

Figure 15. 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 2019 The company expects to turn to its CT assay subsequent to that. As a result, the CT assay will not be available until after 2020.

SAMBA Platform (*Diagnostics for the Real World, UK*)

SAMBA has been developed by a team led by Dr Helen Lee, Director of the Diagnostics Development Unit (DDU) at the University of Cambridge. Diagnostics for the Real World Ltd (DRW), the spinout company of DDU, located in California, is the manufacturer of the SAMBA system. SAMBA manufactures two NAAT-based platforms: SAMBA I, an automated batch testing system, and SAMBA II, a sample-in,

[5] 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.

result-out system. The SAMBA II, pictured below, is comprised of an assay module and a tablet/display module, which are linked via Bluetooth.

Figure 16. SAMBA II System: Assay module (left) and tablet/display module (right)

Each Tablet Module can control up to 4 Assay Modules, which means the system can be tailored to the needs of specific healthcare facilities. In addition, Assay Modules can be added to the system if additional throughput is required. Consistent electricity is required.

There are currently two assays available for the SAMBA II system: (i) HIV-1 Qualitative Whole Blood test (for early infant diagnosis and diagnosis of acute HIV infection, and (ii) HIV-1 Semi-Quantitative Plasma test (for HIV monitoring). A combined CT/NG assay is in the pipeline. An expected launch date has not been set.

ID NOW™ Platform *(Abbott, Inc.)*

The ID NOW™ platform, formerly the Alere™-I platform, (pictured below) from Abbott is an instrument-based, molecular diagnostic test utilizing isothermal nucleic acid amplification technology (iNAAT) for the qualitative detection of 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 ID NOW™ 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. Abbott 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 ID NOW™ platform may use various iNAAT technologies.

Figure 17. ID NOW™ Platform

ID NOW™ 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. ID NOW™ Strep A was launched in 2015, and with test results in 8 minutes or less, is the fastest CLIA-waived molecular Strep A test. ID NOW™ 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. Abbott received 510k clearance and CLIA waiver of ID NOW™ Strep A 2 and ID NOW™ Influenza A & B 2, and both assays were launched in 2018. ID NOW™ 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.

Accula™ System (*Mesa Biotech, Inc., USA*)

Mesa Biotech has developed a sample-in, result-out, easy-to-use PCR testing platform designed specifically for diagnosis of infectious disease at POC. Its Accula™ System is comprised of the Accula Dock, pictured below, a palm-sized, reusable dock and single-use, disposable test cassettes. The Accula Dock is a semi-automated, colorimetric, multiplex RT-PCR NAAT instrument that integrates nucleic acid extraction, reverse transcription, and amplification using a novel Mesa Biotech technology, as well as hybridization-based visual detection into a self-contained and automated system. TAT for existing assays is approximately 30 minutes.

Figure 18. Accula Dock and Cassette

Currently, Mesa Biotech offers two assays, the Accula Flu A/Flu B and respiratory syncytial virus (RSV), both of which are CE-IVD marked and have obtained 501(k) clearance and CLIA waiver from the FDA. The company has a CT/NG/TV assay in its pipeline. No expected launch date is available.

HG Swift (*Hibergene Diagnostics, Ireland*)

Hibergene Diagnostics manufactures the HG Swift instrument, pictured below, for the diagnosis of infectious disease. It is lightweight and compact. The instrument uses isothermal amplification technology, along with dual-channel fluorometric detection and unique results-calling software. The instrument can simultaneously detect across 8 tubes in 2 fluorescent channels. The system features an integrated touchscreen interface and provides real-time display of amplification. The system can be run with mains or battery power. TAT is approximately 40 minutes.

Figure 19. HG Swift Instrument

Currently, Hibergene offers seven assays for the HG Swift system: HG Flu A/B *Combo*, HG *Mycoplasma pneumoniae*, HG Pneumo/Meningo *Combo*, HG RSV A/B *Combo*, HG *C. difficile*, HG Meningococcus, and HG Group B Streptococcus. In the near-term pipeline is a CT/NG combo assay.

ResistancePlus® GC (SpeeDx Pty, Ltd, Australia)

SpeeDx is a private company that manufactures molecular IVD assays, including the *ResistancePlus*® GC assay (pictured below), which is a multiplex qPCR test for the detection of GC and sequences in the *gyr*A gene of the bacteria associated with susceptibility or resistance to ciprofloxacin from a variety of specimen types. Results from the test can be used to guide treatment decisions for gonorrhea infections, giving doctors and patients the option of avoiding the use of ceftriaxone, one of the last remaining antibiotics available for multi-drug resistant infections. Management guidelines in the UK have already recognized the important of utilizing resistance or susceptibility information (resistance guided therapy) when managing infections such as gonorrhoea, opting for preferential use of ciprofloxacin if susceptibility information is available prior to treatment.

SpeeDx reports sensitivity of 96.9% and 99.7% specificity for GC detection using *ResistancePlus*® GC, and sensitivity/specificity of 100%/98.6% for *gyr*A detection. The assay, which is CE-IVD marked, is currently validated for use on two laboratory-based systems: the LightCycler 480 II (Roche, USA) and the ABI 7500 (Thermo Fisher Scientific, USA), with CFX 96 (BioRad, USA) available later in 2019. Although the *ResistancePlus*® GC assay is currently only validated on laboratory-based systems, it could be adapted and validated on POC or near-POC systems.

Figure 20. SpeeDx *ResistancePlus*® GC assay

In addition to the *ResistancePlus*® GC assay, SpeeDx offers the following:

- *ResistancePlus*® MG – detects MG and five macrolide resistance markers from male and female urine and swab specimens, and is validated for use on the LightCycler 480 II (Roche, USA), CFX 96 (BioRad, USA) and the ABI 7500 (Thermo Fisher Scientific, USA). A platform-specific version will also be available in September 2019, for use on the GeneXpert system, a near POC platform discussed earlier in this report.

- *PlexPCR*® VHS – detects and differentiates HSV-1, HSV-2, Varicella Zoster (VZV) and TP from genital and non-genital swabs;
- *PlexPCR*® HSV-1&2, VZV – detects and differentiates HSV-1, HSV-2, and VZV from cutaneous and mucocutaneous lesion swab specimens; and
- *PlexPCR*® RespiVirus – detects 14 targets in 2 wells, representing 11 respiratory viruses, including, Influenza A, Influenza B, Rhinoviruses (A & B), and Respiratory Syncytial Viruses (A & B).

With the exception of the *PlexPCR*® RespiVirus, all of the above assays are CE-IVD marked.

SpeeDx assays utilize qPCR *PlexZyme*® technology during which primers amplify target nucleic acid sequences and produce amplicons that serve as a template for *PlexZyme*® formation. *PlexZyme*® enzymes are catalytic DNA complexes that assemble in the presence of target and cleave universal probes. An active *PlexZyme*® is made up of two DNA oligonucleotide components called partial enzymes or "partzymes". The bi-specificity of the *PlexZyme*® activity minimizes the chance of false positive signals, and the use of universal probes allow for robust and consistent reaction performance. Once the two partzymes have assembled into *PlexZyme®* enzymes, universal probes bind and enzymatic cleavage of the probes between fluorophore and quencher dye pairs generates fluorescence. Changes in fluorescence allow detection and/or quantification of the target nucleic acid in real time. The methodology is both sensitive and highly specific.

In order to detect and/or characterize a suite of mutations linked to antibiotic resistance or sensitivity, *PlexPrime®* technology is incorporated into the SpeeDx *ResistancePlus®* range of assays. *PlexPrime®* is a novel method for nucleic acid amplification that creates amplicons that are distinctly different from the parent sequence. The combination of *PlexPrime®* and *PlexZyme*® technology enables multiplex mutation detection with high sensitivity and specificity.

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 *(82)*. 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 collected and analyzed via desktop computer or a Bluetooth-enabled smartphone that allows the user to control the testing with an app. Because the diagnostic analysis is automated, it does not require a lab technician to process results.

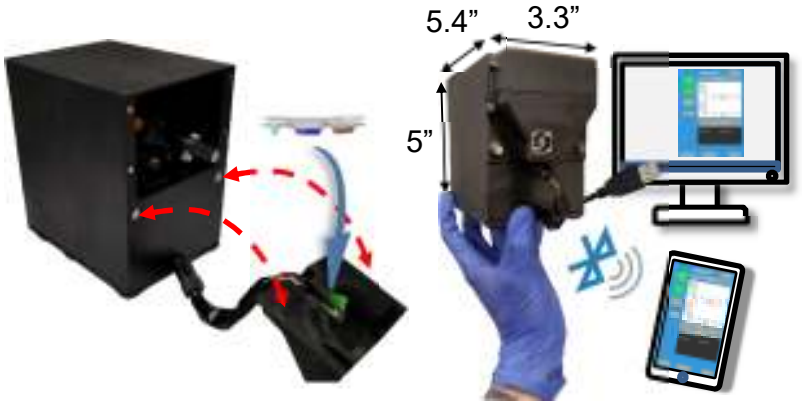

Figure 21. MobiNAAT Platform

The MobiNAAT instrument contains a built-in fluorescence detector with dual target multiplexing capabilities. Each cartridge comes pre-loaded with all necessary reagents for sample purification and nucleic acid amplification with a total cost around $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. An additional NG MobiNAAT cartridge assay for both detection and antibiotic susceptibility testing is currently undergoing validation at Baltimore City Health Department STD clinics.

Trichomonas Vaginalis

Like CT, NG and syphilis, TV is a significant health problem globally. The WHO estimates that there were about 156 million new cases of TV worldwide in 2016 *(1)*. 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 *(83,84)*. Diagnosis of TV in men is typically from wet mount with microscopic visualization of the parasites on slide preparations from urethral secretions *(85)*. 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, USA), which studies have shown performed reasonably well when compared to wet mount and culture *(86,87)*.

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, binx

health has a combined CT/NG/TV assay in its development pipeline for the io® platform. Finally, Quidel Corporation (Quidel) has launched a near-POC TV assay for its Solana® platform, which is described below.

Solana® (*Quidel Corporation, USA*)

Quidel develops and markets immunoassay and molecular diagnostic platforms and assays with a focus on point-of-care testing. Immunoassay platforms include Sofia for rapid respiratory virus and other infectious disease tests and the Triage MeterPro for cardiovascular and toxicology tests. Molecular platforms include AmpliVue a multiplex isothermal NAAT platform for low-volume settings, Solana®, a moderate throughput benchtop system, and Lyra reagents which are higher throughput assays designed to run on the Thermo Fisher 7500 Fast Dx and QuantStudio Dx real-time PCR instruments.

Of interest with respect to tests for TV is Quidel's Solana® platform, pictured below. Quidel offers nine different molecular assays with 15 different pathogens on Solana®, a compact benchtop instrument (9.4" x 9.4"x 5.9") featuring the company's proprietary helicase-dependent amplification (HDA) technology. HDA uses a helicase enzyme to unwind double-stranded DNA into single strands, eliminating the need for a thermocycler. Unlike other isothermal amplification methods, HDA uses a probe-based detection method, resulting in greater specificity. In addition, HDA permits the multiplexing of multiple pathogens in a single reaction tube.

Figure 22. Solana® Platform from Quidel

The Solana® platform can process individual samples or batches up to 12 samples in a single run enabling cost effective testing across a wide range of daily testing volumes. 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. The Solana® platform is designated as moderately complex by the US FDA.

The Solana® TV assay allows for rapid detection of TV from vaginal swabs and female urine specimens obtained from symptomatic and asymptomatic females. TAT for the test is 35 minutes. The workflow for the Solana® is detailed below:

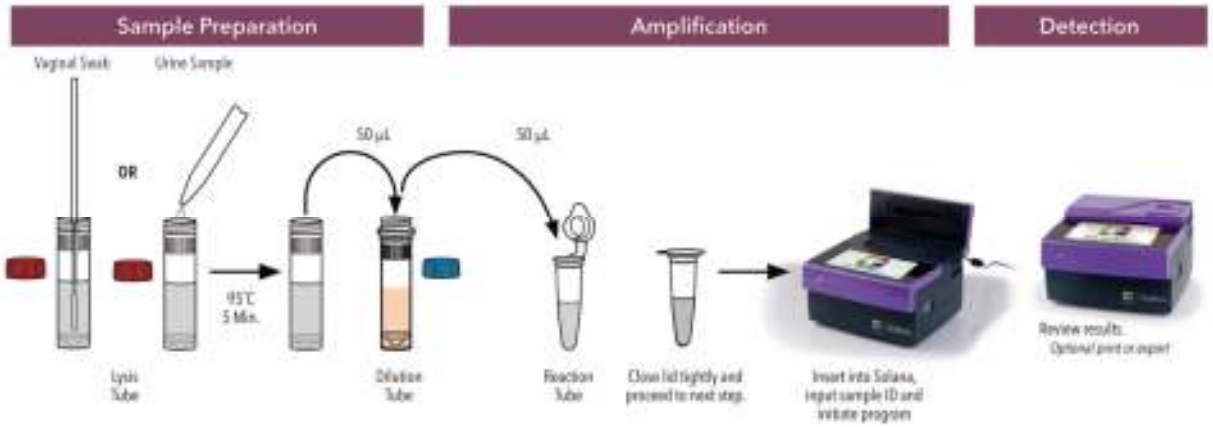

Figure 23. Typical Workflow on Solana® Platform

The Solana® TV assay has been independently evaluated and performed well compared to the reference assay *(88)*. Gaydos et al collected vaginal swabs and urine specimens from 501 asymptomatic and 543 symptomatic women *(88)*. 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 *(88)*.

Solana® also features a qualitative assay for the simultaneous detection and differentiation of HSV 1, HSV 2 and VZV from cutaneous and mucocutaneous lesion samples obtained from symptomatic patients. TAT for this test is 50 minutes.

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) should be the first line of screening in the United States.[6] 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 RealTi*m*e High Risk HPV test from Abbott, the QIAGEN *digene* HC2 HPV Test (QIAGEN, N.V., Germany), or the Onclarity™ HPV assay on the BD Viper™ LT System.[7]

[6] 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.

[7] A recent technology landscape, including HPV testing, in the context of cervical cancer has been published and covers laboratory-based platforms in more detail. See: https://unitaid.org/assets/Cervical_Cancer_Technology-landscape-2019.pdf.

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 24. 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 *(89)*. 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: 16 - 50) and 99.4% (95%CI: 97 - 100), respectively, for CIN2+ detection *(90)*. 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 *(91)*.

_care_HPV™ System

The *care*HPV™ Test, which recently was added to the WHO list of prequalified *in vitro* diagnostics (IVDs), 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. A multicenter clinical study conducted to validate the *care*HPV Test reported a clinical sensitivity of 88% and clinical specificity of 85% for CIN2+, although results may vary depending on specific patient populations, including slightly lower sensitivity and specificity in women living with HIV/AIDS *(91)*.

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 25. _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 materials 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+ (*91*). 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 (91).

Performance of the *care*HPV assay has been good relative to testing using either the Pap test or VIA *(91,92,93,94)*. The assay has also performed better than cervical ctytology *(94)*. Kelly et al conclude that the *care*HPV test performs as well as visual inspection and cervical cytology *(94,95)*, and has the advantage of allowing for self-sampling *(91)*.

Additional Technologies in the Pipeline for STIs

Q-POC™ (QuantuMDx Group, UK)

The QuantuMDx Group is developing a small benchtop diagnostic device, the Q-POC™, test cassette and Sample Caddy (pictured below) that can deliver sample-to-answer results in less than 30 minutes for certain tests.

Figure 26. Q-POC™ Cassette, Sample Caddy and Device

The Q-POC™ device is a portable, simple to use, sample-to-answer molecular platform that runs end-point PCR, real-time PCR, real-time RT-PCR, asymmetric chemistries and includes a microarray for additional detection capabilities. Per the company, it is the first multiplex diagnostic POC platform that combines the ability to quantitate pathogens through its 6-channel real-time reader and allows for additional multiplex detection of ~50 markers with its integrated microarray. The device can run sample-to-answer assays between 7 to 30 minutes, depending on the complexity of the assay. It does this due to its rapid microfluidic thermal cycler that performs a 35-cycle PCR in as fast as two to three minutes.

The first assay being developed on the Q-POC™ platform is an HPV genotyping assay that detects and genotypes 14 high-risk HPV subtypes in under 30 minutes direct from a swab sample. The assay is presently in field evaluations to demonstrate the clinical utility of screen and treat programs in LMICs. The platform and assay are projected for CE-IVD marking in 2020 followed by the WHO PQ process. QuantuMDx's second assay is a CT/NG/TV/MG detection assay, which will include a limited set of resistance markers for NG and MG. The assay will accept swabs and urine samples.

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

NEDxA®, Nano Electronic Diagnostic Array, is a POC platform consisting of a disposable cartridge and an analyzer that is easy to transport, install and maintain. The test results are obtained in 75 minutes without the need of trained personnel. It has the simplicity of a home device (without sample handling and without expensive equipment) and yet meets the requirements of a molecular diagnostic test: sensitive, precise, specific and reproducible. It offers an innovative and affordable solution to the need for early diagnosis and genotyping of multiple targets. It is designed in a modular way, being able to adapt to the processing needs of samples of any laboratory. Artificial Intelligence tools will be available to improve the diagnosis, treatment and follow-up of patients, as well as technical support.

The NEDxA® platform consists of:

- The Instrument (pictured below): light and easy to transport device, for health professionals without the need of specific training. It receives the cartridge with the clinical sample and automatically returns the interpreted results of the test. For the development of the instrument, NEDxA works with a company with extensive experience in the development of medical devices. NEDxA has also designed an accessory that allows the user to flexibly adapt the processing capacity of samples to any range, from a few per day, to up to 64.

Figure 27. NEDxA Platform

- The Cartridge (pictured below): a disposable device with everything necessary to allow the instrument to perform an *in vitro* diagnosis. The cartridge is based on the use of microfluidic technology to miniaturize molecular diagnostic assays. The design allows versatility to develop

future applications. Because devices are integrated for a single use, contamination in handling and human errors are avoided.

- 

Figure 28. NEDxA Cartridge

The demonstrated sensitivity parameters have been achieved due to the combination of a miniaturized multiplex-PCR and an innovative cartridge design, made with microfluidics, and micromechanics. The detection system is based on a stable and sensitive electrochemical reaction due to the specific binding of the product obtained during the multiplex-PCR and the complementary DNA detection probes.

The first application for the platform is the detection and complete genotyping of the 14 high-risk types of human papillomavirus (HR HPV). GENOMICA reports >95% sensitivity and >99% specificity for the HPV assay based on internal validations (published evaluations are not yet available). The following application to be developed will be NEDxA® STI (sexually transmitted infections).

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 2020.

cobas® Liat® System (Roche)

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, and Influenza A/B & 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. In addition, Roche also offers the cobas® Cdiff Nucleic acid test for use on

the cobas® Liat® System, which is intended as an aid in the diagnosis of *Clostridium difficile* infection. The assay is CE-IVD marked.

Figure 30. 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. The graphical user interface provides on-screen prompts for easy-to-follow directions to guide the operator through sample loading and tube insertion. An on-board scanner supports a variety of barcode types for added ease of use. 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 all tests.

Figure 31. cobas® Liat® Test Procedure

The **cobas®** Liat® System has an internal optical system that provides independent optical detection channels, allowing for the detection of multiple targets in each test and providing future expandability for detection of multiple diseases. It is powered by AC mains.

Pricing information is available by contacting local Roche representatives in each country. Global Pricing information is not available. 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.

Savanna (Quidel)

Quidel is developing Savanna, pictured below, a fully integrated sample-to-results molecular diagnostics system originally developed in collaboration with Northwestern Global Health Foundation.

Figure 29. Savanna

Savanna has been designed to meet the accuracy, turnaround time and ease of use requirements for use at POC in both developed and resource-limited settings. It will feature both real-time PCR and isothermal amplification technologies, run qualitative and quantitative assays and multiplex up to 12 targets. Room temperature reagent storage and minimal calibration and maintenance are planned.

According to the company, sample preparation requires limited user interaction depending on the assay. The cartridge-based system will be configured as a small single-bay benchtop instrument that can be expanded with addition of modular bays enabling deployment across a wide range of test settings.

Quidel is currently developing panels for vaginitis, and STIs, as well as for respiratory viruses, pharyngitis and GI pathogens. Market introduction is to be determined. TAT is estimated at about 40 minutes, depending on the assay. Sample types will include direct swab and processed liquid samples. Sample processing will be integrated on Savanna.

Validex System (Prominex, USA)

Prominex is an early stage company focusing on the development and manufacture of molecular diagnostic assays for infectious disease testing at POC. To this end, Prominex has developed a rapid, multiplex molecular diagnostic test platform, the Validex System, which can provide results in less than 5 minutes. The platform incorporates two novel technologies: Coupled Hairpin Amplification System (CHASE) and Evanescent Waveguide Biosensor Array Detection (WaveTech). These technologies work in crude clinical samples (e.g., whole blood, urine and swabs), with no sample processing required, and they require no polymerases, primers, or thermocycling.

The Validex System is designed for use at POC and CLIA waiver. The system has high multiplexing and quantitation capabilities, but also has a simple workflow. One of the initial assays on which the company is focusing is CT/GC. Assays for additional STIs and respiratory tract infections are also under development. No launch date for the CT/GC assay has been set yet.

To the extent information is available for them, 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 and the Vivalytic Analyzer both of which use microfluidic techniques, the Solana® platform from Quidel, which has unique HDA technology, and the IDAlert (discussed below), 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 *(96)*.[8]

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 number 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 *(97).* 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.

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.

[8] This section of the report draws significantly from the publication of Damhorst et al. *(96)*.

Some of the most common optical detection methods are fluorescence, absorbance and chemiluminescence *(98)*. 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, SlipChip (Talis Biomedical, USA), which has been shown to be capable of detecting 37 copies/mL of viral RNA with HIV and HCV samples *(99,100)*. Currently the device is in early stage development; a CT/NG assay is planned. However, many NAAT-based platforms use isothermal amplification (e.g., Ustar's CPA platform and the ID NOW™ 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 (*101*).

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 *(102)*.

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 *(103,104)*. 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 *(105,106)*, and impedance-

based cell counting approaches have also been used in the context of malaria diagnosis *(107,108)*. 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 *(109).*

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), and Talis Biomedical, 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 32. 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 33. Blusense Solution Workflow

Products currently available from BluSense 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 34. Blusense Viro-Track® Acute Dengue Cartridge line

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.

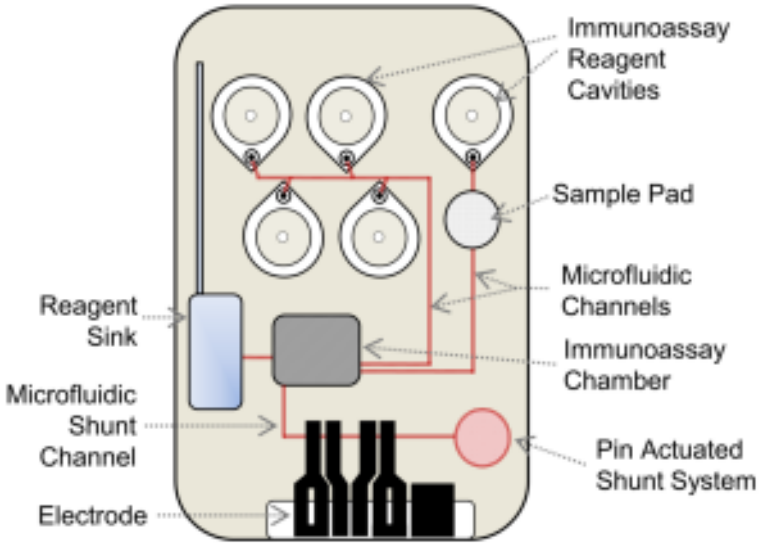

Figure 35. IDAlert Chip Card

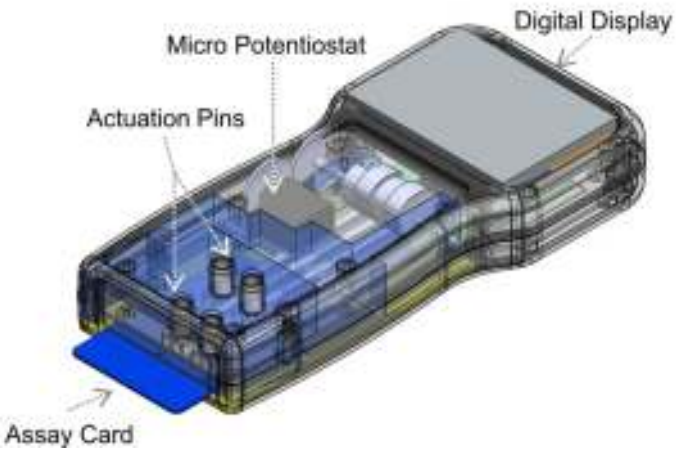

Figure 36. IDAlert Assay Reader

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.[9] 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.

Figure 37. 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 (Thermo Fisher Scientific, USA).

[9] 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.

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 (*110*) 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) (*110*). 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) (*110*). The authors concluded that GynTect® "is a robust and highly reproducible assay for the triage of HPV-positive women" (*110*).

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 ONE (pictured below).

Figure 38. The BLINK ONE Instrument

The BLINK product platform is being developed to support a variety of workflows for the detection of different analytes, such as nucleic acids and proteins, and if there was demand, it could also be adapted to support cellular detection. Moreover, the company is developing a novel assay cartridge that enables digital detection and quantification of individual analyte molecules in a sample and ensures a substantial dynamic quantification range. The system is being designed to process sample sizes from a few microliters (µl) up to large samples > 10mL and to be compatible with all common sample matrices (whole blood, plasma, serum, urine, and sputum swabs). The BLINK One platform is envisioned to be battery operated, mobile and safe for use outside of dedicated laboratory environments.

Further, the underlying architecture of the platform supports the flexible setup of different workflows required by a given assay. Workflows will be assembled from a variety of cartridge modules. With respect to the BLINK One platform, which is currently under development by the company, BLINK

intends to engage multiple assay developers and manufacturers to develop and produce tests for the platform.

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

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 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.[10] 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 or platforms that new tests for STIs will be well positioned to achieve the desired level of uptake and impact in global health.

[10] Available at: https://www.who.int/reproductivehealth/POTC-TPPs-2016.pdf?ua=1. Also, https://www.who.int/reproductivehealth/topics/rtis/pocts/en/.

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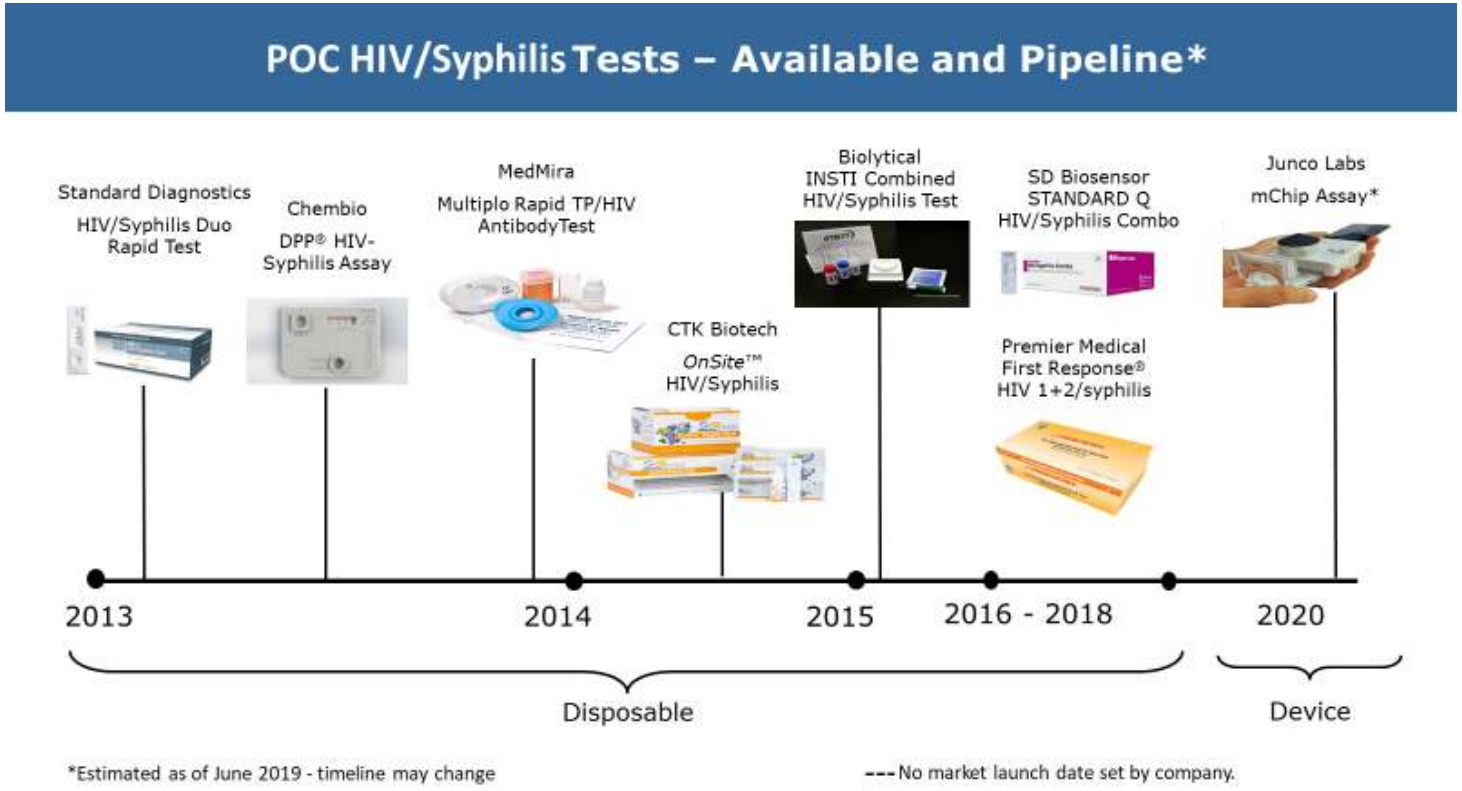
ANNEX A

Combined HIV/Syphilis tests – available and pipeline



ANNEX B and ANNEX C

Combined HIV/Syphilis tests – characteristics of available tests and characteristics of tests in the pipeline

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[11]			
Anti-HIV	100%	98.7%	99.6%
Anti-TP	100%	94.3%	95.8%
Specificity[11]			
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	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

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

	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		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	**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;

		rectangular test and control window should have disappeared; if not, discard the test device and repeat the procedures); (xii) add four drops of running buffer (green cap) to buffer well 2 (a reddish colour should begin to flow across the strip within 2–3 minutes); (xiii) read the test result 10–15 minutes after the addition of the running buffer to buffer well 2 **For venous whole blood, serum or plasma:** (i) remove the DPP® HIV-Syphilis test device from its pouch; (ii) obtain a venous blood, serum or plasma sample according to normal laboratory practices; (iii) before adding the sample, write the sample ID on the sample buffer bottle with the black cap; (iv) remove (unscrew) the white cap, keeping the black cap screwed onto the white part of the cap; (v) add 10 µL venous blood, serum or plasma sample using a calibrated pipette into the sample buffer bottle with the black cap such that the pipette tip is touching the bottom of the bottle; (vi) replace the black/white cap assembly onto the bottle and gently shake the bottle for 10 seconds; (vii–xiii) the remaining steps are the same as for the fingerstick blood sample	(xii) dispense the remaining buffer, in drops, from the vial of universal buffer onto the InstantGold cap and allow the solution to be absorbed; (xiii) remove the InstantGold cap, waiting for the solution to be completely absorbed; (xiv) read test results immediately. **For venipuncture whole blood collection and use**: (i) use standard venous phlebotomy procedures to collect a whole blood sample; (ii) place the sample tube (provided) in a secured rack on a flat surface; (iii) add five drops from the 30 mL bottle of Universal Buffer (provided) to the sample tube; (iv) use the transfer pipette provided to collect specimen from the specimen collection tube; (v) add one drop of whole blood into the sample tube prepared in step iii above; (vi) hold the sample tube and gently tap the side of the tube near the bottom until the mixture becomes a clear reddish colour; (vii) pour the entire contents of the sample tube into the well of the test cartridge; (viii) allow specimen to be absorbed; (ix) place the InstantGold cap on the test cartridge; (x) dispense 12 drops from the 30 mL bottle of Universal Buffer onto the InstantGold cap and allow the solution to be completely absorbed; (xi) remove the InstantGold cap and wait for solution to be completely absorbed; (xii) add three drops of universal buffer to clarify results; (xiii) read test results immediately **For serum/plasma**: (i) apply three drops of Universal Buffer to the centre of the test

			cartridge; (ii) allow the buffer to absorb completely; (iii) apply one drop of serum or plasma specimen to the centre of the test membrane; (iv) wait for the specimen to absorb completely before proceeding to the next step; (v) place the InstantGold cap on the test cartridge; (vi) dispense 12 drops of Universal Buffer onto the InstantGold cap; (vii) allow the solution to be completely absorbed; (viii) remove the InstantGold cap; (ix) wait for the solution to be completely absorbed; (x) add three drops of Universal Buffer to clarify results; (xi) read test results immediately.
Read window	Results should not be read more than 20 minutes after adding assay diluent	15 minutes after running buffer is added to sample	N/A; results should be read immediately.
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 Private Limited (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[11]			
Anti-HIV	100% (136/136 positive)[c]	99%	100%
Anti-TP	96.5% (55/57 positive)	98.1%	100%
Specificity[11]			
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 required	50 µL	20 µL	20 µL
Sample storage	Whole blood collected in EDTA tubes may be stored at 2 °C–8 °C for up to five days; serum or plasma EDTA samples may be stored up to five days at 2 °C–8 °C for up to five days, up to three months at -20 °C and up to one year at -70 °C	Whole blood 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)	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	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

	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 center well; (vi) place the membrane unit on a level surface (for sample identification purposes the tab of the membrane unit may be labelled with the patient's name or number); (vii) remix the sample diluent-specimen mixture and pour the entire contents into the center of the membrane unit well within five minutes after the specimen has been added to the sample diluent vial; (viii) re-suspend the color 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 color developer and add the entire contents to the center of the membrane unit well (the colored solution should flow through completely in about 20 seconds); (x) open the clarifying solution (solution 3 vial) and add the entire contents to the center 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	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 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.	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 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

	containing the membrane unit, and one vial each of the sample diluent (solution 1 vial), colour developer (solution 2 vial), and clarifying solution (solution 3 vial) for each test to be performed; (iii) using a pipette, add 50 µl of whole blood, serum or plasma to the sample diluent vial; (iv) recap the vial and mix by inversion; (v–xi) the remaining steps are the same as for the fingerstick blood sample		
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.

Test name	**Standard Q HIV/Syphilis Combo (SD Biosensor)**
Company	SD Biosensor (Republic of Korea)
Type of technology	Lateral flow
Availability	Commercially available
Output	Qualitative detection of antibodies specific to HIV-1 including subtype O, HIV-2 and Syphilis TP
Antigen type (HIV)	Recombinant HIV-1 gp41 protein/recombinant HIV-1 subtype O gp41 and recombinant HIV-2 GP36 protein
Antigen type (syphilis)	Recombinant p17 *Treponema pallidum* protein
Sensitivity[11]	
Anti-HIV	100% (637/637 positive)[c]
Anti-TP	98.8% (395/400 positive)
Specificity[11]	
Anti-HIV	99.9% (1499/1500 negative)
Anti-TP	100.0% (1500/1500 negative)
Sample type	Whole blood (fingerstick or venous), serum or plasma
Volume of sample required	10µl serum, 20µl plasma
Sample storage	Venous whole blood in an anti-coagulant tube may be stored in a refrigerator at 2-8°C / 36-46°F and can be used for testing within 1-2 days after collection. Capillary blood must be used immediately after collection. Serum in a plain tube may be stored in a refrigerator at 2-8°C / 36-46°F, and the specimen can be used for testing within 1 week after collection. Plasma in an anti-coagulant tube may be stored in a refrigerator at 2-8°C / 36-46°F,

	and the specimen can be used for testing within1 week after collection.
Time to result	15 minutes and not later than 20 minutes
Protocol complexity – steps required	**For fingerstick blood**, (i) clean a fingertip by wiping with an alcohol swab; (ii) dry and pierce the wiped fingertip with a lancet to bleed; and (iii) collect the 20µl of capillary whole blood to the black line of the capillary tube. **For venous blood, serum or plasma:** collect 10µl of serum/plasma and 20µl of venous whole blood specimen using a micropipette. **For all specimens**: (i) add the collected specimen to the sample well of the test device; (ii) add 3 drops of assay diluent into the sample well of the test device; and (iii) read the test results after 15 minutes.
Read window	The test should not be read after 20 minutes.
Shelf life of test kit	N/A
Storage requirements	2-40°C /36-104°F, out of direct sunlight.
Test kit components	Test device; Assay diluent; Capillary tube (20µl); Lancet; Alcohol swab; and Instructions for use
Not included in test kit	Micropipette and tip; Blood collection tube; PPE (Personal Protective Equipment); and Biohazard container
Controls	Test has built-in procedural controls that demonstrate assay validity and adequate sample addition
Regulatory	
Pricing	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[12]	
Anti-HIV	100% (95% CI, 97–100)
Anti-TP	90% (87–93)
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

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

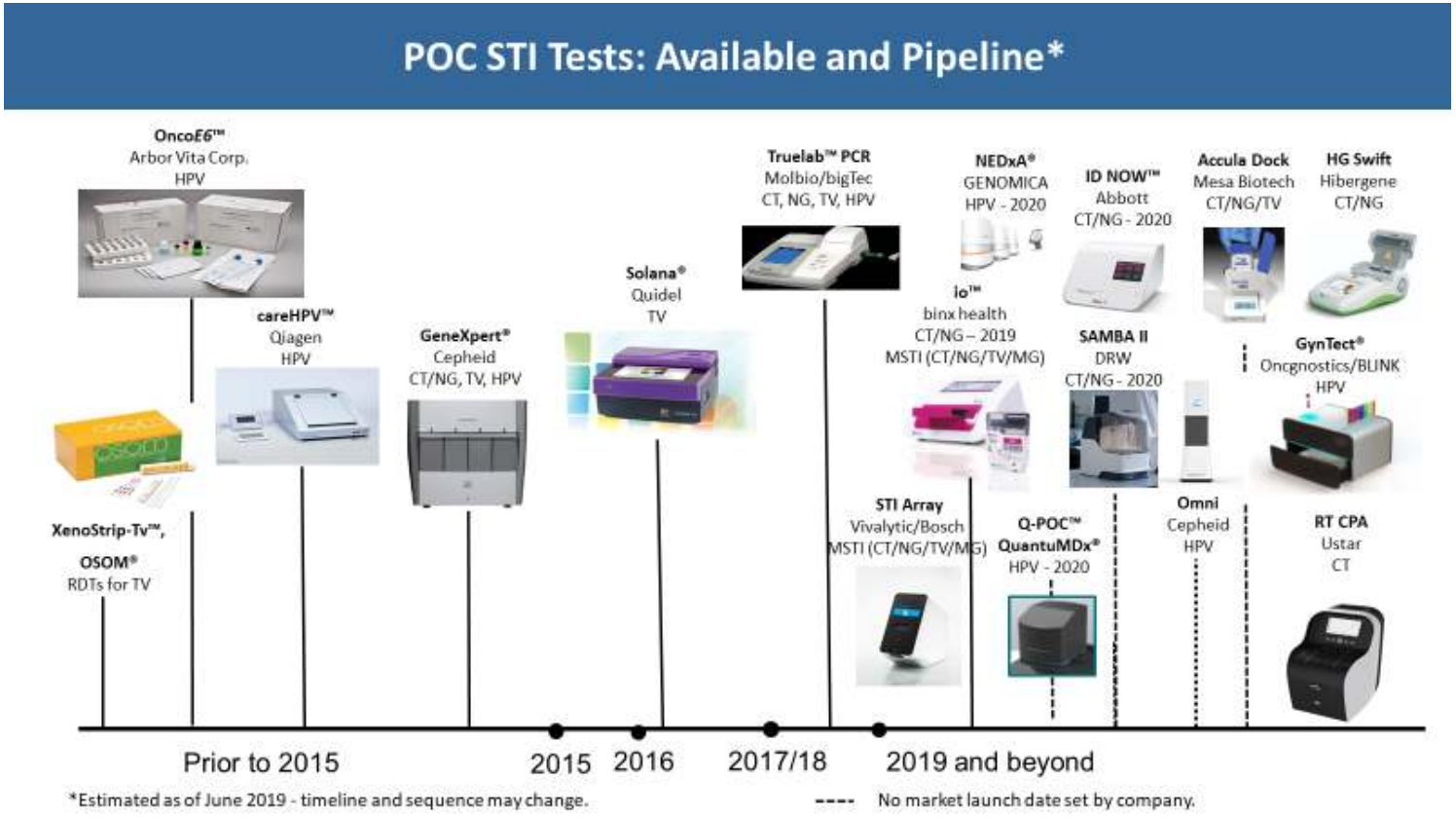
	ambient temperature (15 °C–30 °C) for up to six hours
Time to result	15 minutes
Protocol complexity – steps required	**For fingerstick blood**, (i) obtain a fingerstick blood sample according to normal laboratory practices using a sterile lancet; (ii) wick blood into the sample holder capillary tube; (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



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
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	Singleplex	Nucleic acid hybridization assay	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
io™ Diagnostic System binx health, inc.	Multiplex Level 1 (possible)	NAAT, immunoassay and small molecule chemistry	N/A	N/A	√ CE-IVD FDA	Pipeline	N/A
Vivalytic Randox/Bosch	Multiplex Panels (CT/NG/TV plus 7 others) Level 2	iNAAT	N/A	N/A	CT/NG/TV plus - pipeline CE-IVD (2019, assays only)		N/A
SAMBA II DRW	Multiplex	iNAAT	N/A	N/A	Pipeline	N/A	N/A
RT-CPA CT Test Ustar	Multiplex Level 2	iNAAT – CPA	Pipeline	N/A	N/A	N/A	N/A
ID NOW™ Abbott	Multiplex Level 2, Level 1 (possible)	iNAAT – RPA or NEAR	N/A	N/A	Pipeline	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
Q-POC™ QuantuMDx Group	Multiplex Level 2	Continuous flow PCR			CT/NG/TV/MG Pipeline		2020
HG Swift Hibergene Diagnostics	Multiplex Level 2	LAMP/Fluorometric detection	N/A	N/A	Pipeline	N/A	N/A
Accula System Mesa Biotech	Multiplex Level 1	RT-PCR NAAT	N/A	N/A	CT/NG/TV Pipeline		N/A
NEDxA GENOMICA S.A.U.	Singleplex	PCR-based NAAT	N/A	N/A	N/A	N/A	2020
cobas™ Liat Roche	Multiplex Level 1 (possible), Level 2	PCR-NAAT	Unknown which of these assays will be developed by the company				
GynTect™ ocgnostics/BLINK	Multiplex Level 2, Level 1 (possible)		N/A	N/A	N/A	N/A	Pipeline

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
Savanna Quidel	Multiplex Level 2, possibly Level 1	Real-time PCR and isothermal amplification	Vaginitis and STIs (not currently specified)				
Validex System Prominex	Multiplex	Coupled Hairpin Amplification System (CHASE) and Evanescent Waveguide Biosensor Array Detection (WaveTech).	N/A	N/A	Pipeline	N/A	N/A

Level 1 – primary healthcare center; 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**	**binx health, inc.**	**Abbott**
Assay Name	**GeneXpert®** **CT, CT/NG**	**io™ Diagnostic System** **CT/NG**	**ID NOW™** **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-	Less than ½ day

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

		explanatory user guide and screens on instrument	
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 center; 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**	**Hibergene Diagnostics**	**Ustar**
Assay Name	**Truelab™ PCR** **CT, NG**	**HG Swift** **CT/NG**	**RT CPA CT** **CT (pipeline)**
Use Setting	Level 2; 2 instruments; not portable	Level 2; Table top; not portable	Level 2
Specimen	TBD	Urine or vaginal swab	TBD
Steps	Multiple pipetting steps	~4 - 5 steps	~3 – 5 steps from sample to result
Time to Result		66 minutes	
Cold Chain; Reagent Stability	No; 3 months at temperatures to 40C	No	
Power	Rechargeable Lithium ion battery	AC mains or rechargeable lithium polymer battery	Mains power or rechargeable battery
Training	Less than ½ day	Minimal training required	Approximately ½ day
Connectivity	Yes; wireless connectivity	Yes; wireless connectivity	Will be used with Genie® device; TBD

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

Equipment Cost ($US); Per test	~$8,000; TBD		<$5,000; TBD

Level 1 – primary healthcare center; 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	**Mesa Biotech**	**DRW**	**Randox/Bosch**
Assay Name	**Accula™ System** **CT/NG/TV (pipeline)**	**SAMBA II** **CT/NG (pipeline)**	**Vivalytic STI Multiplex Array** **CT/NG/TV + 7 STIs**: Assay CE-IVD marked; but not for use on Vivalytic platform
Use Setting	Handheld; Level 1	Level 2; Table-top, two modules	Level 2/3; Table-top; Single module
Specimen	TBD	TBD	Urine, swabs
Steps	~3 - 4 steps	~4 – 5 steps	4, excluding sample preparation
Time to Result	30 minutes	TBD	~6 hours
Cold Chain; Reagent Stability	No; TBD	No; TBD	No
Power	AC mains	AC mains	AC mains
Training	Minimal training required	Minimal training required	Minimal training required
Connectivity	No	USB port, Ethernet, SMS	Yes; built-in

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

Equipment Cost ($US); Per test	TBD	TBD	N/A

Level 1 – primary healthcare center; 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**	**binx health, inc.**
Assay Name	**GeneXpert®**	**Solana®**	**io™ Diagnostic System**
Use Setting	Table-top, not portable Level 2	Table-top; not portable Level 2, Level 3	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 - 25C
Power	Mains power required; can use solar	Mains power required for heat block and Solana instrument	Mains power required

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

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
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 center; 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	**Arbor Vita**	**QIAGEN**	**Cepheid**
Assay Name	**Onco*E6*™ Assay**	**careHPV™ System**	**GeneXpert®**
Use Setting	Disposable RDT, but requires centrifuge, tube rotator, and thermometer Level 2, possibly Level 1	Table-top (3 modules) Level 2	Table-top, not portable Level 2
Specimen	Female endocervical swab; liquid ThinPrep	Female endocervical swab, which may be self-collected	Female endocervical swab
Steps	~5 – 6, several of which are manual	~7 steps, a number of which are manual	~4; sample prep automated
Time to result	~2.5 hours	~2.5 hours	~60 minutes
Cold Chain; reagent stability	No	12 months between 4 and 25°C	Kit storage: 2 – 28°C
Power	Mains power for centrifuge and rotator	Mains power	Mains power required; can use solar
Training	Minimal laboratory training required	Some laboratory training required	Less than ½ day

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

Connectivity	No	No	
Equipment Cost ($US); per test	~$2,000 for reusable equipment		

Level 1 – primary healthcare center; 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	**QuantuMDx Group**	**GENOMICA S.A.U.**
Assay Name	**Q-POC™** **(Pipeline); also CT/NG/TV/MG in the pipeline**	**NEDxA** **(Pipeline)**
Use Setting	Table-top; portable Level 2, possibly Level 1	Table-top Level 2
Specimen	Swabs	Female endocervical swab, ThinPrep and SurePath™ liquid samples
Steps	~4	~4; no sample prep
Time to result	<30 minutes	~75 minutes
Cold Chain; reagent stability	N/A	Kit storage: 4 and 25°C
Power	Battery powered	Mains power required. Low power consumption.
Training	Minimal training required	No training needed

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

Connectivity	N/A	Yes; compatible with LIMS systems
Equipment Cost ($US); per test	TBD	7.500€ per instrument. 16-20€ per cartridge (per sample including 14 targets); pricing is volume-based.

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