Sexually transmitted infections (STIs) continue to be a significant global public health issue, with an estimated 357 million people becoming ill each year with one of 4 STIs: syphilis, *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Trichomonas vaginalis* (TV) (1). In addition, more than 290 million women have a human papillomavirus (HPV) infection (1).

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

There are now a variety of tests available for use at or near the point of patient care (POC) for STIs (2). These include a wide range of rapid diagnostic tests (RDTs) for human immunodeficiency virus (HIV), hepatitis C and syphilis, among others, with which it is possible to detect infection using fingerprick blood, or in some cases, oral fluid. 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 The material in this landscape was gathered by the author from publicly available information, published and unpublished reports and prospectuses, and interviews with developers and manufacturers.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 28 February 2017.

**Syphilis**

The WHO estimates that in 2012, the most recent year for which such statistics are available, there were approximately 6 million new cases of syphilis worldwide (3). The highest disease burden for syphilis is in sub-Saharan Africa and South and Southeast Asian countries (4).

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1 Note that RDTs are a subset of POC tests. RDTs are generally lateral fluid or immunofiltration strip or cassette-enclosed tests that are disposable, easy-to-use and have short time to test result. They can be used at all levels of the healthcare system.
Syphilis has particularly profound consequences for pregnant women, considered to be a vulnerable population, and for individuals in certain key populations where its prevalence is high: men who have sex with men (MSM) and sex workers. With respect to pregnant women, maternal syphilis is a significant cause of infant mortality. In 2008, the WHO estimated that about 1.86 million cases of syphilis occur worldwide among pregnant women each year, many of whom are either untreated or inadequately treated (5). It is estimated that more than 500,000 perinatal deaths (i.e., deaths that occur from 22 weeks gestation through the first 7 days of life) occur each year as a result of untreated maternal syphilis. 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 (7).

With respect to MSM, the WHO estimates that syphilis infects 5% or more of MSM in at least 42 countries, 10% or more in 20 countries and more than 20% in 8 countries (8). In the United States, the United States Centers for Disease Control and Prevention (CDC) estimates that 75% of syphilis cases are among MSM (9). In addition, the WHO reports an increasing trend of syphilis in MSM, with 48% of all new syphilis cases in 2012 reported among that population (3). Untreated, syphilis can lead not only to serious complications, but it also increases the risk of acquiring and transmitting HIV.

Finally, per the WHO, syphilis infects more than 5% of sex workers in 34 countries, more than 10% in 21 countries and more than 20% in 7 countries (10). 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 (10).

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 these are CE-marked rapid tests from Alere, Inc. (Alere Determine™), Alere/Standard Diagnostics (SD Syphilis 3.0), The Tulip Group/Qualpro (Syphicheck® - WB) and Omega Diagnostics (Visitect® Syphilis). These tests are summarized below.

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2 This compares to annual death rates for other important infections in pregnancy, such as HIV, which is estimated to cause between 250 000 and 290 000 perinatal deaths worldwide, and malaria in pregnancy, which is estimated to cause about 200 000 perinatal deaths (6).

3 Note that to date the WHO prequalification program for diagnostics has not prequalified syphilis only assays. Therefore, manufacturers primarily rely on European Union approval through the CE-marking process.
<table>
<thead>
<tr>
<th>Test (Manufacturer)</th>
<th>Specimen</th>
<th>Volume of whole blood or other specimen</th>
<th>Time to result (minutes)</th>
<th>Storage Temperature (°C)</th>
<th>Test life (months)</th>
<th>Test type</th>
</tr>
</thead>
</table>
| Alere Determine™ Syphilis TP  
Alere, Inc. (USA) | Whole blood (fingerstick), plasma or serum | 50 µL | 15 minutes (up to 24 hours) | 2 - 30° | NA | Lateral flow strip |
| SD Syphilis 3.0  
Alere/SD Bioline (South Korea) | Whole blood (venous or fingerstick), plasma or serum | 20 µL (whole blood)  
10 µL (plasma or serum) | 5 - 20 minutes | 2 - 30° | NA | Cassette enclosed test card |
| Syphicheck® - WB  
The Tulip Group/Qualpro (India) | Whole blood (venous or fingerstick), plasma or serum | 25 µL | 15 minutes | 4 - 30° | NA | Cassette enclosed test card |
| Visitect® Syphilis  
Omega Diagnostics (UK) | Whole blood (venous or fingerstick), plasma or serum | 50 µL | 30 minutes | 4 - 30° | NA | Cassette enclosed test card |

NA = Not Available

Table 1. Select RDTs for Detection of Syphilis

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

The other concern about TP RDTs has been performance. However, a recent meta-analysis on their performance demonstrates that rapid TP tests for syphilis report sensitivity and specificity estimates comparable to laboratory-based tests, for which there is no gold standard (11). In this review, adjustments were made for imperfect reference standards using the Bayesian Hierarchical Summary Receiver Operating Characteristic Curve method. The result is point estimates of sensitivity and specificity for each test, using serum and whole blood, around a 95% credible interval (as opposed to a confidence interval), as shown in the table following.
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 (TPAAs), 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).4

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:

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<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Specimen</th>
<th>Volume of whole blood or other specimen</th>
<th>Time to result (minutes)</th>
<th>Storage Temperature (°C)</th>
<th>Test life (months)</th>
<th>Test type</th>
</tr>
</thead>
</table>
| 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 |
| 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 both OnSite™ 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). No peer reviewed evaluations of the Uni-Gold™ Treponemal 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 the point of care 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, 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.

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

There are several peer-reviewed publications of the performance of the DPP assay, each of which found that the test performed favorably against laboratory-based reference tests. In a multi-center field study in China, three kinds of specimens (whole blood [WP], fingerprick blood [FP] and blood plasma [BP]) were used to evaluate the sensitivity and specificity of the DPP® platform. The TPPA assay and the toluidine red unheated serum test (TRUST) were used as reference standards. A total of 3,134 specimens (WB 1,323, FB 488, and BP 1,323) from 1,323 individuals were collected. The sensitivities as 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 (17).

The second study of the DPP assay®, a laboratory-based evaluation, used 1,601 banked serum samples. The TPPA assay and a quantitative RPR test were used as reference standards. Compared to the RPR test, the sensitivity of the DPP® non-TP line was 98.4% when the RPR titers of sera were ≥1:2, and the specificity of the non-TP line was 98.6%. Compared to the TPPA assay, the reactive and nonreactive concordances of the TP line were 96.5% and 95.5%, respectively (18).

Causer et al performed another study in Australia. Of the 1005 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%) (19). 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%

5 Note that Span Diagnostics had also developed TP/Non-TP immunofiltration rapid test, but it is not currently a product that Span is offering commercially. See Castro AR, Mody HC, Parab SY, et al. An immunofiltration device for the simultaneous detection of non-treponemal and treponemal antibodies in patients with syphilis. Sex Transm Infect. 2010; 86: 532-536 (16).
of 121 low-titer infections, 27.5% of 211 past/treated infections, and 78.1% of 242 infections classified as not being syphilis (19). Of the 211 past/treated infections, 49.8% were incorrectly identified as active infection and a further 22.8% as not syphilis (19). 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™ (14,19). 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 (19).

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.

Figure 1. DPP® Syphilis Screen & Confirm Assay

Other than the Chembio, 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 are significant causes of death in infants and young children globally each year. Of the approximately 2.0 million new HIV infections among adults and children in 2014, for example, it is estimated that about 220,000 children became newly infected with HIV (20). There are effective interventions to prevent these adverse outcomes for infants and young children who are at risk of HIV and/or syphilis caused by MTCT. The CDC estimates that key interventions, including HIV testing and counselling of all pregnant women and provision of antiretroviral drugs to all HIV-positive women during pregnancy, among other interventions, can reduce MTCT of HIV from 35% to 5% (21). 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 (22,23,24).

WHO has long supported screening of all pregnant women for HIV (25), 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 (26,27,28,29), WHO launched a global initiative for the elimination of congenital syphilis in 2007 (30). Yet, despite this call and the launch of the Global Congenital Syphilis Project (GCSP) to advocate for, and invest in, the fight against congenital syphilis, syphilis screening programs for pregnant women still have not been widely implemented in resource-limited settings.

In summary, in the case of MTCT of both HIV and syphilis, testing pregnant women is a critical intervention for prevention, care and treatment of both mother and child. In addition, such tests can be a particularly 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 several combined HIV/syphilis tests that are in the pipeline. The available and pipeline tests are shown in summary form in Annex A.

There are currently five combination HIV/syphilis (TP) RDTs on the market: the SD Bioline HIV/Syphilis Duo Rapid Test (Alere, Inc.), the DPP® HIV-Syphilis Assay (Chembio Diagnostics Systems, Inc.), the Multiplo Rapid TP/HIV Antibody Test (MedMira, Inc.), the INSTI™ HIV/Syphilis Multiplex Test (biolytical Laboratories Inc.) and the OnSite™ HIV/Syphilis Ab Combo Rapid Test (CTK Biotech Co).

Of the five tests, three of them – the tests from Alere, Chembio and MedMira – have been the subject of recently-published, independent laboratory evaluations. In the first of these evaluations, the performance of the three tests was compared using sera specimens in the United States. All three RDTs were tested in parallel by trained laboratory technicians. The results of the RDTs for HIV were compared to those via routine testing (EIA and Western blot); while the results of the TP assay were compared to TPPA test results. One hundred and fifty samples were included in the study. The performance of the RDTs was good. Sensitivity for HIV antibody detection by the RDTs ranged from 98 to 99%, and the specificity ranged from 94 to 100%, compared to the Siemens Advia HIV EIA. The authors characterized the performance of the three RDTs as excellent for the detection of TP, ranging from 93 to 95% sensitivity and 97 to 100% specificity, compared to TPPA. The authors concluded that overall the evaluations “showed performance by the RDTs that was comparable to the reference methods, with excellent sensitivity and specificity” (31).

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

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

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

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

Yin et al suggest that further research on the above RDTs is needed to evaluate their performance on whole blood samples in primary healthcare settings – i.e., in the hands of less well trained users in target use settings (32).

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

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

One of five HIV/syphilis rapid diagnostic tests currently on the market is the SD Bioline HIV/Syphilis Duo Rapid Test from Alere (pictured below).
Figure 2. SD Bioline HIV/Syphilis Duo Rapid Test

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

There are a number of peer-reviewed, published performance evaluations of the SD Bioline HIV/Syphilis Duo Rapid Test, several of which were done in field settings in Uganda, Ethiopia, Peru and Haiti, respectively. 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 (33,34,35,36,37,38).

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

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

Figure 3. DPP® HIV-Syphilis Assay
In addition to the three simultaneous evaluations of combined HIV/syphilis RDTs, one independent, peer-reviewed study was 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 the HIV antibody detection was 100% and the specificity was 98.7% (with or without the use of an electronic reader) (39). For visual TP antibody detection, the sensitivity of the assay was 94.7% and the specificity was 100.0%; using the electronic reader, the sensitivity of the test was 94.7% and the specificity was 99.7% (39).

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

The Multiplo Rapid TP/HIV Antibody Test from MedMira, Inc. (POC format pictured below) is commercially available in Colombia, and the company is pursuing product registration in a number of other countries. The combination assay is built on the MedMira Rapid Vertical Flow Technology platform and is sold in in the same packaging formats as the company’s rapid HIV antibody test.

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**Figure 4. Multiplo Rapid TP/HIV Antibody Test**

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

Two peer-reviewed, published performance evaluations of the Multiplo Rapid TP/HIV Antibody Test were found in the literature. A total of 200 stored serum specimens collected from MSM and transgender women presenting in one of two STI clinics in Lima, Peru were tested in a laboratory setting by Bristow et al (40). The estimated sensitivity of the HIV component of the Multiplo test was 100% with a 95% CI of 95.1% - 100%, and the specificity was estimated to be 91.9% (95%CI, 85.7% - 96.1%) (40). With respect to the TP antibody component of the test, the sensitivity and specificity estimates were 94.6% (95% CI, 88.5% - 98.0%) and 92.8% (95% CI 84.9% - 97.3%) (40). Subsequently, Bristow et al. conducted a field evaluation...
of the Mixplo 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 (41). 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%) (41).

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

The INSTI™ HIV/Syphilis Multiplex test, pictured below, is designed to provide rapid qualitative detection of HIV-1 and HIV-2 as well as Syphilis TP in a rapid test format using immunofiltration. Turnaround time is about 60 seconds.

Figure 5. INSTI™ HIV/Syphilis Multiplex Test

The INSTI™ combo test has only recently been introduced onto the market. Nevertheless, 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 (42). 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 (42). 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%) (42).

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

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

![OnSite™ HIV/Syphilis Ab Combo Rapid Test](image)

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

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

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

The detailed performance (sensitivity and specificity), as reported by the companies in product inserts, and operational characteristics of the five assays described above are detailed in Annex B. Both the SD Bioline and DPP dual assays have been evaluated by the CDC and are on the USAID Waiver List. The SD Bioline and INSTI assays are CE-IVD marked. All but the CTK assay have been submitted to WHO for prequalification, but to date, only the SD Bioline assay has been prequalified; however, the INSTI™ is currently under review by WHO.

**Combined HIV/syphilis tests in the pipeline**

In addition to the assays described above, two more combined HIV/syphilis tests for use at the point of care are in the pipeline. (See Annex C for operational characteristics of assays in the pipeline for which such data are available.) These include assays from Junco Labs and Columbia University in collaboration with OPKO Health, Inc. and an assay from ChipCare Corporation.
mChip Assay (*Junco Labs and Columbia University in collaboration with OPKO Health, Inc.*)

The first of these assays, the mChip assay (pictured below) from Junco Labs and Columbia University in collaboration with OPKO Health, Inc., will go beyond existing combination HIV/syphilis TP assays and may include qualitative detection of non-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 (43). No commercial launch date for the HIV/syphilis TP assay has been set.

![mChip Platform with Smart Phone](image)

**Figure 7. mChip Platform with Smart Phone**

**ChipCare Platform (*ChipCare Corporation*)**

ChipCare Corporation is developing a portable, easy-to-use diagnostic platform for laboratory-quality blood testing at the point of care (pictured below). The platform utilizes novel microfluidic technologies to provide multiple cell surface and blood analyte tests on a disposable cartridge. ChipCare’s initial assay
is for absolute CD4 counting, but the company is developing a combined HIV/syphilis test, which will also provide the option of other assays such as HIV early infant diagnosis and HIV viral load or HCV.

Figure 8. ChipCare Platform

Designed for use by healthcare workers in resource-limited settings, the ChipCare device weighs less than 2 kg and will be sufficiently small to be carried in a backpack. The platform does not require continuous power or running water for operation. The assays do not require cold chain. The ChipCare platform is powered by a lithium ion battery that can be recharged via AC mains, a car battery or solar panel. The platform has built-in connectivity.

The price of the ChipCare device is expected to be about $5,000. Test cartridges are expected to cost about $6-8 per test. The combined HIV/syphilis assay is expected to be launched in mid-2018.

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.6

6 The TPP for HIV/syphilis dual test is available on the website of the International Diagnostics Centre: http://www.idc-dx.org/resources/target-product-profile-combined-hivsyphilis-test.
Chlamydia trachomatis, Neisseria gonorrhoeae

Both CT and NG are significant global health problems. The WHO estimates that in 2012 approximately 131 million and 78 million new cases of CT and NG, respectively, were diagnosed worldwide (3). It has been concluded by many researchers that current POC diagnostic tests for both CT and NG do not perform adequately and that improved assays are required (44,45,46), especially for women, where the syndromic approach to managing STIs is inadequate (47). Traditional STI 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), Hologic, Roche Diagnostics, and others. However, these platforms are laboratory-based and require significant infrastructure, including continuous power, clean running water and climate control. In order to reach patients in resource-limited settings, patient samples must be transported from urban, peri-urban and rural settings to the laboratory for processing. This is done using sample transport networks in-country. But, frequently, these services are not well developed, leading to long delays in returning sample results to patients and loss to follow-up. The conclusion is that what is needed in resource-limited settings is more sensitive, easier-to-use and cheaper tests for both CT and NG the results of which can be delivered in a single patient visit (48,49).

There is currently one NAAT-based platform available for near-patient diagnosis of CT, NG and CT/NG combined – the GeneXpert® system from Cepheid. Additional CT, NG and combination CT/NG tests are in the pipeline. (See Annex D for existing and pipeline tests for CT, NG and CT/NG for use at or near the point of care, including those for TV and HPV.) These are discussed below.

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 24 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.
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. The assay may be used on the following specimens from both asymptomatic and symptomatic patients: female and male urine, endocervical swab, and patient-collected vaginal swab (collected in a clinical setting). The test process is straightforward, with total hands-on time estimated to be less than one minute. The operator (i) obtains either urine or swab samples, which are previously collected and stored in the Cepheid Transport Reagent; (ii) transfers the sample to the Xpert® cartridge; and (iii) inserts the cartridge into the Xpert® system and starts the assay. Time to result is approximately 90 minutes.

The 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 specifically calls out high risk types 16 and 18/45 in separate detection channels with 11 other high risk types detected in combined channels.

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 (50).

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 (51).

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. Wireless data connections via satellite phone networks are in development, as is a secure, hosted platform called Cepheid C360. Cepheid C360 allows for systems monitoring to observe instrument performance data, and disease surveillance to aggregate and monitor disease state testing data. An early version of this software, called Remote Xpert, has been in use in South Africa for tuberculosis test monitoring for more than 2 years. More than 10 countries in sub-Saharan Africa are currently scaling up their GeneXpert program with Cepheid C360.

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

Arguably, the GeneXpert® platform is best used at district hospitals and above in the tiered laboratory system in-country. It is not as well suited to use at health centers and below for reasons including cost, the need for stable electricity and remote calibration requirements. However, the GeneXpert® has been used quite successfully at remote Aboriginal healthcare facilities in Australia and many other decentralized settings (52,53,54).

The performance of the Xpert® CT/NG assay has been evaluated and found to be very good relative to established laboratory based assays (55,56,57) and Xpert® TV (58).

Platforms/Assays in the Pipeline

Atlas Genetics io® Platform (Atlas Genetics)

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

Figure 10. Atlas Genetics io® Platform and Cartridge

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

Atlas Genetics considers tests for STIs to be one of its core focus areas. In addition to a CE-marked test for CT, the company has several other STI assays in development, including combination CT/NG, NG antibiotic resistance, combination CT/NG/TV, and *Mycoplasma genitalium* (MG). Each of the assays has undergone, or is undergoing, preliminary clinical evaluations (primarily in the United States through the Johns Hopkins STD POC Centre). The CT assay was CE-IVD marked in early 2016, and Atlas expects to launch the assay and the io® system, both of which will be CE-IVD marked, in Europe in 2017. A clinical trial for the CT/NG assay was expected to be conducted in the United States starting in the first quarter
of 2017, with FDA approval and product launch to follow. Evaluation of the CT assay has demonstrated clinical sensitivity of 94.1% and specificity of 99.0%.\(^7\)

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

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

**GeneXpert® Omni (Cepheid)**

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

![GeneXpert® Omni Platform](image)

**Figure 11. GeneXpert® Omni Platform**

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

The projected cost of the platform, including the GeneXpert® Omni single module, the dedicated mobile device, an AC/DC power cord (country specific), and the supplemental rechargeable battery power supply is expected to be US$2,895.

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\(^7\) Results from an unpublished study with 215 female participants by Widdice L, MD, Cincinnati Children’s Hospital Medical Center, which was shared by the company in personal correspondence.
The GeneXpert® Omni system is expected to be available outside the US in the second half of 2017. The initial assays planned for availability on the system will be the Xpert® MTB/RIF, Xpert® MTB/RIF Ultra, Xpert® HIV-1 Qual, Xpert® HIV-1 Viral Load, and Xpert® HCV Viral Load. Xpert® CT/NG and Xpert® HPV are expected to be available on Omni in 2018. Over time, it is Cepheid’s intent to have the majority of the Xpert menu available on the GeneXpert® Omni.

**RT CPA CT Test (Ustar Biotechnologies)**

Ustar Biotechnologies has developed Cross Priming Amplification (CPA), a novel isothermal NAAT with multiple iterative designs that can address a wide variety of key obstacles to traditional amplification technologies such as PCR. By using multiple crossing primers and probes, target DNA sequences can be rapidly and precisely amplified at a uniform temperature (typically 63°C) in an easy-to-use protocol with high sensitivity and specificity. By utilizing its CPA technology on its dedicated platform (pictured below), Ustar is now developing assays for HIV, hepatitis C virus (HCV), CT and polio virus (the latter two together with PATH).

![Figure 12. RT CPA-CT Platform (left) and Cartridge (right)](image)

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 plans to modify the commercially-available Genie®, a portable instrument developed by OptiGene Ltd., in order to integrate an automated sample preparation instrument with the existing Genie® instrument for a fully automated sample-in, answer-out system. For this purpose, Ustar is now working with 2 professional medical device design companies, one specializing in instrument design, and the other specializing in consumable design, in order to produce a high quality, fully integrated and automated (sample-in, answer-out) molecular diagnostic system for POCT settings.

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8 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 the portable OptiGene instrument, which is being designed for use at or near the point of patient care.
The final Ustar diagnostic test kit is expected to be 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 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 finalization of its TB assay, which is expected to be launched by Q3 of 2018. The company expects to turn to its CT assay subsequent to that. As a result, the CT assay will not be available until after 2018.

**Truelab™ Real Time micro PCR System (Molbio Diagnostics Pvt. Ltd. (A Tulip Group – Bigtec labs partnership))**

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, and malaria (both P. falciparum) are currently available, and assays for CT, NG, and HIV viral load, among others, are in development. The company may also develop assays for TV and HPV.

The testing process begins with sample collection (blood, serum or plasma) followed by extraction, which uses the Trueprep™ MAG Sample Prep Device and Trueprep Mag sample prep kits. The extraction process takes about 20–25 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 Real Time micro PCR Analyser, pictured below. Thermal cycling takes place automatically within the analyser.

During amplification, the Truenat micro PCR chip exponentially releases fluorophores. 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 Truenat 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.
Test results are automatically stored in the analyser memory (up to 5,000 results), can be printed, and transported wirelessly to any server/compatible device by Wi-Fi, GPRS, Bluetooth or even SMS.

Although tests for CT and NG are in the pipeline, no launch date has been set by the company.

**Alere™-i Platform (Alere Inc.)**

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

Figure 14. Alere™-i Platform

Alere™-i Influenza A & B was the first molecular test to be granted CLIA waiver in the U.S and delivers actionable, lab-accurate results in less than 15 minutes on a user-friendly platform. Alere™-i Strep A was launched in 2015, and with test results in 8 minutes or less, is the fastest CLIA-waived molecular Strep A test. Alere™-i RSV (respiratory syncytial virus) was launched in October 2016, and is the first molecular test that can be used at the point-of-care to detect RSV in 13 minutes or less.

Tests for *C. difficile* and CT/NG are currently in development.

**Microwave-Accelerated Metal-Enhanced Fluorescence Test (MAMEF)**

Scientists at the University of Maryland Baltimore County and Johns Hopkins, led by Chris D. Geddes, have developed a MAMEF test for CT and gonorrhea. The test combines lower-power microwave acceleration to hasten biological reactions, reducing assay run times over 1000-fold, with metal-enhanced fluorescence, whereby the close proximity of silver nanoparticles amplifies the fluorescence of labels in the near field (59). Per the developers, it is this combination of enhanced fluorescence emission coupled with a significantly reduced assay turnaround time that makes MAMEF a powerful technology for point-of-care testing.

**CT MAMEF-based detection**

Two MAMEF assays have been evaluated for the detection of CT DNA from vaginal swabs compared to those of NAATs. The overall rates of agreement with NAAT results for the assays were 89.3% (16S rRNA assay) and 91.0% (cryptic plasmid assay), and the authors of the study concluded that the “sensitivity,
specificity, and rapid detection of the plasmid-based MAMEF assay appear to be suited for clinical POC testing” (60).

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

![Flow Chart of Sequence of Steps and Timeline for a MAMEF-based CT Test](image)

**Figure 15. Flow Chart of Sequence of Steps and Timeline for a MAMEF-based CT Test**

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

While the MAMEF-based test is clearly fast and inexpensive, in its present configuration, it would likely not be appropriate for use at the point of care in resource-limited settings. In general, one of the advantages of NAAT-based approaches in those settings is that many such assays have been evaluated and are well-validated; the assays are available in quality-assured kits; and clinicians are comfortable interpreting the results. In order for the MAMEF-based assays to be suitable for use in resource-limited settings, they would likely need to be further optimized, simplified and standardized for use in laboratories that are very basic.

In this regard, Dr. Geddes and his team launched “Lyse-it LLC™”, a Maryland-based biotechnology company that has successfully commercialized the lysing component of the technology. Lyse-it’s commercialization has enabled the lysing component of the technology to be significantly simplified and readily available as a tool for researchers and clinicians alike. Lyse-it™ LLC sells starter lysing kits, including the microwave hardware, for about US$700. Disposable lysing slides are subsequently

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9 Images and description from Melendez et al, pp 2914-15. (60)
available to lyse samples, as a consumable when the hardware is in place. Per the company, this enables the rapid and efficient lysing of samples, at significantly lower cost, more easily and more quickly than any other lysing technology available today.

**Gonorrhea MAMEF-based detection**

A MAMEF-based assay, similar to the CT assay, has now been developed for detection of gonorrhea. The assay targets the *PorA* gene of *N. gonorrhoeae* and has shown excellent specificity against pathogens commonly found in genital specimens. Preliminary studies with dry vaginal swabs suggest that the sensitivity of the assay is approximately 83% and specificity is 93% (61). A blinded comparison evaluation of the NG MAMEF-based assay with vaginal and urethral swabs is currently underway.

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

**MobiNAAT (Johns Hopkins University BioMEMS lab)**

Researchers at Johns Hopkins University BioMEMS laboratory have created a new smartphone DNA test capable of diagnosing CT. Early results in a small study on the platform are very promising. Additional validation testing at Johns Hopkins is the next step for this device followed by field testing.

The device, called MobiNAAT, is a low-cost NAAT platform that integrates sample preparation, DNA amplification and data processing into one instrument the size of a coffee mug (pictured below). The device is battery-powered and uses a microfluidics cartridge to identify CT DNA in genital swab samples. Results are analyzed via a smartphone that allows the user to control the testing and process data with an app. Because the smartphone diagnostic is automated, it does not require a lab technician to process results.
Figure 16. MobiNAAT Platform

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

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

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

*Trichomonas Vaginalis*

Like CT, NG and syphilis, TV is a significant health problem globally. The WHO estimates that there were about 143 million new cases of TV worldwide in 2012 (3). Diagnosis of TV infection has traditionally been performed by microscopy of vaginal secretions, but this technique requires immediate evaluation of a wet preparation and is only about 50% sensitive when compared with culture or NAAT (62,63).1

Diagnosis of TV in men is typically from wet mount with microscopic visualization of the parasites on slide preparations from urethral secretions (64). Modern nucleic acid-based testing for TV is convenient, accurate, and more sensitive than traditional methods. Like testing for CT and NG, there are a number of reliable laboratory-based molecular systems for TV testing. These include the APTIMA *Trichomonas vaginalis* Assay (Hologic), the BD ProbeTec *Trichomonas Vaginalis* Qx Amplified DNA Assay (BD) and the Affirm VPIII Microbial Identification Test (BD), which also detects *Gardnerella vaginalis* and *Candida albicans*.

In addition to the above molecular tests, there is at least one rapid diagnostic test for detection of TV: the OSOM® *Trichomonas* Test (Sekisui Diagnostics), which studies have shown performed reasonably well when compared to wet mount and culture (65).

Of the companies developing assays for molecular platforms discussed earlier in this report in connection with tests for CT and NG, GeneXpert® (Cepheid) has launched an assay for TV (discussed
earlier in this report). In addition, Atlas io™ has a TV assay in its development pipeline for 2017. Finally, Quidel Corporation (Quidel) has launched two near POC TV assays, one for its AmpliVue® platform and another for its Solana® platform. These are described below.

AmpliVue® (Quidel Corporation)

Quidel is developing assays based on the company’s “instrument-free” molecular diagnostic platform (the AmpliVue®). The platform (pictured below) combines the company’s proprietary helicase-dependent amplification (HDA) technology (which uses a helicase enzyme to unwind double-stranded DNA into single strands, thus eliminating the need for a thermocycler and providing a method for assay development) with qualitative lateral-flow detection housed in a cassette. The company emphasizes that as compared to other methods of isothermal amplification, HDA uses a probe-based detection method, thereby resulting in greater specificity. In addition, because HDA only detects amplicons, rather than turbidity caused by amplification as with Loop Mediated Amplification (LAMP), it provides assurance that amplification of only the intended target will be identified as positive. HDA can also multiplex in a single tube.

Per Quidel, HDA is both simple because it is isothermal, requiring only one temperature (64°C), and robust because it tolerates crude samples. At the same time, the platform is low-cost as it only requires a heat-block for amplification.

Figure 17. AmpliVue® Platform

The typical workflow for a test (C. difficile, in this example) on the AmpliVue® platform is illustrated below. It should be emphasized that this workflow is essentially identical to that for the TV assay except that the amplification time for that assay is 25 minutes, which means that the total turnaround time for the test is 45 minutes.
The testing process uses manual specimen preparation (dilution of sample and pipetting of same followed by heat lysis), with the entire process, including heat lysis, taking about 60 – 90 minutes (depending on the assay). The platform is designated as moderately complex by the FDA. The company believes the platform is ideal for small to medium-sized microbiology labs not currently performing molecular tests as well as labs in physicians’ offices that can operate moderately-complex assays. In resource-limited settings, this would likely translate into use at district hospitals and above.

The first assay developed for the AmpliVue platform is for *C. difficile*, which is both CE marked and FDA approved. Similarly, five additional assays are CE marked and FDA cleared – assays for herpes simplex (cutaneous and mucocutaneous) lesion specimens (HSV 1+2), Group B Streptococcus, Group A Streptococcus, TV and Bordetella pertussis. The assay for detection of TV in women (using vaginal specimens) was launched in 2015 and is FDA cleared.

The AmpliVue® TV assay has been independently evaluated and performed well against saline microscopy, TV culture (InPouch) and the APTIMA TV assay, identifying all of the culture and wet mount positive cases of TV. Specificity for all women was 98.2% (66). The overall agreement against the APTIMA TV assay was 97.8% (66). Sensitivity of the AmpliVue® assay compared with the APTIMA TV assay was 90.7%; specificity was 98.9% (66).

**Solana® (Quidel)**

In addition to the TV assay for its AmpliVue® platform, Quidel has also developed a qualitative TV assay for its Solana® platform, pictured below.
Like the AmpliVue® platform, the Solana® platform uses the company’s proprietary HDA technology described above. The Solana® is a compact benchtop instrument measuring 9.4” x 9.4” x 5.9” that allows for rapid detection of TV from vaginal swabs and female urine specimens obtained from symptomatic and asymptomatic females. Turnaround time for the test is 35 minutes. In addition, the platform permits operators to batch up to 12 samples in a single run, allowing for testing scale-up as needed.

As illustrated below, the workflow for the Solana® is similar to that for the AmpliVue® platform.

The Solana® platform is designated as moderately complex by the FDA. The company believes the platform is ideal for small to medium-sized microbiology labs not currently performing molecular tests as well as labs in physicians’ offices that can operate moderately-complex assays. In resource-limited settings, this would likely translate into use at district hospitals and above.

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

The Solana® TV assays has been independently evaluated and performed well compared to the reference assays. Gaydos et al collected vaginal swabs and urine specimens from 501 asymptomatic and
32 symptomatic women (67). 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 (67).

**Human Papilloma Virus**

In the United States, the Papanicolaou (Pap) test has been the gold standard for detecting cervical cancer in women over 30 years of age, most of which is caused by HPV. However, the U.S. Food and Drug Administration (FDA) recently recommended that the cobas® HPV Test (Roche Diagnostics) should be the first line of screening in the United States. 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 RealTime High Risk HPV test from Abbott or the Qiagen digene HC2 HPV Test. However, Qiagen has also introduced an HPV assay, the careHPV™ Test, a molecular diagnostic test for HPV that is designed for use in low resource settings. Performance of the assay has been good relative to testing using either the Pap test or VIA (68,69).

Of the diagnostic platforms discussed earlier in this report, GeneXpert® (Cepheid) has a commercially available HPV assay. Several peer-reviewed, published studies of the assay found strong performance relative to laboratory-based reference standards in women referred for colposcopy (70,71,72,73). Cepheid also has an HPV assay in the pipeline for its new, smaller Omni platform, which is likely to be in the market in 2018.

In addition, GENOMICA has developed an HPV assay for use on its NEDxA platform, which is described below

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

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

---

10 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.
The NEDxA platform accepts dry endocervical swabs from females; no DNA extraction is required. Commencing in June, the system will also accept ThinPrep and SurePath™ liquid samples. The NEDxA cartridge, pictured below, is based on microfluidic technology.

NEDxA is a closed system; all reagents are included in the cartridge, which can be shipped and stored at room temperature (from 4°C to 25°C). The sample is inserted directly into the cassette; no pipetting is required. The sample is then moved to the PCR chamber where the DNA amplification takes place. Following the PCR process, the amplified product moves to the detection chamber for hybridization of targets with specific probes. Reagents are then pumped into the detection chamber for incubation of the solution. Finally, an electric potential is applied through 64 Au electrodes to obtain an instant electrochemical detection. Turnaround time for the HPV test is approximately 75 minutes.
The NEDxA system is easy to use. The operator selects the analysis to be done, places the cartridge on a tray, and starts the test run. The running test screen displays the test status and test time remaining. Results are reported automatically. The NEDxA platform provides LAN connection and a USB port; the system is compatible with LIMS systems.

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

Additional Technologies in the Pipeline for STIs

**cobas® Liat® System (Roche Molecular Diagnostics)**

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

The System currently has assays clinically validated, CE-IVD marked and FDA cleared for the detection of Influenza A/B, Strep A, & Influenza A/B and RSV. All three assays have received CLIA Waiver from the FDA. A CLIA Waiver determines that there is little risk of error due to the simple use of the test, and that no special training is required. Additional assays are under development, including for HIV.

![cobas® Liat® System](image)

**Figure 23. cobas® Liat® System.** *The cobas® Liat® Analyzer compresses the assay tube to move the sample and selectively release reagents from tube segments, under temperature controlled conditions.*

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

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

![Figure 24. cobas® Liat® Test Procedure](image)

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

No price information is available on either the instrument or the individual assays for resource-limited settings. Roche launched the system in the US at the end of 2014 and is planning to expand globally. Although the company is considering STI assays for the cobas® Liat® System, no decision has yet been made about which assays might be added and when. The stability profile for future assays is also not available at this time.
PanNAT® Platform – (Micronics, Inc.)

Micronics, Inc., a subsidiary of Sony Corporation of America, has developed the PanNAT® system (pictured below), which is a small, portable microfluidic platform for near-patient use in in vitro molecular diagnosis of infectious diseases in resource-limited settings. It is a fluorescent-based reader capable of processing individual, disposable, assay-specific test cartridges, each of which is designed to perform a single and/or multiplexed nucleic acid assay. The cartridge includes all necessary reagents on board. The system is lightweight, mains-powered, can store up to 1000 test results before prompting the user to download or delete results, and can provide results in approximately 1 hour, depending upon assay parameters. Battery-operation and WiFi-enabled options will be available with results output to USB, local network, LIS/HIS and printer.

Figure 25. PanNAT® Platform and Cartridge

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

Micronics has a number of tests in development, including certain STIs, respiratory and healthcare-associated Infections (the specifics of which are confidential), an assay for Shiga toxin-producing E-coli, as well as other infectious disease diagnostics. Commercial launch of a first test and system is targeted for 2017.

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 and ChipCare assays for simultaneous detection of HIV and syphilis and the PanNAT® platform, all of which use microfluidic techniques, the MAMEF test for CT, which uses microwave acceleration and metal-enhanced fluorescence, the AmpliVue® platform from Quidel, which has unique HDA technology, and the AlertID, 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 (74).¹¹

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

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

Microfluidic Sample Preparation

There are a variety of microfluidic sample preparation approaches. These include mechanical, magnetic, electrokinetic, immunoaffinity, and chemical techniques. The approach used for any particular diagnostic will depend on both the sample type (e.g., whole blood, sputum) and the target analyte. As an example, immunoaffinity techniques can be used in microfluidic sample processing when an antibody with specificity for the target is available. Micro- or nanoparticles, particularly magnetic particles, functionalized with antibodies can be mixed with the sample to bind the analyte and then separated downstream. For example, one piece of recent research has demonstrated that magnetic particle binding can detect HIV capsid protein p24 as low as 0.1 pg/ml as part of a bio-barcode detection system (75). 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.

¹¹ This section of the report draws significantly from the publication of Damhorst et al. (74).
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. For example, researchers at the University of California Berkeley have developed a mobile phone-based microscope by pairing a smartphone with a 3D-printed plastic base. Called the video CellScope, the device, pictured below, uses video to detect and quantify infection by parasitic worms in blood. Turnaround time is approximately 2 minutes (76).

![Figure 26. Video CellScope Device](image)

Some of the most common optical detection methods are fluorescence, absorbance and chemiluminescence (77). Fluorescence is the most common of these used in diagnostics, including in microscopy, flow cytometry and PCR. Micro-scale approaches often use fluorescence detection, frequently incorporating a laser or light emitting diode (LED) for excitation of the tag. For example, fluorescence microscopy has been a standard method of detecting mycobacterium bacteria (TB) in sputum samples. More recently, LED-based microscopy has increased access to microscopy in resource-limited settings.

Fluorescence is also commonly used as an indicator in NAAT-based testing, either as a DNA intercalating dye or as part of a fluorophore-quencher system conjugated to probe DNA. One example is the digital PCR device from SlipChip, which has been shown to be capable of detecting 37 copies/mL of viral RNA with HIV and HCV samples (78,79). Currently the device is used only in research in clinical labs and has not been commercialized. However, many NAAT-based platforms use isothermal amplification (e.g., Ustar’s CPA platform and the Alere™-i platform), and these have been commercialized, although none is yet an ultra-portable, handheld device.
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 (80).

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 (81,82). 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 (83,84), and impedance-based cell counting approaches have also been used in the context of malaria diagnosis (85,86). 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 (87).

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 three integrated, next-generation diagnostic technologies that have demonstrated proof of principle, and in the case of two of the products, are on the verge of commercialization for use at the point of care.

**Nanotechnology-based POC Diagnostic (BD)**

BD is developing a nanotechnology-based diagnostic platform in an antibody-based (immunoassay) format that provides core-lab (ELISA) sensitivity with a workflow suitable for use at the POC. The company indicates that the platform can detect bacteria, parasites, proteins and oligomers in a multiplexed way in complex samples, including blood or stool. The platform requires little or no sample preparation or wash steps, and provides a “sample in, results out” workflow that can be used by minimally-trained operators.

The company points out the following key attributes of the platform:

- **Simple sample processing**: Technology is able to detect targets in complex samples without additional washing or purification steps. Near-infrared light is used to acquire the signal, which reduces or eliminates fluorescent background from complex or dirty biological samples.
- **Multiplexing**: Technology has the ability to simultaneously detect multiple targets within the same sample.
- **Quantitation**: Technology is fully quantitative; quantitation over 4 logs of target concentration has been demonstrated.
- **Inexpensive**: Assay uses dried immunoassay reagents in a simple, tube-based disposable that can be produced at very low cost; the technology incorporates a low-cost reader that is compatible with a large test menu and multiple sample types.
- **Environmental Impact**: The nanoparticles used in the technology are based on standard chemistries with no toxicity concerns and will be contained in a closed vessel, reducing the risk of human contact with the particles and the sample.

BD has also developed prototype instruments (one of which is pictured below) to support its clinical assay development efforts.
At present, BD has developed a wide array of assays for its nanotechnology platform, including a CT assay that uses urine as its sample. BD indicates that these assays typically perform better than (or at least equivalent to) immunoassays conducted in central laboratories. However, the assays have been developed for research use only, and there is no current plan to commercialize any of them.

**IDAAlert (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.
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.\textsuperscript{12} The diagnostic performance of the HSV-2 biochip tested was determined by testing a panel of serum samples \((n = 60)\) and comparing results to data generated on clinically validated HSV-2 serological assays (DiaSorin LIAISON® HSV-2 and Focus HerpeSelect® 2 IgG ELISA). The sensitivity and specificity of the IDAlert HSV-2 biochip test was 100\% compared to the LIAISON® test. The sensitivity and specificity of the system were 96.7\% and 100\%, respectively, compared to the HerpeSelect® 2 assay.

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

**Paper-Based Diagnostic (Diagnostics for All)**

Diagnostics for All (DFA) uses patterned paper technology to create diagnostic devices for use in resource-limited settings. The technology, developed by Professor George Whitesides at Harvard University, patterns channels and assay zones (or wells) of water-repellant materials into a piece of paper about the size of a postage stamp, as illustrated below. Biological and chemical assay reagents are deposited into the wells. When biological specimens (e.g., blood, urine, saliva) are applied to the device, the paper wicks the sample through the channels to the assay zones. No external pumps or power are required. Upon contact with the specimen, the assay zone quickly changes color and results are read by comparing the color change with a reference scale printed on the device. After use, the device can be disposed of by incineration. It will also be possible to embed DFA’s patterned paper-based devices with electrical circuitry to enable resistive heating, electrochemical assays or for initial processing of assay results.

![Image of DFA technology](image)

**Figure 30. Patterns channels and assay zones of DFA technology.**

\textsuperscript{12} The study results were presented by Aalto Bio Reagents in a poster at the Lab-on-a-Chip Microfluidics and Microarrays World Congress held from 26 – 28 September 2016 in San Diego, California and shared with the author in personal correspondence.
Advantages of the technology include that it is low cost, as paper is less expensive than other materials typically used in diagnostic devices. In addition, the technology is lightweight and durable. There are no mechanical parts and no auxiliary equipment, electricity or laboratory infrastructure required to run the test. Tests can generally be run using fingerstick whole blood, with no need for syringes, clean water, etc.

Per DFA, the patterned paper devices can be manufactured at scale using existing high-volume, low capital techniques and equipment. In addition, the technology can be applied to a variety of tests: immunoassays, electrochemical assays, clinical chemistry assays and molecular diagnostics.

There has been a field evaluation of a fingerstick version of DFA’s alanine aminotransferase (ALT)-only test on 600 patients in Vietnam. The goals of the study were to assess the operational feasibility, inter-operator variability, lot-to-lot variability, device failure rate, and device accuracy in order to utilize the results to modify the device as needed. The results of the study, which were published in 2013, found high inter-operator agreement for visual reading of results obtained in a target clinical population, as performed by local healthcare workers, which indicated that the device operation and reading process were both feasible and reproducible. However, there were issues around ALT bin cut-off accuracy and lot-to-lot variability that suggested further optimization of the device is required (87).

Further, and very important for implementation and use of the DFA device in resource-limited settings, the training required for the healthcare workers in this study was intensive, which is unlikely to be feasible in real-world operation of the test. The authors of the study concluded that: “[a] thorough understanding of the minimal training requirements for novice users will ultimately be key to understanding the range of clinical environments in which this test can be used – whether that be in centralized clinics as performed by trained staff, decentralized clinical settings as performed by minimally trained health-care workers, or even at home as performed by patients themselves” (88).

The Limits of Diagnostic Technology

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

These weaknesses suggest not only the in-country need for training of test operators and service and maintenance contracts for diagnostics, but also suggest that the following operational specifications for POC diagnostic assays/platforms should be prioritized:

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

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

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

**Data Capture, Connectivity and Data Export.** If combined with a reader (either internal or external), the reader must store patient results, and its output needs to be compatible with centralized data aggregation and analysis. In order to monitor test performance, a GPS/GPRM modem, preferably internal to the reader, should be incorporated, and full data export capabilities over mobile phone networks should be a minimal standard.

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

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

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

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

**Sample Capacity, Throughput and Time to Result.** These are important specifications for new POC diagnostic assays, but there is no single specification for capacity, throughput and turnaround time (TAT) that will fit all settings. Rather, these specifications will depend on the volume of testing and TAT for each assay at the target use setting (e.g., district hospital, health center). The ability to give same-day results is critical and must be considered with respect to each assay; otherwise the value of a POC test is substantially diminished. The working day in many health center settings is greatly abbreviated (6 hours or less), and the TAT for a diagnosis must also allow time for the pre-analytic activities (e.g., patient registration) and post-analytic activities (e.g., clinical interpretation and treatment) necessary to provide a complete service to the patient within one working day.
These factors, along with required technical performance, must be considered and prioritized by developers of diagnostics intended for use at or near the point of patient care in resource-limited settings.

Conclusions

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

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

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

REFERENCES


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


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


ANNEX A

Combined HIV/Syphilis tests – available and pipeline
POC HIV/Syphilis Tests – Available and Pipeline*

<table>
<thead>
<tr>
<th>Year</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>Standard Diagnostics HIV/Syphilis Duo Rapid Test</td>
</tr>
<tr>
<td></td>
<td>Chembio DPP® HIV-Syphilis Assay</td>
</tr>
<tr>
<td></td>
<td>MedMira Multiplo Rapid TP/HIV Antibody Test</td>
</tr>
<tr>
<td></td>
<td>Biolytical INSTI Combined HIV/Syphilis Test</td>
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<tr>
<td>2014</td>
<td>CTK Biotech OnSite™ HIV/Syphilis</td>
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<tr>
<td>2015</td>
<td>Junco Labs mChip Assay</td>
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<tr>
<td>2016-2017</td>
<td>ChipCare HIV/syphilis Assay</td>
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<td>2018</td>
<td></td>
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</table>

*Estimated as of March 2017 - timeline and sequence may change

--- No market launch date set by company.
ANNEX B

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

<table>
<thead>
<tr>
<th>Test name</th>
<th>HIV/Syphilis Duo Rapid Test</th>
<th>DPP® HIV-Syphilis Assay</th>
<th>Multiplo Rapid TP/HIV Antibody Test</th>
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</thead>
<tbody>
<tr>
<td>Company</td>
<td>Standard Diagnostics, Inc. (Republic of Korea)</td>
<td>Chembio Diagnostic Systems, Inc. (United States)</td>
<td>MedMira, Inc. (Canada)</td>
</tr>
<tr>
<td>Type of technology</td>
<td>Rapid immunochromatographic assay, using lateral flow (RDT)</td>
<td>Rapid immunochromatographic assay, using immunofiltration (RDT)</td>
<td>Rapid Vertical Flow (RVF)</td>
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<tr>
<td>Availability</td>
<td>Commercially available</td>
<td>Commercially available</td>
<td>Pipeline (available for research use)</td>
</tr>
<tr>
<td>Output</td>
<td>Qualitative detection of HIV-1, including subtype O, and HIV-2 (combined) and/or syphilis TP</td>
<td>Qualitative detection of HIV-1 and HIV-2 (combined) and/or syphilis TP</td>
<td>Qualitative detection of HIV-1, including subtype O, and HIV-2 (combined) and/or syphilis <em>Treponema pallidum</em> (TP)</td>
</tr>
<tr>
<td>Antigen type (HIV)</td>
<td>Recombinant HIV-1 capture antigen (gp41), recombinant HIV-2 capture antigen (gp36) and recombinant HIV-subtype O antigen</td>
<td>Unspecified mix of HIV-1/2 antigens</td>
<td>Synthetic HIV peptides gp36, gp41, gp120 and HIV group O</td>
</tr>
<tr>
<td>Antigen type (syphilis)</td>
<td>Recombinant TP antigens (17kDa)</td>
<td>Unspecified recombinant TP antigen</td>
<td>Recombinant TP antigens 15kDa, 17kDa, 47kDa</td>
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<tr>
<td><strong>Sensitivity</strong></td>
<td><strong>Anti-HIV</strong> 100%</td>
<td>98.7%</td>
<td>99.6%</td>
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<td></td>
<td><strong>Anti-TP</strong> 100%</td>
<td>94.3%</td>
<td>95.8%</td>
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1 As reported by the company in product insert.
### Specificity

<table>
<thead>
<tr>
<th></th>
<th>Anti-HIV</th>
<th>Anti-TP</th>
<th>Sample type</th>
<th>Sample storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100%</td>
<td>100%</td>
<td>Whole blood (fingerstick or venous), serum or plasma</td>
<td>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</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>100%</td>
<td>Whole blood (fingerstick or venous), serum or plasma</td>
<td>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)</td>
</tr>
<tr>
<td></td>
<td>98.2%</td>
<td>98.0%</td>
<td>Whole blood (fingerstick or venous), serum or plasma</td>
<td>Fingerstick blood must be tested immediately; venous blood may be stored for up to five days at 2 °C–8 °C (36 °F–46 °F); if storage of venipuncture whole blood specimen is required for more than five days, plasma should be separated from the blood and stored at -20 °C (-4 °F or) below.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serum/Plasma:</td>
<td>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 more than 5 days, serum/plasma specimens should be stored at -20°C or below.</td>
</tr>
</tbody>
</table>

### Sample type

- 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 from the blood and stored at -20 °C (-4 °F or) below.

### Time to result

- ~15–20 minutes
- ~10 minutes
- ~3-minute test procedure; results must be read immediately.
| Protocol complexity – steps required | For fingerstick blood: (i) remove the DPP® HIV-Syphilis test device from its pouch; (ii) before collecting sample, write sample ID on the sample buffer bottle with the black cap; (iii) remove (unscrew) the white cap, keeping the black cap screwed onto the white part of the cap; (iv) obtain a fingerstick blood sample according to normal laboratory practices; (v) touch the sample loop to the drop of blood allowing the opening of the loop to fill with blood; (vi) insert the sample loop into the sample buffer bottle with the black cap such that the loop is touching the bottom of the bottle; (vii) snap and twist the shaft at the break notch to dislodge the loop into the bottle; (viii) replace the black/white cap assembly onto the bottle and gently shake the bottle for 10 seconds; (ix) remove (unscrew) the black cap keeping the white cap screwed onto the sample buffer bottle; invert the sample buffer bottle containing the collected sample and hold it vertically (not at an angle) over the sample + buffer well 1 on the test kit; (x) slowly add two drops into the sample + buffer well 1; (xi) wait five minutes (by which time the blue and green coloured lines in the rectangular test and control window should have disappeared; if not, discard the test device and repeat the procedures); (xii) add four drops of the reaction mixture into the sample + buffer well 1; (xiii) dispense the remaining buffer, in drops, from the vial of universal buffer onto the InstantGold cap and allow the solution to be absorbed; (xiv) read test results immediately. | For fingerstick whole blood collection and use: (i) place sample tube in a secured rack on a flat surface; (ii) add five drops from the vial of Universal Buffer to the sample tube (included); (iii) obtain a fingerstick blood sample according to normal laboratory practices using the sterile lancet provided with the test; (iv) use the auto-fill pipette provided with the test to collect one drop of blood from the fingerstick site by touching the tip of the pipette to the blood sample in a horizontal position (the blood sample is automatically drawn to the black fill line); (v) place the tip of the auto-fill pipette into the universal buffer in the sample tube [prepared in step (ii) above]; (vi) squeeze the bulb to empty the blood sample into the tube; (vii) discard the auto-fill pipette; (viii) hold the sample tube and gently tap the side of the tube near the bottom until the mixture becomes a clear reddish colour; (ix) pour the entire contents of the sample tube into the well of the test cartridge; (x) allow the specimen to be absorbed; (xi) place the InstantGold cap on the test cartridge; (xii) dispense the remaining buffer, in drops, from the vial of universal buffer onto the InstantGold cap and allow the solution to be absorbed; (xiii) remove the InstantGold cap, waiting for the solution to be completely absorbed; (xiv) read test results immediately. |
drops of running buffer (green cap) to buffer well 2 (a reddish colour should begin to flow across the strip within 2–3 minutes); (xiii) read the test result 10–15 minutes after the addition of the running buffer to buffer well 2

For venous whole blood, serum or plasma:
(i) remove the DPP® HIV-Syphilis test device from its pouch; (ii) obtain a venous blood, serum or plasma sample according to normal laboratory practices; (iii) before adding the sample, write the sample ID on the sample buffer bottle with the black cap; (iv) remove (unscrew) the white cap, keeping the black cap screwed onto the white part of the cap; (v) add 10 µL venous blood, serum or plasma sample using a calibrated pipette into the sample buffer bottle with the black cap such that the pipette tip is touching the bottom of the bottle; (vi) replace the black/white cap assembly onto the bottle and gently shake the bottle for 10 seconds; (vii–xiii) the remaining steps are the same as for the fingerstick blood sample

For venipuncture whole blood collection and use: (i) use standard venous phlebotomy procedures to collect a whole blood sample; (ii) place the sample tube (provided) in a secured rack on a flat surface; (iii) add five drops from the 30 mL bottle of Universal Buffer (provided) to the sample tube; (iv) use the transfer pipette provided to collect specimen from the specimen collection tube; (v) add one drop of whole blood into the sample tube prepared in step iii above; (vi) hold the sample tube and gently tap the side of the tube near the bottom until the mixture becomes a clear reddish colour; (vii) pour the entire contents of the sample tube into the well of the test cartridge; (viii) allow specimen to be absorbed; (ix) place the InstantGold cap on the test cartridge; (x) dispense 12 drops from the 30 mL bottle of Universal Buffer onto the InstantGold cap and allow the solution to be completely absorbed; (xi) remove the InstantGold cap and wait for solution to be completely absorbed; (xii) add three drops of universal buffer to clarify results; (xiii) read test results immediately

For serum/plasma: (i) apply three drops of Universal Buffer to the centre of the test cartridge; (ii) allow the buffer to absorb completely; (iii) apply one drop of serum or plasma specimen to the centre of the test membrane; (iv) wait for the
specimen to absorb completely before proceeding to the next step; (v) place the InstantGold cap on the test cartridge; (vi) dispense 12 drops of Universal Buffer onto the InstantGold cap; (vii) allow the solution to be completely absorbed; (viii) remove the InstantGold cap; (ix) wait for the solution to be completely absorbed; (x) add three drops of Universal Buffer to clarify results; (xi) read test results immediately.

<table>
<thead>
<tr>
<th>Read window</th>
<th>Results should not be read more than 20 minutes after adding assay diluent</th>
<th>15 minutes after running buffer is added to sample</th>
<th>N/A; results should be read immediately.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shelf life of test kit</td>
<td>24 months</td>
<td>24 months</td>
<td>18 months</td>
</tr>
<tr>
<td>Storage requirements</td>
<td>1 °C–30 °C (test devices and diluent)</td>
<td>2 °C–30 °C (test devices and buffers)</td>
<td>2 °C–30 °C (test devices and buffers)</td>
</tr>
<tr>
<td>Test kit components</td>
<td>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</td>
<td>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</td>
<td>Multiple 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</td>
</tr>
<tr>
<td>Not included in test kit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>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</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>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”</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>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”</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
External Test Controls are available as an accessory Cat. No. 815311006074

<table>
<thead>
<tr>
<th>Regulatory</th>
<th>WHO Prequalified USAID Waiver List CE-IVD Marked</th>
<th>Submitted to WHO Prequalification Programme in March 2013 USAID Waiver List CE-IVD Marked</th>
<th>Submitted to WHO Prequalification Programme in February 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated pricing</td>
<td>US$ 1.30 – 1.50 per test</td>
<td>US$ 3.50 per test</td>
<td>US$ 2.20 to $4.50 per test. This range is dependent on the packaging format and available volume discount.</td>
</tr>
</tbody>
</table>

N/A = Not available.
As reported in the respective package inserts for the tests.
<table>
<thead>
<tr>
<th>Test name</th>
<th>INSTI HIV/Syphilis Multiplex Test</th>
<th>OnSite™ HIV/Syphilis Ab Combo Rapid Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company</td>
<td>BioLytical Laboratories (Canada)</td>
<td>CTK Biotech, Inc. (United States)</td>
</tr>
<tr>
<td>Type of technology</td>
<td>Immunofiltration (flow through)</td>
<td>Lateral flow chromatographic immunoassay</td>
</tr>
<tr>
<td>Availability</td>
<td>Commercially available</td>
<td>Commercially available</td>
</tr>
<tr>
<td>Output</td>
<td>Qualitative detection of HIV-1 and HIV-2 (combined) and/or syphilis TP</td>
<td>Qualitative detection of HIV-1 and HIV-2 (combined) and/or syphilis TP</td>
</tr>
<tr>
<td>Antigen type (HIV)</td>
<td>Recombinant gp36 (HIV-2) and gp41 (HIV-1)</td>
<td>Unspecified antigens to HIV 1 &amp; 2</td>
</tr>
<tr>
<td>Antigen type (syphilis)</td>
<td>Recombinant p17-p47 fusion protein</td>
<td>Unspecified recombinant TP antigens</td>
</tr>
<tr>
<td>Sensitivity¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HIV</td>
<td>100% (136/136 positive)</td>
<td>99%</td>
</tr>
<tr>
<td>Anti-TP</td>
<td>96.5% (55/57 positive)</td>
<td>98.1%</td>
</tr>
<tr>
<td>Specificity¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HIV</td>
<td>100% (874/874 negative)</td>
<td>99.2%</td>
</tr>
<tr>
<td>Anti-TP</td>
<td>99.8% (991/993 negative)</td>
<td>100%</td>
</tr>
<tr>
<td>Sample type</td>
<td>Whole blood (fingerstick or venous), serum or plasma</td>
<td>Whole blood (fingerstick or venous), serum or plasma</td>
</tr>
<tr>
<td>Volume of sample required</td>
<td>50 µL</td>
<td>20 µL</td>
</tr>
<tr>
<td>Sample storage</td>
<td>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.</td>
<td>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.</td>
</tr>
<tr>
<td>Time to result</td>
<td>60 seconds, from addition of sample to sample diluent</td>
<td>15 minutes from addition of sample diluent</td>
</tr>
<tr>
<td>Protocol complexity – steps required</td>
<td><strong>For fingerstick blood,</strong> (i) obtain a fingerstick blood sample according to normal laboratory practices and instructions in package insert using the sterile lancet provided; (ii) as the blood bubbles up, hold the pipette (provided) horizontally and touch the tip of the pipette to the blood; (iii) transfer the blood held in the pipette to the sample diluent vial (solution 1); (iv) align the tip of the pipette with the sample diluent vial and squeeze the bulb to dispense the sample; (v) tear open the pouch and carefully remove the membrane unit without touching the centre well; (vi) place the membrane unit on a level surface (for sample identification purposes the tab of the membrane unit may be labelled with the patient’s name)</td>
<td><strong>For whole blood,</strong> (i) collect specimen (either venous or fingerstick blood) into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®); (ii) when ready to test, open the pouch at the notch and remove the device and place the test device on a clean, flat surface; (iii) label the device with the specimen’s ID number; (iv) fill the capillary tube with specimen (about 20 µL) not to exceed the specimen line on the tube; for better precision, transfer specimen using a pipette capable of delivering a 20 µL volume; (v) holding the capillary tube vertically, dispense the entire specimen into the center of the sample well making sure that there are no air bubbles; (vi) immediately add 2 drops (60 – 80 µL) of sample diluent to the</td>
</tr>
</tbody>
</table>
or number); (vii) remix the sample diluent-specimen mixture and pour the entire contents into the centre of the membrane unit well within five minutes after the specimen has been added to the sample diluent vial; (viii) re-suspend the colour developer (solution 2 vial) by slowly inverting to mix the solution thoroughly, continuing this process until careful visual observation confirms that the reagent is evenly suspended; (ix) open the colour developer and add the entire contents to the centre of the membrane unit well (the coloured solution should flow through completely in about 20 seconds); (x) open the clarifying solution (solution 3 vial) and add the entire contents to the centre of the membrane unit well; (xi) immediately read the result while the membrane is still wet

For venous blood, serum or plasma:
(i) obtain a venous blood, serum or plasma sample according to normal laboratory practices; (ii) gather one sealed test pouch containing the membrane unit, and one vial each of the sample diluent (solution 1 vial), colour developer (solution 2 vial), and clarifying 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.
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

<table>
<thead>
<tr>
<th>Read window</th>
<th>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</th>
<th>Fifteen minutes; results should not be read after 20 minutes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shelf life of test kit</td>
<td>15 months</td>
<td>N/A</td>
</tr>
<tr>
<td>Storage requirements</td>
<td>15 °C–30 °C</td>
<td>2 °C–30 °C (unopened pouches)</td>
</tr>
<tr>
<td>Test kit components</td>
<td>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)</td>
<td>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).</td>
</tr>
<tr>
<td>Not included in test kit</td>
<td>HIV-1, HIV-2, TP and negative controls available</td>
<td>HIV Ab positive control; HIV Ab negative control; Syphilis Ab positive control; Syphilis Ab negative control.</td>
</tr>
<tr>
<td>Controls</td>
<td>Test has built-in procedural controls that demonstrate assay validity and adequate sample addition</td>
<td>Test has a built-in procedural control.</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Regulatory</td>
<td>CE-IVD marked</td>
<td></td>
</tr>
<tr>
<td>Pricing</td>
<td>To be determined</td>
<td>N/A</td>
</tr>
</tbody>
</table>

N/A = Not available.
ANNEX C

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

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

<sup>2</sup> As reported by the company from preliminary studies.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-cardiolipin</td>
<td>95% (92–98)</td>
</tr>
<tr>
<td>Anaemia (haemoglobin)</td>
<td>0.2 g/dL (0–25 g/dL measurement range)</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
</tr>
<tr>
<td>Anti-HIV</td>
<td>100% (95% CI, 97–100)</td>
</tr>
<tr>
<td>Anti-TP</td>
<td>90% (87–93)</td>
</tr>
<tr>
<td>Anti-cardiolipin</td>
<td>95% (92–98)</td>
</tr>
<tr>
<td>Anaemia</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Sample type</strong></td>
<td></td>
</tr>
<tr>
<td>Whole blood (fingerstick or venous)</td>
<td></td>
</tr>
<tr>
<td><strong>Volume of sample required</strong></td>
<td>1 µL</td>
</tr>
<tr>
<td><strong>Sample storage</strong></td>
<td>Whole blood is stored in a sample holder; once blood is in mChip holder, it should be tested immediately, but can be stored at ambient temperature (15 °C–30 °C) for up to six hours</td>
</tr>
<tr>
<td><strong>Time to result</strong></td>
<td>15 minutes</td>
</tr>
<tr>
<td><strong>Protocol complexity – steps required</strong></td>
<td>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</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Read window</th>
<th>N/A; results are shown on smartphone screen and may be stored or sent to cloud</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shelf life of test kit</td>
<td>6 months</td>
</tr>
<tr>
<td>Storage requirements</td>
<td>15 °C–30 °C</td>
</tr>
<tr>
<td>Test kit components</td>
<td>Single-use sample holders; individually-pouched, single-use microfluidic chips on which reagents are pre-stored; lancet; package insert</td>
</tr>
<tr>
<td>Not included in test kit</td>
<td>Dongle; smartphone</td>
</tr>
<tr>
<td>Controls</td>
<td>Internal negative and positive control for each test; external quality control kit is available separately</td>
</tr>
<tr>
<td>Regulatory</td>
<td>Pricing</td>
</tr>
<tr>
<td>------------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>US$ 2 per test, US$ 30 mChip device (dongle)</td>
</tr>
</tbody>
</table>

N/A = Not available.

b As reported by the company from preliminary studies.
c Data from field testing in Bangalore, India, 2012–2013.
ANNEX D
POC STI Tests: available and pipeline
POC STI Tests: Available and Pipeline*

**GeneXpert®**
- Cepheid
  - CT, NG, CT/NG

**GeneXpert®**
- Cepheid
  - TV, HPV

**AmpliVue®**
- Quidel
  - TV

**Solana®**
- Quidel
  - CT/NG

**Truelab PCR**
- Molbio/bigTec
  - CT, NG

**NEDxA®**
- GENOMICA
  - HPV

**XenoStrip-Tv™**, **OSOM®**
- RDTs for TV

**Atlas io™**
- Atlas Genetics
  - CT - 2016
  - CT/NG, TV - 2017

**Alere™-i**
- Alere
  - CT/NG

**cobas® Liat Analyser**
- Roche

**PanNAT®**
- Micronics

**GeneXpert®**
- Omni
  - CT/NG, HPV

**RT CPA**
- Ustar
  - GT

---

*Estimated as of March 2017 - timeline and sequence may change.  
----- No market launch date set by company.
ANNEX E

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

________________________
<table>
<thead>
<tr>
<th>PLATFORM</th>
<th>SYSTEM LEVEL</th>
<th>TECHNOLOGY</th>
<th>CT</th>
<th>NG</th>
<th>CT/NG</th>
<th>TV</th>
<th>HPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneXpert®</td>
<td>Multiplex Level 2</td>
<td>PCR-based NAAT</td>
<td>√</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CE-IVD</td>
<td></td>
<td></td>
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<td>FDA</td>
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<td></td>
<td></td>
<td></td>
<td>(FDA, 2018)</td>
</tr>
<tr>
<td>Cepheid</td>
<td></td>
<td>CE-IVD</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>AmpliVue®</td>
<td>Multiplex Level 2</td>
<td>iNAAT-HDA</td>
<td>N/A</td>
<td>N/A</td>
<td>Pipeline</td>
<td></td>
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</tr>
<tr>
<td>Quidel Corporation</td>
<td></td>
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</tr>
<tr>
<td>Solana®</td>
<td>Multiplex Level 2</td>
<td>iNAAT-HDA</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quidel Corporation</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Atlas io™</td>
<td>Multiplex Level 1 (possible)</td>
<td>NAAT, immunoassay and small molecule chemistry</td>
<td>CE-IVD (2016)</td>
<td>N/A</td>
<td>2017</td>
<td>Pipeline</td>
<td>N/A</td>
</tr>
<tr>
<td>Atlas Genetics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEDxA</td>
<td>Multiplex Level 2</td>
<td>PCR</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
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<tr>
<td>GENOMICA S.A.U.</td>
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</tr>
<tr>
<td>PLATFORM</td>
<td>SYSTEM LEVEL</td>
<td>TECHNOLOGY</td>
<td>CT</td>
<td>NG</td>
<td>CT/NG</td>
<td>TV</td>
<td>HPV</td>
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</tr>
<tr>
<td><strong>Alere™ i Platform</strong> Alere Inc.</td>
<td>Multiplex Level 2, Level 1 (possible)</td>
<td>iNAAT – RPA or NEAR</td>
<td>N/A</td>
<td>N/A</td>
<td>2017/2018</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>RT-CPA CT Test</strong> Ustar</td>
<td>Multiplex Level 2</td>
<td>iNAAT – CPA</td>
<td>2019</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Truelab™ RT micro PCR</strong> Molbio</td>
<td>Multiplex Level 2</td>
<td>RT-PCR</td>
<td>Pipeline</td>
<td>Pipeline</td>
<td>N/A</td>
<td>Unknown which of these assays will be developed by the company</td>
<td></td>
</tr>
<tr>
<td><strong>cobas™ Liat</strong> Roche</td>
<td>Multiplex Level 1 (possible), Level 2</td>
<td>PCR-NAAT</td>
<td>Unknown which of these assays will be developed by the company</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PanNAT®</strong> Micronics, Inc.</td>
<td>Multiplex Level 2</td>
<td>NAAT</td>
<td>Unknown which of these assays will be developed by the company</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Level 1 – primary healthcare centre; Level 2 – district hospital; N/A = Not applicable
<table>
<thead>
<tr>
<th>Company</th>
<th>Cepheid</th>
<th>Atlas Genetics</th>
<th>Alere</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Name</td>
<td>GeneXpert® CT, CT/NG</td>
<td>Atlas io™ CT; CT/NG (pipeline)</td>
<td>Alere™ i CT/NG (pipeline)</td>
</tr>
<tr>
<td>Use Setting</td>
<td>Table-top, not portable</td>
<td>Table-top; portable</td>
<td>Table-top; portable</td>
</tr>
<tr>
<td>Specimen</td>
<td>Female and male urine,</td>
<td>Self-collected and clinician-</td>
<td>Female and male urine,</td>
</tr>
<tr>
<td></td>
<td>endocervical swab/</td>
<td>collected vaginal swabs from</td>
<td>endocervical swab/</td>
</tr>
<tr>
<td></td>
<td>patient-collected vaginal swab</td>
<td>symptomatic and asymptomatic</td>
<td>patient-collected vaginal swab</td>
</tr>
<tr>
<td>Steps</td>
<td>~4; sample prep automated</td>
<td>~4; automated sample prep on</td>
<td>~6 simple steps; raw sample added to device</td>
</tr>
<tr>
<td>Time to result</td>
<td>~90 minutes</td>
<td>instrument</td>
<td></td>
</tr>
<tr>
<td>Cold Chain; Reagent stability</td>
<td>No; TBD</td>
<td>Cartridges with reagents stable at 2 – 25°C</td>
<td>No; &gt;12 months</td>
</tr>
<tr>
<td>Power</td>
<td>Mains power required;</td>
<td>Mains power required</td>
<td>AC mains and DC from</td>
</tr>
<tr>
<td></td>
<td>solar power possible</td>
<td></td>
<td>external AC/DC supplied plug pack</td>
</tr>
<tr>
<td>Training</td>
<td>Less than ½ day</td>
<td>Less than one hour; no formal</td>
<td>Less than ½ day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>training required; self-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>explanatory user guide and</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>screens on instrument</td>
<td></td>
</tr>
<tr>
<td>Connectivity</td>
<td>Yes; computer/Internet required; remote calibration</td>
<td>Yes, via middleware</td>
<td>Yes; USB and Ethernet outlets</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>----------------------------------------------------</td>
<td>---------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Equipment Cost ($US); Per test cost</td>
<td>~$17,000 (with 4 modules), but could be higher; $16.20 (CT/NG)</td>
<td>TBD</td>
<td>TBD</td>
</tr>
</tbody>
</table>

Level 1 – primary healthcare centre; Level 2 – district hospital; N/A – Not available; TBD = To be determined
## STI POC Diagnostics Available and in the Pipeline
### Summary Chart
#### Platforms for CT and CT/NG

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

*Level 1 – primary healthcare centre; Level 2 – district hospital; N/A – Not available; TBD = To be determined*
<table>
<thead>
<tr>
<th>Company</th>
<th>Cepheid</th>
<th>Quidel</th>
<th>Atlas Genetics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay Name</strong></td>
<td><strong>GeneXpert®</strong></td>
<td><strong>AmpliVue®</strong></td>
<td><strong>Atlas io™</strong></td>
</tr>
<tr>
<td><strong>Use Setting</strong></td>
<td>Table-top, not portable</td>
<td>Table Top; not portable</td>
<td>Table Top; portable</td>
</tr>
<tr>
<td></td>
<td>Level 2</td>
<td>Level 2</td>
<td>Level 1</td>
</tr>
<tr>
<td><strong>Specimen</strong></td>
<td>Female and male urine, endocervical swab, patient-collected vaginal swab</td>
<td>Vaginal swabs from symptomatic and asymptomatic women</td>
<td>Vaginal swabs and female urine specimens obtained from symptomatic and asymptomatic females</td>
</tr>
<tr>
<td><strong>Steps</strong></td>
<td>~4; sample prep automated</td>
<td>Moderately complex; 12 steps</td>
<td>Moderately complex; 13 steps</td>
</tr>
<tr>
<td><strong>Time to result</strong></td>
<td>~60 minutes</td>
<td>45 minutes</td>
<td>35 minutes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Batch up to 12 samples in a single run</td>
</tr>
<tr>
<td><strong>Cold Chain; reagent stability</strong></td>
<td>Kit storage: 2 – 28C</td>
<td>Cartridge/cassettes: 2 to 30C Buffers: 2 to 8C</td>
<td>Kit storage: 2 to 8C</td>
</tr>
<tr>
<td>Power</td>
<td>Mains power required; can use solar</td>
<td>Mains power required for heat block</td>
<td>Mains power required for heat block and Solana instrument</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------------------------------</td>
<td>------------------------------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>Training</td>
<td>Less than ½ day</td>
<td>Less than ½ day</td>
<td>Less than ½ day</td>
</tr>
<tr>
<td>Connectivity</td>
<td>Yes; computer/Internet required; remote calibration</td>
<td>No</td>
<td>Yes; bi-directional</td>
</tr>
<tr>
<td>Equipment Cost ($US); per test</td>
<td>~$17,000 (with 4 modules, but could be higher; $19.00)</td>
<td>TBD</td>
<td>TBD</td>
</tr>
</tbody>
</table>

Level 1 – primary healthcare centre; Level 2 – district hospital; N/A – Not available; TBD = To be determined
## STI POC Diagnostics Available and in the Pipeline
### Summary Chart
#### Platforms for HPV

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

Level 2 – district hospital