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Multiplexed technologies for sexually transmitted infections: global evidence on patient-centered and clinical health outcomes

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ABSTRACT

Introduction Conventional care packages around screening for sexually transmitted infections (STIs) entail multiple clinic visits and precipitate losses to follow-up. To prevent these losses, multiplexed technologies for STIs (immunochromatographic tests/devices/assays and molecular assays that can screen multiple pathogens or multiple strains of one STI) can yield same-day results in a single visit. Research evidence of patient-centred (preference, satisfaction) and clinical health outcomes (feasibility, case positivity, uptake, impact) has not been synthesised. We conducted a systematic review to fill this gap.

Methods For the period 2009–2020, two independent reviewers searched PubMed and Embase, retrieved 4440 citations and abstracted data from 42 relevant studies.

Results Of 42 studies, 10 (23.8%) evaluated multiplexed immunochromatographic and 32 (76.2%) molecular assays. Outcomes were reported as follows: preference (n=3), satisfaction (n=2), uptake (n=1), feasibility (n=2), case positivity (n=42) and impact (n=11). Screened populations included various at-risk groups. A majority (86.1%–92.4%) of participants preferred (60.2%–97.2%) multiplexed technologies (over conventional testing). Compared with conventional lab-based testing, test uptake improved by 99.4% (hepatitis C), 99.6% (*Trichomonas vaginalis*), 78.6% (hepatitis B) and 42.0% (HIV). Varying case positivities were documented depending on populations screened: HIV (1.8%–29.3%), hepatitis B (1.1%–23.9%), hepatitis C (0.5%–42.2%), *Chlamydia trachomatis* (2.8%–30.2%), *Neisseria gonorrhoeae* (0.0%–30.3%) and *T. vaginalis* (0.0%–32.7%). Regarding impact, 70.0%–100.0% of screened participants were linked to care, with result turnaround times ranging from 14 min (immunochromatographic assays) to 300 min (molecular assays).

Conclusions Compared with conventional lab-based testing, rapid multiplexed technologies were preferred by testees and led to quicker turnaround times for many STIs yielding same-day results thereby allowing to initiate rapid linkages to care. They were further shown to be highly feasible and impactful for detection and treatment facilitation. Based on these promising results, multiplexed technologies offer potential to screen at-risk populations to reduce onward STI transmission worldwide.

Key questions

What is already known?

- A majority of sexually transmitted infections (STIs) are asymptomatic and if left undetected and untreated, they can lead to long-term health complications.
- Multiplexed technologies include both immunochromatographic tests and molecular assays.

What are the new findings?

- Multiplexed technologies were preferred by participants, operationally feasible, impacted detection and treatment of various STIs with same-day results and rapid linkages to care.

What do the new findings imply?

- Immunochromatographic and molecular assays are able to address gaps in the care cascade for screening and treating STIs.
- Our consolidation of research evidence on outcomes that are patient-centred and that can support implementation will aid a variety of stakeholders including healthcare professionals and policymakers.

INTRODUCTION

Diagnosing, treating and managing sexually transmitted infections (STIs) represent key pillars to reduce STI transmission and significant morbidity, and thus represent crucial targets across the spectrum of STI management. Approximately 1 million curable STIs are acquired globally each day leading to on average 376 million STIs being acquired annually worldwide, primarily in resource-limited settings.[1] While a vast majority of these infections are asymptomatic, when left untreated, they can cause lifelong and often serious complications. Evidence from epidemiological studies has shown that commonly occurring STIs such as chlamydia, gonorrhoea and syphilis increase transmission of HIV. Moreover, behaviours associated with acquiring HIV infection increase the risk of acquiring

additional STIs and of worsening severity among those with existing infections.[2]

STIs are conventionally diagnosed and confirmed using laboratory-based tests, considered the reference (gold standard) on account of their high diagnostic accuracy.[3 4] However, they include culture, often entail multiple patient visits due to the longer turnaround time to test results (at minimum two visits: to collect samples and to communicate test results to individuals), and sample transportation; collectively, these multiple requirements associated with lab-based testing precipitate inaction and consequent losses to follow-up.[4 5] Furthermore, they often require substantial laboratory infrastructure used by trained laboratory personnel making them difficult to carry out in remote settings. This puts rural communities with high rates of STI transmission at risk.[6–8] With a rise in the global prevalence of STIs, a shift towards efficient technologies such as multiplexed technologies is needed to enable healthcare providers to screen several STIs both rapidly and accurately and return the result to the patient often in one visit.

Multiplexed rapid screening technologies are of two types primarily: (a) antibody-based immunochromatographic tests/assays/handheld devices and (b) molecular tests/assays. Multiplexed technologies also meet the needs and preferences of testees, primarily by reducing the number of clinic visits, with incumbent time and cost savings. As a result, multiplexed rapid testing, if integrated into routine testing, can optimise treatment linkages thereby minimising losses to follow-up and patient anxiety associated with conventional STI testing.[9]

While a number of published STI-related reviews have described technologies and their use both in clinical and field settings worldwide,[10–12] a systematic review of evidence to support implementation (ie, impact, uptake, feasibility) and to collect end users' preferences beyond diagnostic accuracy has not been synthesised. With a view to plug the knowledge gap, we conducted a systematic review. Diagnostic accuracy outcomes (sensitivity, specificity) have been separately synthesised and are being peer reviewed.[13]

METHODS

Search strategy and study selection

We followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses (http://www.prisma-statement.org/). We registered the protocol with The International Prospective Register of Systematic Reviews (registration number: CRD4202179218).[14]

For the period 1 January 2009–20 April 2020, two independent reviewers (FN and AK) searched two electronic databases, PubMed and Embase, to retrieve relevant primary articles and conference abstracts. In addition, we searched bibliographies of included studies. We included abstracts only if full-text articles were unavailable.

Our objective was to consolidate evidence on clinical outcomes pertaining to multiplexed technologies, including immunochromatographic point-of-care devices and molecular assays used to screen populations for various bacterial, viral and parasitic STIs. FN and AK independently reviewed all citations to achieve a final subset of studies for inclusion (figure 1). Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

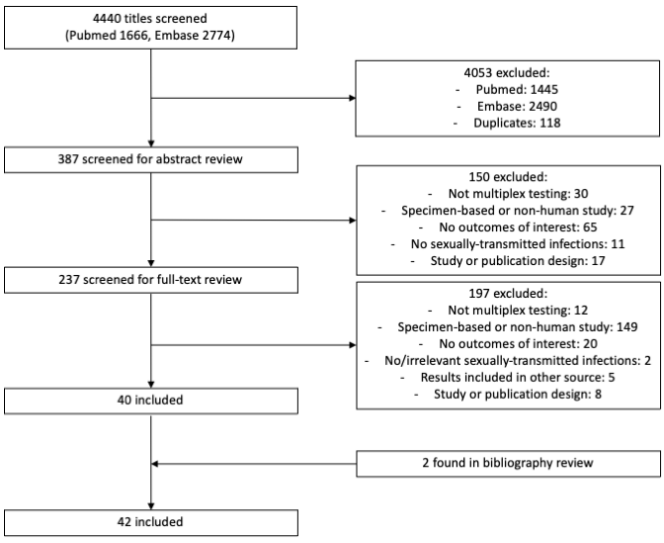

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow chart.

Search string

Our search string included multiplex*, duplex*, triplex*, quadruplex*, simultaneous*, point-of-care, rapid, platform, molecular, sexually transmitted infection*, sexually transmitted disease*, human immunodeficiency virus, acquired immunodeficiency syndrome, chlamydia, gonorrh*, hepatitis, syphilis, Treponema pallidum, human papillomavirus, herpes simplex and trichomonas.

Eligibility criteria

Studies that were included for review consisted of full-text primary articles or abstracts that assessed rapid multiplexed technologies where multiplexed was defined as testing for more than one STI or more than one strain of the same STI. Reviews, reports, editorials, commentaries and studies that used precollected specimens (not for the purposes of STI testing) or studies not based on human subjects were excluded (figure 1).

Outcomes

Four outcomes were analysed: impact, feasibility and preference, and the number of positive cases for each STI.

The impact of multiplexed technologies included their ability to detect new infections, turnaround times to test results and linkages to care. A change in uptake was also used to assess impact; this was defined as the increase in STI testing once multiplexed rapid testing was made available and quantified by calculating the absolute difference between the percentage of test usage from baseline,

that is, conventional lab-based testing, to follow-up, that is, multiplexed rapid testing.

Testee preference was assessed in one of several ways, depending on the manner reported in each article: (1) participants' preference to undergo multiplexed testing using a rapid test (immunochromatographic or molecular assay) over conventional testing; (2) participants' satisfaction or acceptance of multiplexed technologies; and (3) participants' willingness to recommend multiplexed testing to others. Preference outcomes were quantified by dividing the number of participants who indicated preference of rapid multiplexed testing by the total number of participants who were surveyed. Feasibility was quantified by metrics such as completion rate of the multiplex rapid testing strategy.

Finally, we aimed to identify the case positivity of the specific STIs based on the positive test results generated by multiplexed technologies. The number of positive STI cases was ascertained by determining the number of laboratory-confirmed positive cases divided by the total number of participants tested for the specific STI. When available, the positive (PPV) or negative (NPV) predictive value of each index test used to ascertain STI positivity was recorded or calculated.

Data abstraction

The data abstraction was performed independently by two reviewers (FN and AK). Abstraction items were tabulated in a data abstraction form and included general study characteristics, participant information, types of index and reference tests, diagnostic accuracy and the above-stated four outcomes.

Quality assurance

The quality of included studies was assessed using a revised tool for the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) by the two reviewers (FN and AK).[15]

RESULTS

As shown in figure 1, we reviewed a total of 4440 citation titles. After deduplication and our initial review of titles, we reviewed 387 abstracts. Of 387, 150 citations were excluded such that a total of 237 citations were eligible for full-text review. Of 237 citations, 197 did not meet our eligibility criteria, leading to 40 eligible publications. Two additional sources were identified from bibliography review and added to the final set, therefore a total of 42 publications were included in the final set. The reasons of exclusion as well as the counts for each are detailed in figure 1. Online supplemental table 1 provides a description (including author/year of publication; study design, setting and population; STIs and the type of diagnostic test used) of the 42 studies included.

Description of included studies

Of the 42 studies included, 10 (23.8%) studies reported the use of multiplexed immunochromatographic tests, while 32 (76.2%) studies reported using multiplexed molecular assays (online supplemental table 1).

Our review includes data from high-income, middle-income and low-income countries and includes key populations at a higher risk of acquiring STIs (such as men who have sex with men (MSM), sex workers, injection drug users (IDUs) as well general STI clinic attendees).

All 42 (100.0%) studies reported on case positivity.[3 5 16 17] About 11 studies reported on impact outcomes; 3 (7.1%) studies reported on preference[17–19]; 2 (4.8%) on patient satisfaction[19 20]; 2 (4.8%) on acceptance of multiplexed testing[17 18] and 1 (2.9%) on recommending multiplexed testing[19] (table 1).

Impact

The impact of multiplexed technologies was defined in one of several ways including their ability to detect new infections, increase uptake and to improve turnaround times to test results and linkages to care. Impact was reported by 11 (25.6%) where 3 (6.8%) studies reported on the increased detection of new/previously undiagnosed infections as a result of multiplexed testing, 1 (2.3%) study reported the increase in multiplexed testing from baseline,[21] 6 (13.9%) studies reported the turnaround time to test results,[22–27] and 3 (6.9%) reported on linkages to/retention in care[18 21 28] (table 1).

One study reported the detection of a single new infection of syphilis and HIV using an immunochromatographic test, respectively.[19] In another study, 30 new infections of hepatitis B virus (HBV) and 11 new infections of *Trichomonas vaginalis* (TV) were detected with immunochromatographic assays.[21] Finally, one study determined that 3.2% more infections of TV were detected by a molecular assay than culture and 71.4% more infections were detected compared with wet mount[29] (table 1).

Increased uptake of multiplexed testing from baseline was reported for four STIs: HIV (58.0% at baseline to 100.0% at follow-up, an overall 42.0% increase), HBV (21.0% at baseline and 100.0% at follow-up, an overall 78.6% increase), hepatitis C virus (HCV) (0.6% at baseline and 100.0% at follow-up, an overall 99.4% increase) and TV (0.4% at baseline and 100.0% at follow-up, an overall 99.6% increase).[21] The turnaround time for immunochromatographic devices ranged from 15 min to 20 min, whereas for molecular assays it ranged from 14 min to 300 min.[22–27] Most participants who underwent multiplexed testing were linked to care (70.0%–100.0%)[18 21 28] (table 1).

Preference and feasibility

Testee preference for multiplexed testing varied from a low of 60.2% to a high of 97.2% among study participants.[18 19 21] Overall, participants reported high satisfaction with being tested by multiplexed technologies (92.0%–99.5%)[19 21] and high acceptance of multiplexed technologies (100.0%).[18 21] With regard to

Table 1 Impact outcomes (detection of new infections/uptake/turnaround time/linkage to care) associated with the use of rapid multiplexed STI diagnostic devices

Author, year	Impact outcome	Multiplex test type	Result
Pai *et al*, 2014[19]	New infection	IMT	1/109 (0.9%) new infection of syphilis and 1/109 (0.9%) new infection of HIV detected with IMT
Pai *et al*, 2014*[19]	New infection	IMT	56/375 (14.9%) diagnosed with HIV, 75/375 (20.0%) with HBV, (37/375) 9.9% with syphilis, 2/375 (0.5%) with HCV
Pant Pai, *et al* 2019[21]	New infection	IMT	30/510 (5.9%) new infections of HBV and 11/510 (2.2%) new infections of TV detected with IMT
Van Der Pol *et al*, 2017†[29]	New infection	Molecular assay	3.2% more infections of TV detected by molecular assay than culture and 71.4% more than wet mount
Pant Pai *et al*, 2019[21]	Uptake	IMT	99.4% increase for HCV IMT 79.0% increase for HBV IMT 42.0% increase for HIV IMT 99.6% increase for TV IMT
Le Roy *et al*, 2012[23]	TAT	Molecular assay	4.5–5 hours for 50 samples (CT)
Longo *et al*, 2018[24]	TAT	IMT	15 min (HIV, HBV and HCV)
Mboumba Bouassa *et al*, 2018[25]	TAT	IMT	15 min (HIV, HBV, HCV)
Nuñez-Forero *et al*, 2016[26]	TAT	Molecular assay	14 min (CT and NG)
Omoding *et al*, 2014[27]	TAT	IMT	20 min (syphilis and HIV)
Causer *et al*, 2015[22]	TAT	Molecular assay	91 min (CT and NG)
Pant Pai *et al*, 2019[21]	Retention in care	IMT	95.0% patients retained in care (HIV, HBV, HCV, TV)
Pant Pai *et al*, 2019[21]	Linkage to care	IMT	70.0% patients linked to care (HIV, HBV, HCV, TV)
Kalla *et al*, 2019[28]	Linkage to care	IMT	100.0% patients linked to care (HIV, HBV, HCV)
Menzato *et al*, 2018[18]	Linkage to care	IMT	100.0% patients linked to care (HIV)

*Study conducted in Canadian and Indian populations.
†Raw data were unavailable in the publication.
CT, *Chlamydia trachomatis*; HBV, hepatitis B virus; HCV, hepatitis C virus; IMT, immunochromatographic test; NG, *Neisseria gonorrhoeae*; STI, sexually transmitted infection; TAT, turnaround time; TV, *Trichomonas vaginalis*.

recommendation, up to 99.1% of study participants would recommend multiplexed technologies to others.[19]

Finally, with respect to feasibility, two (4.7%) studies reported on completion rates[19 21] (table 2). In terms of feasibility, among participants, completion rate of multiplexed testing procedures ranged between 86.1% and 92.4%[19 21] (table 2).

Positivity (case), PPV and NPV

Varying prevalence rates of STIs were documented in studies, conducted in diverse populations; we computed PPVs and NPVs across studies and technologies (both molecular and immunochromatographic assays) in online supplemental tables 2 and 3.

The high PPVs and NPVs of both immunochromatographic tests and molecular assays support the usage of multiplexed technologies in the detection of numerous STIs whether disease prevalence is low or high in a variety of populations and settings worldwide.

For molecular assays, the ranges of PPVs and NPVs for assessing STIs were calculated or abstracted. These included: *Chlamydia trachomatis* (91.2%–100.0% and 98.5%–100.0%, respectively), *Neisseria gonorrhoeae* (NG) (50.0%–100.0% and 98.4%–100.0%, respectively), TV (37.5%–100.0% and 98.9%–100.0%, respectively), *Treponema pallidum* (syphilis) (100.0% and 93.3%–100.0%, respectively), herpes simplex virus (HSV)-1 (75.0%–100.0% and 12.5%–100.0%, respectively), and HSV-2 (40.0%–100.0% and 36.1%–100.0%, respectively).

Similarly, for immunochromatographic tests, the ranges of PPVs and NPVs for assessing the most commonly reported STIs were: *T. pallidum* (93.3%–100.0% and 86.0%–100.0%, respectively), HCV (97.1%–100.0% and 95.7%–98.2%, respectively), and HIV (94.1%–100.0% and 99.5%–100.0%, respectively).

Twenty pathogens were identified by these tests. These included: adenovirus, *C. trachomatis, Gardnerella vaginalis,*

Table 2 Preference and feasibility outcomes associated with the use of rapid multiplexed STI diagnostic devices

Author, year	Preference/feasibility outcome	Test type	STI(s) tested	Result
Pai *et al*, 2014[19]	Preference for multiplexed testing	IMT	HIV, HBV, HCV, syphilis	106/109=97.2% preference rate
Pai *et al*, 2014*[19]	Preference for multiplexed testing	IMT		226/374=60.2% preference rate
Pant Pai *et al*, 2019[21]	Preference for multiplexed testing	IMT	HIV, HBV, HCV, TV	73.0% preference rate
Pant Pai *et al*, 2019[21]	Satisfaction with multiplexed testing	IMT		453/491=92.0% satisfaction rate
Pai *et al*, 2014[19]	Satisfaction with multiplexed testing	IMT	HIV, HBV, HCV, syphilis	373/375=99.5% satisfaction rate
Menzato *et al*, 2018[18]	Acceptance of multiplexed testing	IMT	HIV	898/898=100.0% acceptance rate
Pant Pai *et al*, 2019[21]	Acceptance of multiplexed testing	IMT	HIV, HBV, HCV, TV	510/510=100.0% acceptance rate
Pai *et al*, 2014[19]	Recommend multiplexed testing to others	IMT	HIV, HBV, HCV, syphilis	108/109=99.1% recommendation rate
Pai *et al*, 2014*[19]	Recommend multiplexed testing to others	IMT	HIV, HBV, HCV, syphilis	125/375=33.0% recommendation rate
Pant Pai *et al*, 2019[21]	Completion	IMT	HIV, HBV, HCV, TV	466/510=91.6% completion rate
Pai *et al*, 2014[19]	Completion	IMT	HIV, HBV, HCV, syphilis	109/118=92.4% completion rate
Pai *et al*, 2014*[19]	Completion	IMT	HIV, HBV, HCV, syphilis	323/375=86.1% completion rate

*Study conducted in Canadian and Indian populations.
HBV, hepatitis B virus; HCV, hepatitis C virus; IMT, immunochromatographic test; STI, sexually transmitted infection; TV, *Trichomonas vaginalis*.

Haemophilus ducreyi, H. influenzae, HBV, HCV, HIV, HSV-1 and HSV-2, human papillomavirus (HPV), *Mycoplasma genitalium, M. hominis,* NG, *N. meningitidis, Streptococcus pneumoniae, T. pallidum* (syphilis), TV, *Ureaplasma parvum, U. urealyticum* and other *Ureaplasma* spp.

Across studies, the most commonly reported STIs were: *C. trachomatis* in 22 (51.1%) studies, NG in 21 (48.8%), TV in 11 (25.6%), HIV in 6 (13.9%), *T. pallidum* in 6 (13.9%), HSV-1/2 in 8 (18.6%), HBV in 3 (6.9%), and HCV in 4 (9.3%) studies, respectively.

For the more commonly reported STIs, case positivity estimates varied within populations studied: *C. trachomatis* (2.8%–30.2%), NG (0.3%–30.3%), TV (0.1%–32.7%), *T. pallidum* (syphilis) (0.9%–27.0%), HSV-1/2 (0.8%–90.2%), HCV (0.5%–42.2%), HIV (1.8%–29.9%), HBV (1.1%–23.9%), and HPV (12.3%–98.2%).

Quality assessment of included studies

The QUADAS-2 checklist was used to evaluate quality of the included studies. In general, the majority of studies (83.7%) used appropriate reference tests to ascertain patient disease status, and most studies (60.5%) included all patients in their respective analyses. Figure 2 provides a breakdown of the included studies according to the quality checklist.

DISCUSSION

Multiplexed technologies fill many gaps in the spectrum of STI diagnostic care.[24 30] These technologies offer the ability to screen for many pathogens including those that do not present any clinical manifestations and those that are not commonly identified in standard STI diagnostic practices yet are able to give rise to coinfections. The test results also help catalyse the process of diagnosis and the process of seeking subsequent linkages to care. In fact, modelling studies have shown that immunochromatographic tests with adequate sensitivity can reduce prevalence of disease in communities where it is otherwise high.[22] Particularly now, during the COVID-19 pandemic, rapid testing is more relevant than ever and would offer several benefits, such as to destigmatise testing, whether related to STI or COVID-19 testing, and to expedite turnaround time for both types of pathogens. Certain multiplexed test devices are now available that can test for both COVID-19 and STIs, such as Cepheid's GeneXpert.[31 32] The ability to conduct simultaneous testing of various pathogens presents as an additional advantage in diagnostic evaluations.[33]

The evidence presented herein suggests that over the past decade, multiplexed technologies are increasingly being developed and used to address the burden of the most common viral, bacterial and parasitic STIs in both high-income and low-income countries. While we found high feasibility of execution of multiplexed rapid testing (86.1%–92.4%),[4 19 21] the uptake of these technologies for less commonly screened STI was very high: HBV (79.0%), HCV (99.4%) and TV (99.6%). The

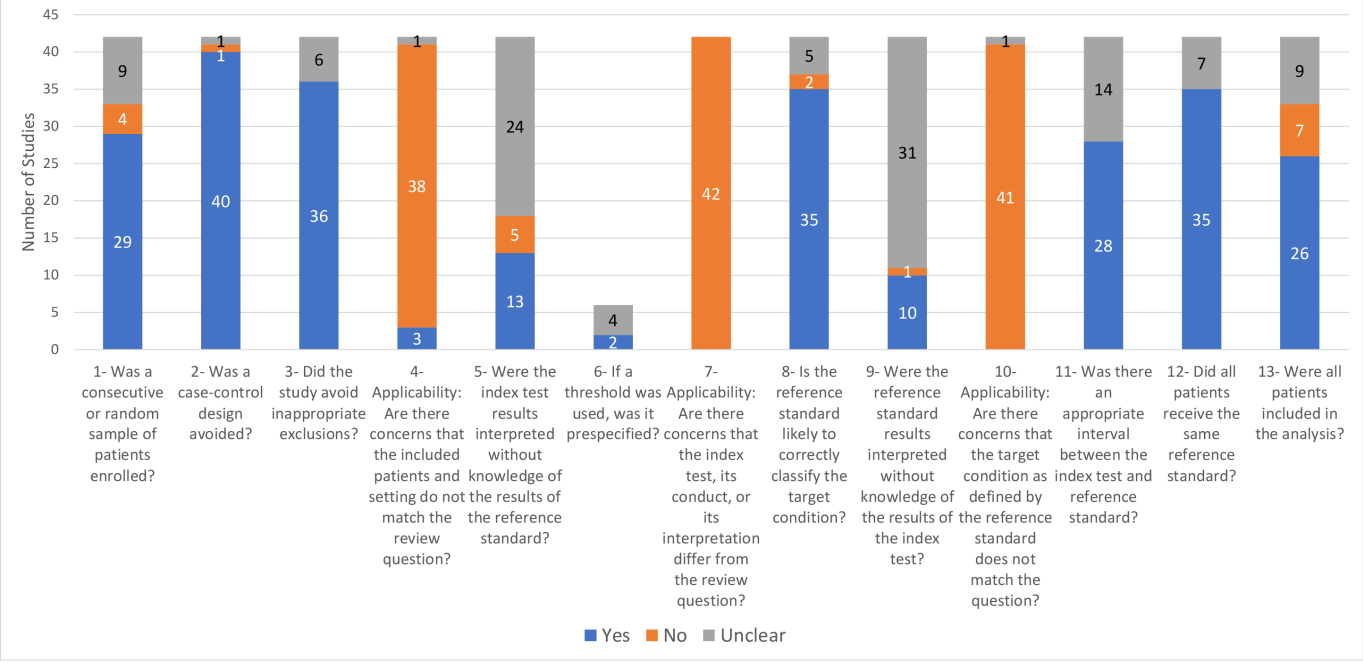

Figure 2 Quality assessment of included studies.

high PPVs and NPVs of both immunochromatographic and molecular assays support the usage of multiplexed technologies in the detection of numerous STIs whether disease prevalence is low or high in a variety of populations and settings worldwide. Unsurprisingly, with respect to case positivity and new cases detected with these multiplexed technologies, we found that the highest rates were reported for asymptomatic *C. trachomatis*, NG, *T. pallidum* and TV.[16 34–36] Our findings suggest that asymptomatic STIs may be left undetected and untreated thereby emphasising the need for routine STI screening for at-risk populations, including MSM, IDUs, sex workers and transgender populations residing in endemic settings. Given their high feasibility and rapid turnaround time, multiplexed technologies have the potential to accelerate the screening and treatment process of these key populations and other asymptomatic individuals.[37] These results further support implementation of multiplexed rapid tests in clinical care, particularly as 41 out of the 42 studies included in our final set were observational in nature and therefore alluded to real-world implementation of these test devices.

We also found evidence in favour of rapid turnaround time, and that multiplexed rapid testing was preferred by participants over conventional lab-based testing (60.2%–97.2%). Published findings corroborate these results; Rompalo *et al* and Widdice *et al* reported that patients are in favour of rapid and user-friendly diagnostic tests.[12 38] With respect to HIV, multiplexed technologies addressed various barriers to testing including having to wait for test results.[39] Long wait times can induce feelings of prolonged anxiety and fear among test seekers thereby discouraging individuals from seeking testing.

As the turnaround times ranged between 15 and 20 min for immunochromatographic devices and between 14 min and 5 hours for molecular assay-based testing, rapid tests have the potential to eliminate the time barrier in getting tested for STIs by yielding same-day results. While a 5-hour turnaround time for a molecular assay may be considered a lengthy period to receive test results, it nonetheless obviates the need for additional patient visits, therefore facilitating the testing and linkage to care process for individuals with limited healthcare access, such as those residing in rural areas.

Additional published findings further support the feasibility and usefulness of rapid testing into clinical care. For instance, in a qualitative research study, Fuller *et al* concluded that patients and clinicians across six sexual health clinics in the UK expressed their acceptance in regard to point-of-care testing, particularly if information is provided prior to testing regarding the changes they may expect in services rendered.[40] Furthermore, Harding-Esch *et al* demonstrated the high feasibility associated with a 'sample first' clinical pathway where patients provided samples on arrival at a London sexual health clinic, subsequently to be tested in the point of care. Their findings illustrated that over 90% of patients reported high satisfaction with the evaluated strategy, and that all results were available prior to patients leaving the clinic which led to high linkage to treatment rates.[41]

Despite the benefits associated with rapid multiplex testing, we must however acknowledge that certain devices are not fully optimisable at the point of care. While the design of handheld immunochromatographic tests fully support point-of-care usage, the majority of molecular tests are conducive for laboratory testing. The

sole molecular assay recommended for use near testees is the Cepheid GeneXpert.[22 42–45]

Strengths and limitations

We were unable to conduct a meta-analysis due to the heterogeneity of settings, populations and pathogens screened and outcomes that could not be pooled due to the lack of data in clinically relevant subgroups defined by pathogens, populations and technologies. This limitation needs to be addressed in future research. Moreover, the wide range of pathogens evaluated across studies present as a limitation and as a strength. A number of studies assessed STIs that are not generally included in STI testing guidelines such as various *Mycoplasma* and *Ureaplasma* spp. As a result, these infections were omitted from analysis as they demonstrate limited clinical utility. Also, while our review focused on the preference, case positivity, uptake, feasibility and impact of multiplexed rapid testing, the examination of these devices to detect antimicrobial resistance may be warranted, in particular with respect to gonorrhoea testing.

Study limitations, as identified by the study authors, included variability in disease prevalence in the settings and populations screened that led to limited case finding.[19 26 46–48] Convenience sampling potentially introduced biases (namely, volunteer, selection and/or confounding),[19 21 49] and missing data generated potential for information bias.[50] Technological challenges were reported in regard to the multiplexed devices. For instance, the simultaneous molecular amplification performed by STDFinder used by Muvunyi *et al* reduced the device's capability to amplify and detect singleton targets.[51] Molecular assays reported PCR drift.[52] Moreover, skilled healthcare staff were required to perform testing with molecular assays and venous blood was required for confirmatory tests.[19 27 29 46]

Implications

Several important implications of timely screening ensue as a result of ascertaining disease status early: the mitigation of transmission of disease and disease-specific complications, such as chronic pelvic pain, ectopic pregnancies, stillbirths, infertility, hepatic failure or cirrhosis.[19 53] These time savings allow people who test positive to immediately be linked to counselling and care, as evidenced by the 70.0%–100.0% of tested patients in three identified studies being linked to care after multiplexed rapid testing.[18 21 28] An additional benefit of using these tests is the non-invasive nature of specimen collection, such as blood samples. While our findings support the numerous clinical benefits associated with rapid multiplexed devices in STI management, the importance of conventional lab-based testing should not be overlooked to confirm preliminary positive test results as these still constitute the gold standard of testing to diagnose STIs.

Our systematic review findings add value to the current body of literature as it consolidates data relevant for healthcare professionals, policymakers, decision makers, and government officials, academics and researchers to make decisions on an offer of multiplexed testing to meet the needs of integrated testing agenda of public health organisations.

CONCLUSION

With the issues related to routine STI testing, particularly the high rates of loss to follow-up and the lengthy turnaround time to test results, multiplexed rapid testing (both immunochromatographic and molecular assays) offer the potential to fill many early and timely screening gaps in the spectrum of care.

We conclude that both multiplexed technologies were found to be feasible and preferred by participants, impacted detection and treatment of many STIs, with provision of same-day test results and rapid linkages to care. Given the increasing incidence of STIs worldwide, multiplexed technologies can safely be the future of integrated screening initiatives for STI diagnosis and treatment worldwide. Based on these findings, we recommend the incorporation of multiplex rapid tests into clinical care, whether they may be in the form of immunochromatographic or molecular assays. Testees' high satisfaction of multiplexed rapid testing as well as the added benefit and impact support their usage in the spectrum of STI diagnostic care, particularly to complement conventional lab-based testing which may at times present as a suboptimal testing approach to reach vulnerable at-risk populations.

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Contributors FN, AK and NP designed, drafted and reviewed the initial manuscript, while the remaining authors (SN, J-PR, CPY, JK) provided critique on subsequent drafts. The search strategy was developed and executed by FN, AK and NP. The quality assessment was performed by FN and AK. All authors approved and contributed to the final written manuscript.

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Supplementary Table 1: Study Characteristics

Reference	Study Design	Country	Infections Assessed	Type of Test	Test Name	Population	Sample Size
Bercot, 2015[1]	Cross-sectional	France	*C trachomatis*, *N gonorrhoeae*, *T vaginalis*, *M genitalium*, *M hominis*, *U urealyticum*, *U parvum*	Molecular Assay	Anyplex II STI-7 Detection Kit PCR	Symptomatic and paucisymptomatic patients	202
Brosh-Nissimov, 2018[2]	Retrospective cohort	Israel	*C trachomatis*, *N gonorrhoeae*, *T vaginalis*, *M genitalium*, *M hominis*, *U urealyticum*, *U parvum*	Molecular Assay	Anyplex II STI-7 Detection Kit PCR	Female and male Israeli soldiers	2816
Causer, 2015[3]	Cross-sectional	Australia	*C trachomatis*, *N gonorrhoeae*	Molecular Assay	GeneXpert CT/NG Test	Aboriginal populations	198
Causer, 2018[4]	Cluster RCT crossover	Australia	*C trachomatis*, *N gonorrhoeae*	Molecular Assay	GeneXpert CT/NG Test	Individuals presenting for STI testing	2486
Choe, 2013[5]	Cross-sectional	South Korea	*C trachomatis*, *N gonorrhoeae*, *T vaginalis*, *M genitalium*, *M hominis*, *U urealyticum*	Molecular Assay	Anyplex II STI-7 Detection Kit PCR, Seeplex PCR, BD ProbeTec strand displacement amplification, AmpliSens PCR, Mycoplasma IST 2 Kit	Symptomatic patients and asymptomatic volunteers	897

Reference	Study Design	Country	Infections Assessed	Type of Test	Test Name	Population	Sample Size
De Baetselier, 2018[6]	Cross-sectional	Belgium	*C trachomatis*, *N gonorrhoeae*	Molecular Assay	Abbott Real-Time (RT) CT/NG assay	MSM	98
De Baetselier, 2017[7]	Cross-sectional	Belgium	*M genitalium*, T vaginalis	Molecular Assay	S-DiaMGTV multiplex kit of Diagenode	MSM	1768
Fernandez, 2016[8]	Cross-sectional	Spain	*C trachomatis*, *M genitalium*, *M hominis*, *U urealyticum*, *U parvum*	Molecular Assay	Anyplex II STI-7 Detection Kit PCR	Individuals seeking care suspected of having an STI, HIV-negative men who have sex with men	267
Fisher, 2015[9]	Cross-sectional	United States	HCV	Immunochromatographic Test	MedMira HIV/HCV, MedMira HIV/HCV/HBV, Chembio HIV/HCV, Chembio HIV/HCV/syphilis	At-risk individuals (>15 years of age), including injection drug users; women with at least two recent partners; men who have sex with men/women; transgender individuals	1048
Foschi, 2017[10]	NA	Italy	*C trachomatis*, *N gonorrhoeae*	Molecular Assay	Aptima Combo2® for CT and NG detection	Women attending outpatient STI clinics complaining of genital STI-related symptoms or reporting unsafe intercourse	100

Reference	Study Design	Country	Infections Assessed	Type of Test	Test Name	Population	Sample Size
Gimenes, 2014[11]	Cross-sectional	Brazil	*C trachomatis*, *N gonorrhoeae*, *M genitalium*, HSV 1, HSV-2, *T pallidum*, HPV	Molecular Assay	PCR-Restriction Fragment Length Polymorphism (PCR-RFLP)	Infertile men	76
Han, 2014[12]	Cross-sectional	China	*C trachomatis*, *N gonorrhoeae*	Molecular Assay	Abbott RealTime CT/NG assay on the automated m2000 molecular platform	Female sex workers	997
Ho, 2015[13]	Cross-sectional	Taiwan	HPV	Molecular Assay	Multiplex real-time quantitative reverse transcriptase PCR	Women	684
Jahan, 2014[14]	Cross-sectional	Bangladesh	*C trachomatis*, *N gonorrhoeae*	Molecular Assay	PCR	Males suspected of having urethritis	185
Kalla, 2019[15]	NA	Cameroon	HIV, HBV, HCV	Immunochromatographic Test	HIV/HCV/HBsAg (Triplex, Biosynex, France)	Volunteers	1206
Le Goff, 2010[16]	NA	Central African Republic	HSV-1, HSV-2	Molecular Assay	BioPlex 2200 immunoassay system	Adults clinically asymptomatic for herpes disease	51
Le Roy, 2012[17]	Cross-sectional	France	*C trachomatis*, *N gonorrhoeae*, *M genitalium*	Molecular Assay	Bio-Rad Dx CT/NG/MG assay	Males and females attending an STI clinic	453
Lodiongo, 2018[18]	Cross-sectional	Sudan	HIV, *T pallidum*	Immunochromatographic Test	SD Bioline HIV/Syphilis Duo RDT	Pregnant women	442

Reference	Study Design	Country	Infections Assessed	Type of Test	Test Name	Population	Sample Size
Longo, 2018[19]	Cross-sectional	Central African Republic	HIV, HBV, HCV	Immunochromatographic Test	HIV/HCV/HBsAG Combo Rapid Test Cassette (ITHD-C43)	Patients with unknown HIV status	71
Lorea, 2018[20]	Cross-sectional	Belgium	*C trachomatis*, *M genitalium*	Molecular Assay	Taqman Array Card	Female students and MSM	129
Loubinoux, 2012[21]	Prospective cohort	NA	*C trachomatis*, *N gonorrhoeae*, *M genitalium*	Molecular Assay	Dx CT/ NG/MG real-time multiplex PCR	Men and women	840
Mawu, 2009[22]	NA	Indonesia	*C trachomatis*, *N gonorrhoeae*, *M genitalium*, *T vaginalis*	Molecular Assay	Multiplex PCR	Female sex workers	221
Mboumba Bouassa, 2018[23]	Cross-sectional	Chad	HIV, HBV, HCV	Immunochromatographic Test	HIV/HCV/HBsAG Combo Rapid Test Cassette (ITHD-C43)	Childbearing aged women in resource limited settings	266
McKechnie, 2009[24]	Cross-sectional	Australia	*C trachomatis*, *N gonorrhoeae*, *M genitalium*, HSV-1, adenovirus, *T vaginalis*, *M hominis*, N meningitidis, *U urealyticum*, *U parvum*	Molecular Assay	Multiplex PCR-based reverse line blot (mPCR/RLB)	Male patients with and without urethral symptoms	529

Reference	Study Design	Country	Infections Assessed	Type of Test	Test Name	Population	Sample Size
Menzato, 2018[25]	Cross-sectional	Guinea Bissau	HIV	Immunochromatographic Test	Abbott Determine	Inhabitants of rural Guinea Bissau, West Africa	898
Muvunyi, 2011[26]	Case-control	Rwanda	*N gonorrhoeae*, *C trachomatis*, *T vaginalis*, *M genitalium*, HSV-2	Molecular Assay	Multiplex ligation-dependent probe amplification (STDFinder assay)	infertile women	242
Nateghi Rostami, 2017[27]	Cross-sectional	Iran	*N gonorrhoeae*, *T vaginalis*, *C trachomatis*	Molecular Assay	Multiplex PCR	Women seeking care for genital complaints	300
Nunez-Forero, 2016[28]	Cross-sectional	Colombia	*N gonorrhoeae*, *C trachomatis*, *T pallidum*	Molecular Assay	Acon Duo (for NG and CT)	Sexually active women aged 14-49 years with lower urinary tract infection symptoms	1444
Omoding, 2014[29]	Cross-sectional	Uganda	HIV, *T pallidum*	Immunochromatographic Test	SD Bioline HIV/Syphilis Duo RDT	Pregnant women	220
Pant Pai, 2014[30]	Cross-sectional	Canada and India	HIV, HBV, HCV *T pallidum*	Immunochromatographic Test	Miriad rapid TP/HBV/HIV/HCV antibody test (MedMira)	Injection drug users/ STI clinic attendees with an at-risk profile (migrants, commercial sex workers, labourers who have paid for sex)	484

Reference	Study Design	Country	Infections Assessed	Type of Test	Test Name	Population	Sample Size
Pant Pai, 2019[31]	Cross-sectional	India	HIV, HBV, HCV	Immunochromatographic Test	Multiplo HBc/HIV/HCV	Pregnant women presenting to care to outreach rural service units	510
Parnell, 2014[32]	Cross-sectional	NA	HSV, *T pallidum*	Molecular Assay	Abbott Architect	Patients with syphilis	47
Roberts, 2011[33]	Cross-sectional	NA	HPV	Molecular Assay	Internally developed multiplex HPV PCR system	Women aged 16-23 years	377
Rumyantseva, 2015[34]	Cross-sectional	Sweden	*N gonorrhoeae*, *C trachomatis*, *M genitalium*, *T vaginalis*	Molecular Assay	AmpliSens PCR assay	STI clinic attendees	1261
Sachdev, 2013[35]	Cross-sectional	India	*N gonorrhoeae*, *C trachomatis*	Molecular Assay	Internally developed multiplex PCR system	Women visiting gynaecology departments	412
Sednaoui, 2011[36]	Prospective cohort	France	*C trachomatis*, *N gonorrhoeae*, *M genitalium*	Molecular Assay	Bio-Rad Dx CT/NG/MG Assay	Individuals who undergo STI screening, medical consultation or biological check-up	955
Stafylis, 2019[37]	Cross-sectional	United States	HIV, *T pallidum*	Immunochromatographic Test	INSTI HIV-1/HIV-2/syphilis rapid antibody test kit	Individuals presenting for outpatient care at an AIDS Healthcare Foundation clinic	274

Reference	Study Design	Country	Infections Assessed	Type of Test	Test Name	Population	Sample Size
Suntoke, 2009[38]	Cross-sectional	Uganda	H ducreyi, *T pallidum*, HSV-1, HSV-2	Molecular Assay	In-house PCR	Patients with genital ulcer disease	100
Vahidnia, 2014[39]	Cross-sectional	The Netherlands	*C trachomatis*, *N gonorrhoeae*, *T vaginalis*	Molecular Assay	Aurora FLOW	Individuals with clinical suspicion of STI	896
Van der Pol, 2017[40]	Cross-sectional	United States	*C trachomatis*, *N gonorrhoeae*, *T vaginalis*	Molecular Assay	BD Max CT/GC/TV	Individuals presenting for routine STI symptom evaluation or screening	2689
Vaughn, 2010[41]	Cross-sectional	United States	*T pallidum*, *Ureaplasma* spp, *M genitalium*, *T vaginalis*, HSV-1	Molecular Assay	FilmArray STD Panel	NA	101
Zhao, 2012[42]	Cross-sectional	China	HPV-16, HPV-18, HSV-1, HSV-2	Molecular Assay	In-house multiplex PCR	Individuals with suspected HPV and HSV infection	187

Supplementary Table 2. STI Case Positivity, Positive Predictive Values and Negative Predictive Values as obtained from Screening Utilizing Molecular Assays

A. Chlamydia trachomatis

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Vahidnia, 2014[39]	Males and females with clinical suspicion of STI	7.1%	NA	Aurora FLOW	Vaginal (female) Urine (urethral), rectal & throat (male)	98.4 (NA)	100.0 (NA)
Rumyantseva, 2015[34]	STI clinic attendees	6.3%	NA	AmpliSens PCR	Vaginal & urine (female) Urine (male)	100.0 (95.3-100.0)	99.8 (99.4-100.0)
Bercot, 2015[1]	Symptomatic and paucisymptomatic patients	30.2%	82% with another STI	Anyplex II STI-7 Detection Kit PCR	Urine, endocervical, vaginal, pelvic fluid	95.5 (92.6-98.3)	92.5 (88.9-96.1)
Choe, 2013[5]	Symptomatic patients and asymptomatic volunteers	8.0%	21.7% with another STI		Urine, endocervical	100.0 (NA)	100.0 (NA)
Nunez-Forero, 2016[28]	Sexually active females aged 14-49 years with lower urinary tract infection symptoms	9.7%	NA	Acon Duo	Endocervical	94.7 (NA)	91.3 (NA)
Han, 2014[12]	Female sex workers	19.0%	NA	Abbott RealTime CT/NG	Cervical	100.0 (97.3-100.0)	98.5 (97.4-99.2)
Choe, 2013[5]	Symptomatic	NA	NA	BD ProbeTec	Urine,	91.2 (NA)	98.8 (NA)

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
	patients and asymptomatic volunteers			strand displacement amplification	endocervical		
Choe, 2013[5]	Symptomatic patients and asymptomatic volunteers	NA	NA	Seeplex PCR	Urine, endocervical	92.3 (NA)	99.8 (NA)
Causer, 2015[3]	Aboriginal populations	8.3%	NA	GeneXpert CT/NG Test	Urine	94.1 (NA)	99.5 (NA)
Sednaoui, 2011[36]	STI clinic attendees	8.1%	NA	Bio-Rad Dx CT/NG/MG Assay	Urogenital, anorectal	100.0 (95.3-100.0)	99.8 (99.4-100.0)
Van der Pol, 2017[40]	Male STI clinic attendees	21.8%	4.2% with NG	BD Max CT/GC/TV	Urine (male)	96.1 (NA)	99.4 (NA)
	Female STI clinic attendees	7.1%	1.8% with 2 or more organisms		Vaginal (female)	99.3 (NA)	98.6 (NA)
Muvunyi, 2011[26]	Infertile females	2.9%	NA	STDFinder (multiplex ligation-dependent probe amplification)	Vaginal	100.0 (NA)	100.0 (NA)
Nateghi Rostani, 2017[27]	Females presenting with genital complaints	11.7%	0.7% with NG; 1.0% with TV	Multiplex PCR	Vaginal	100.0 (NA)	100.0 (NA)
Gimenes, 2014[11]	Infertile males	8.0%	NA	PCR-Restriction Fragment Length	Semen	100.0 (NA)	100.0 (NA)

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
				Polymorphism (PCR-RFLP)			
Brosh-Nissimov, 2018[2]	Female and male Israeli soldiers	6.7%	14.0% for MG; 5.0% for NG; 15.4% for TV; 11.7% for UU; 10.2% for UP; 16.2% for MH	Anyplex II STI-7 Detection Kit PCR	NA	NA	NA
Causer, 2018[4]	Individuals presenting for STI symptom testing	8.5%	NA	GeneXpert CT/NG Test	Urine	NA	NA
De Baetselier, 2018[6]	Men who have sex with men	8.5%	NA	Abbott Real-Time (RT) CT/NG assay	Urine, anorectal, pharyngeal	NA	NA
Fernandez, 2016[8]	Individuals seeking care, young adults (25 years or less) suspected of having an STI, and HIV-negative men who have sex with men	28.8%	3.4% with NG	Anyplex II STI-7 Detection Kit PCR	Urine	NA	NA
Foschi, 2017[10]	Females attending outpatient STI clinics	25.0%	1.0% with NG; 2.0% with MG	Aptima Combo2® for CT and NG detection	Urine, vaginal	NA	NA
Jahan, 2014[14]	Males suspected of having	14.6%	NA	PCR	Urethral discharge	NA	NA

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
	urethritis						
Le Roy, 2012[17]	Asymptomatic females	10.2%	0.7% with MG; 0.4% with NG	Bio-Rad Dx CT/NG/MG assay	Urine, vaginal, endocervical	NA	NA
Le Roy, 2012[17]	Symptomatic females	11.1%	NA		Urine, vaginal, endocervical	NA	NA
Le Roy, 2012[17]	Asymptomatic males	8.1%	NA		Urine	NA	NA
Le Roy, 2012[17]	Symptomatic males	8.3%	NA		Urine, urethral	NA	NA
Lorea, 2018[20]	Female students	7.7%	NA	Taqman Array Card	NA	NA	NA
Loubinoux, 2012[21]	Males	4.9%	0.8% with another STI	Dx CT/ NG/MG real-time multiplex PCR	Urine, other swabs	NA	NA
Loubinoux, 2012[21]	Females	6.9%	NA	Dx CT/ NG/MG real-time multiplex PCR	Urine, vaginal, other swabs	NA	NA
Mawu, 2009[22]	Female sex workers	27.0%	NA	Multiplex PCR	Urine, vaginal	NA	NA
McKechnie, 2009[24]	Males with urethral symptoms	17.3%	0.2% with MG; 0.2% with UU; 0.2% with HSV-1 and UP; 0.2% with NM; 0.2% with HI; 0.4% with	Multiplex PCR-based reverse line blot (mPCR/RLB)	Urine, urethral	NA	NA
McKechnie, 2009[24]	Males without urethral	2.8%		Multiplex PCR-based	Urine, urethral	NA	NA

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
	symptoms		MH	reverse line blot (mPCR/RLB)			
Sachdev, 2013[33]	Females visiting gynaecology departments	26.3%	11.3% with NG	Internally developed multiplex PCR system	endocervical	NA	NA

CI, confidence interval; CT, *Chlamydia trachomatis*; GC or NG, *Neisseria gonorrhoeae*; HI, *Haemophilus influenzae*; HIV, human immunodeficiency virus; HSV, herpes simplex virus; kPCR, kinetic polymerase chain reaction; MG, *Mycoplasma genitalium*; MH, *Mycoplasma hominis*; NA, not available; NM, *Neisseria meningitidis*; STI, sexually-transmitted infection; PCR, polymerase chain reaction; STI, sexually-transmitted infection; TV, *Trichomonas vaginalis*; UP, *Ureaplasma parvum*; UU, *Ureaplasma urealyticum*.

B. Neisseria gonorrhoeae

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Rumyantseva, 2015[34]	STI clinic attendees	0.3%	NA	AmpliSens PCR	Vaginal & urine (female) Urine (male)	100.0 (40.2-100.0)	100.0 (99.7-100.0)
Bercot, 2015[1]	Symptomatic and paucisymptomatic patients	13.9%	61% with another STI	Anyplex II STI-7 Detection Kit PCR	Urine, endocervical, vaginal, pelvic fluid	90.0 (85.9-94.1)	98.4 (NA)
Choe, 2013[5]	Symptomatic patients and asymptomatic	4.1%	21.7% with another STI		Urine, endocervical	79.4 (NA)	100.0 (NA)

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
	volunteers						
Han, 2014[12]	Female sex workers	2.2%	NA	Abbott RealTime CT/NG	Cervical	95.5 (75.1-99.2)	99.9 (99.3-100.0)
Sednaoui, 2011[36]	STI clinic attendees	3.5%	NA	Bio-Rad Dx CT/NG/MG Assay	Urine, vaginal, endocervical, urethral	93.8 (NA)	100.0 (NA)
Choe, 2013[5]	Symptomatic patients and asymptomatic volunteers	NA	NA	Seeplex PCR	Urine, endocervical	90.0 (NA)	100.0 (NA)
Choe, 2013[5]	Symptomatic patients and asymptomatic volunteers	NA	NA	BD ProbeTec SDA	Urine, endocervical	96.0 (NA)	99.7 (NA)
Causer, 2015[3]	Aboriginal populations	3.5%	NA	GeneXpert CT/NG Test	Urine	100.0 (NA)	100.0 (NA)
Nunez-Forero, 2016[28]	Sexually active females aged 14-49 years with lower urinary tract infection symptoms	1.4%	NA	Acon Duo	Endocervical	50.0 (NA)	98.6 (NA)
Van der Pol, 2017[40]	Female STI clinic attendees	2.3%	NA	BD Max CT/GC/TV	Vaginal (female)	95.5 (NA)	99.8 (NA)
	Male STI clinic attendees	12.9%	NA		Urine (male)	99.1 (NA)	100.0 (NA)
Jahan, 2014[14]	symptoms suggestive of urethritis having urethral discharge	30.3%	NA	PCR	Urethral discharge	87.5 (NA)	100.0 (NA)

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Muvunyi, 2011[26]	Infertile females	4.1%	NA	STDFinder (multiplex ligation-dependent probe amplification)	Vaginal	100.0 (NA)	100.0 (NA)
Nateghi Rostami, 2017[27]	Females presenting with genital complaints	5.7%	0.7% with CT; 1.7% with TV	Multiplex PCR	Vaginal	81.0 (NA)	100.0 (NA)
Gimenes, 2014[11]	Infertile males	4.0%	NA	PCR-Restriction Fragment Length Polymorphism (PCR-RFLP)	Semen	100.0 (NA)	100.0 (NA)
Brosh-Nissimov, 2018[2]	Female and male Israeli soldiers	0.6%	0.5% with CT; 3.5% with MG; 0.6% with UU; 0.5% with UP; 0.5% with MH	Anyplex II STI-7 Detection Kit PCR	NA	NA	NA
Causer, 2018[4]	Individuals presenting for STI symptom testing	5.8%	NA	GeneXpert CT/NG Test	Urine, vaginal	NA	NA
De Baetselier, 2018[6]	Men who have sex with men	6.8%	NA	Abbott Real-Time (RT) CT/NG assay	Urine, anorectal, pharyngeal	NA	NA
Foschi,	Females attending	4.0%	NA	Aptima	Urine,	NA	NA

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
2017[10]	outpatient STI clinics			Combo2® for CT and NG detection	vaginal		
Le Roy, 2012[17]	Asymptomatic females	0.6%	NA	Bio-Rad Dx CT/NG/MG assay	Urine, endocervical, vaginal	NA	NA
Le Roy, 2012[17]	Symptomatic females	3.7%	NA		Urine, endocervical, vaginal	NA	NA
Le Roy, 2012[17]	Asymptomatic males	0.4%	NA		Urine	NA	NA
Le Roy, 2012[17]	Symptomatic males	16.7%	NA		Urine, urethral	NA	NA
Loubinoux, 2012[21]	Males	1.2%	NA	Dx CT/ NG/MG real-time multiplex PCR	Urine, other swabs	NA	NA
Loubinoux, 2012[21]	Females	1.4%	NA		Urine, vaginal, other swabs	NA	NA
Mawu, 2009[22]	Female sex workers	11.0%	NA	Multiplex PCR	Urine, vaginal	NA	NA
McKechnie, 2009[24]	Males with urethral symptoms	2.5%	0.2% with HI; 0.4% with CT	Multiplex PCR-based reverse line blot (mPCR/RLB)	Urine, urethral	NA	NA
Sachdev,	Females visiting	27.8%	NA	Internally	Endocervical	NA	NA

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
2013[33]	gynaecology departments			developed multiplex PCR system			
Vahidnia, 2014[39]	Males and females with clinical suspicion of STI	1.2%	NA	Aurora FLOW	Urine, vaginal, urethral, rectal, throat	NA	NA

CI, confidence interval; CT, *Chlamydia trachomatis*; GC or NG, *Neisseria gonorrhoeae*; NA, not available; MG, *Mycoplasma genitalium*; MH, *Mycoplasma hominis*; HI, *Haemophilus influenzae*; SDA, strand displacement amplification; STI, sexually-transmitted infection; PCR, polymerase chain reaction; TV, *Trichomonas vaginalis*; UP, *Ureaplasma parvum*; UU, *Ureaplasma urealyticum*.

C. Trichomonas vaginalis

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Choe, 2013[5]	Symptomatic patients and asymptomatic volunteers	0.1%	21.7% with another STI	Anyplex II STI-7 Detection Kit PCR	Urine, endocervical	75.0 (NA)	100.0 (NA)
Choe, 2013[5]	Symptomatic patients and asymptomatic volunteers	NA	NA	Seeplex PCR	Urine, endocervical	100.0 (NA)	100.0 (NA)
Choe, 2013[5]	Symptomatic patients and asymptomatic volunteers	NA	NA	AmpliSens PCR	Urine, endocervical	37.5 (NA)	100.0 (NA)
Rumyantsev	STI clinic attendees	0.1%	NA		Vaginal &	100.0 (16.5-	100.0 (99.7-

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
a, 2015[34]					urine (female) Urine (male)	100.0)	100.0)
Nateghi Rostami, 2017[27]	Females presenting with genital complaints	32.7%	1% with CT; 32.7% with NG	Multiplex PCR	Vaginal	100.0 (NA)	100.0 (NA)
Van Der Pol, 2017[40]	Female STI clinic attendees	13.5%	NA	BD Max CT/GC/TV	Vaginal	96.1 (NA)	98.9 (NA)
Muvunyi, 2011[26]	Infertile females	19.4%	NA	STDFinder (multiplex ligation-dependent probe amplification)	Vaginal	55.3 (NA)	100.0 (NA)
Bercot, 2015[1]	Symptomatic and paucisymptomatic patients	3.5%	100.0% with another STI	Anyplex II STI-7 Detection Kit PCR	Urine, endocervical, vaginal, pelvic	NA	NA
Brosh-Nissimov, 2018[2]	Female and male Israeli soldiers	0.4%	1.0% with CT; 0.9% with UU; 1.0% with UP; 2.2% with MH	Anyplex II STI-7 Detection Kit PCR	NA	NA	NA
Mawu, 2009[22]	Female sex workers	23.0%	NA	Multiplex PCR	Urine, vaginal	NA	NA
McKechnie, 2009[24]	Males with urethral symptoms	0.4%	NA	Multiplex PCR-based reverse line blot	Urine, urethral	NA	NA

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
				(mPCR/RLB)			
Vahidnia, 2014[39]	Males and females with clinical suspicion of STI	1.1%	NA	Aurora FLOW	Urine, vaginal, urethral, rectal, throat	NA	NA
Vaughn, 2010[41]	STD clinic attendees	4.0%	NA	FilmArray STD Panel	Urine	NA	NA

CI, confidence interval; CT, *Chlamydia trachomatis*; GC, *Neisseria gonorrhoeae*; MH, *Mycoplasma hominis*; NA, not available; PCR, polymerase chain reaction; TV, *Trichomonas vaginalis*; UP, *Ureaplasma parvum*; UU, *Ureaplasma urealyticum*.

D. Treponema pallidum

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Gimenes, 2014[11]	Infertile males	5.3%	NA	PCR-Restriction Fragment Length Polymorphism (PCR-RFLP)	Semen	100.0 (NA)	100.0 (NA)
Suntoke,	Genital ulcer	5.0%	71.0% with	In-house	Ulcer, blood	100.0 (NA)	93.3 (NA)

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
2009[38]	disease patients		another pathogen	PCR			
Nunez-Forero, 2016[28]	Sexually active females aged 14-49 years with lower urinary tract infection symptoms	0.9%	NA	Acon Duo	Endocervical	NA	NA
Vaughn, 2010[41]	STD clinic attendees	1.0%	9.0% co-infected with two pathogens; 1.0% co-infected with three pathogens	FilmArray STD Panel	Urine	NA	NA

CI, confidence interval; NA, not available; PCR, polymerase chain reaction; STD, sexually-transmitted disease.

E. Herpes simplex virus

Reference	Strain	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Gimenes, 2014[11]	HSV-1	Infertile males	8.0%	NA	PCR-Restriction Fragment Length Polymorphism	Semen	75.0 (NA)	100.0 (NA)

Reference	Strain	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
					(PCR-RFLP)			
Suntoke, 2009[38]		Genital ulcer patients	3.0%	64% with HIV or HSV-2	In-house PCR	Ulcer, blood	100.0 (NA)	12.5 (NA)
Le Goff, 2010[16]		Clinically asymptomatic adults	90.2%	NA	BioPlex 2200 immunoassay system	Serum	NA	NA
McKechnie, 2009[24]		Males with urethral symptoms	2.2%	0.2% with HI; 0.2% with UP	Multiplex PCR-based reverse line blot (mPCR/RLB)	Urine, urethral	NA	NA
McKechnie, 2009[24]		Males without urethral symptoms	0.8%			Urine, urethral	NA	NA
Vaughn, 2010[41]		STD clinic attendees	3.0%	NA	FilmArray STD Panel	Urine	NA	NA
Zhao, 2012[42]		Patients with suspected HPV and HSV infection	10.2%	1.6% with HSV-2; 1.1% with HPV-16	In-house multiplex PCR	Genital, cervical	NA	NA
Gimenes, 2014[11]	HSV-2	Infertile males	8.0%	NA	PCR-Restriction Fragment Length Polymorphism (PCR-RFLP)	Semen	100.0 (NA)	100.0 (NA)
Muvunyi, 2011[26]		Infertile females	6.2%	NA	STDFinder (multiplex ligation-dependent probe	Vaginal	40.0 (NA)	100.0 (NA)

Reference	Strain	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
					amplification)			
Suntoke, 2009[38]		Genital ulcer patients	61.0%	64.0% with HIV or HSV-1	In-house PCR	Ulcer, blood	84.5 (NA)	36.1 (NA)
Le Goff, 2010[16]		Clinically asymptomatic adults	45.1%	NA	BioPlex 2200 immunoassay system	Serum	NA	NA
Zhao, 2012[42]		Patients with suspected HPV and HSV infection	17.1%	1.6% with HSV-1; 2.7% with HPV-16; 2.1% with HPV-18; 1.1% with HPV-16 and HPV-18	In-house multiplex PCR	Genital, cervical	NA	NA
Parnell, 2014[32]	Unspecified HSV strain	NA	2.1%	2.1% with TP	Abbott Architect	NA	NA	NA

CI, confidence interval; HI, *Haemophilus influenzae*; HPV, human papillomavirus; HSV, herpes simplex virus; NA, not available; PCR, polymerase chain reaction; TP, *Treponema pallidum*; UP, *Ureaplasma parvum*.

Supplementary Table 3. STI Case Positivity, Positive Predictive Values and Negative Predictive Values as obtained from Screening Utilizing Immunochromatographic Tests

A. Treponema pallidum

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Omoding, 2014[29]	Pregnant females	8.6%	1.4% with HIV	SD Bioline HIV/Syphilis Duo Test	Venous blood (plasma)	100.0 (79.1-100.0)	100.0 (97.7-100.0)
Stafylis, 2019[37]	STI clinic attendees	27.0%	NA	INSTI Multiplex HIV-1/HIV-2/syphilis antibody test kit	Fingerstick blood	93.3 (NA)	86.0 (NA)
Lodiongo, 2018[18]	Pregnant females	3.2%	NA	SD Bioline HIV/Syphilis Duo RDT	Venous blood	NA	NA
Pant Pai, 2014[30]	Injection drug users	1.8%	NA	Miriad rapid TP/HBV/HIV/HCV antibody test (MedMira)	Fingerstick blood	NA	NA
Pant Pai, 2014[30]	STD clinic attendees	9.9%	NA			NA	NA

CI, confidence interval; HIV, human immunodeficiency virus; NA, not available.

B. Hepatitis C virus

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Fisher, 2015[9]	Adult injection drug users, females who engage in risky sexual encounters, mwn who have sex with men and/or women, transgender adults	NA	NA	Chembio HIV/HCV/syphilis	Whole blood	97.1 (93.0-98.9)	98.2 (96.9-99.0)
Fisher, 2015[9]	Adult injection drug users, females who engage in risky sexual encounters, mwn who have sex with men and/or women, transgender adults	NA	NA	MedMira HIV/HCV/HBV	Whole blood	100.0 (96.1-100.0)	96.2 (94.4-97.4)
Fisher, 2015[9]	Adult injection drug users, females who engage in risky sexual encounters, mwn who have sex with men	19.2%	NA	MedMira HIV/HCV	Whole blood	100.0 (96.2-100.0)	95.7 (94.0-97.0)

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
	and/or women, transgender adults						
Fisher, 2015[9]	Adult injection drug users, females who engage in risky sexual encounters, mwn who have sex with men and/or women, transgender adults	NA	NA	Chembio HIV/HCV	Whole blood	97.4 (93.1-99.2)	98.0 (96.6-98.9)
Kalla, 2019[15]	Volunteers	2.2%	NA	HIV/HCV/HBsAg (Triplex, Biosynex, France)	Blood	100.0 (84.5-100.0)	100.0 (99.6-100.0)
Longo, 2018[19]	Patients with unknown HIV status	4.2%	NA	HIV/HCV/HBsAG Combo Rapid Test Cassette (ITHD- C43)	Capillary blood	NA	NA
Mboumba Bouassa, 2018[23]	Childbearing aged females in resource limited settings	7.5%	NA	HIV/HCV/HBsAG Combo Rapid Test Cassette (ITHD- C43)	Capillary blood	NA	NA

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Pant Pai, 2014[30]	Injection drug users	42.2%	NA	Miriad rapid TP/HBV/HIV/ HCV antibody test (MedMira)	Fingerstick blood	NA	NA
Pant Pai, 2014[30]	STD clinic attendees	0.5%	NA			NA	NA

CI, confidence interval; HIV, human immunodeficiency virus; HCV, hepatitis C virus; NA, not available; STD, sexually-transmitted disease.

C. Human immunodeficiency virus

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Omoding, 2014[29]	Pregnant females	7.3%	NA	SD Bioline HIV/Syphilis Duo test	Venous blood (plasma)	94.1 (69.2-99.7)	100.0 (97.7-100.0)
Lodiongo, 2018[18]	Pregnant females	1.8%	NA		Venous blood	100.0 (63.1-100.0)	100.0 (99.2-100.0)
Stafylis, 2019[37]	STI clinic attendees	29.9%	NA	INSTI Multiplex HIV-1/HIV-2/syphilis antibody test kit	Fingerstick blood	100.0 (NA)	99.5 (NA)
Kalla, 2019[15]	Volunteers	2.1%	0.1% with HBV	HIV/HCV/HBsAg (Triplex, Biosynex, France)	Blood	100.0 (83.4-100.0)	100.0 (99.6-100.0)

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Longo, 2018[19]	Patients with unknown HIV status	7.1%	1.4% with HBV	HIV/HCV/HBsAG Combo Rapid Test Cassette (ITHD- C43)	Capillary blood	NA	NA
Mboumba Bouassa, 2018[23]	Childbearing aged females in resource limited settings	3.7%	0.4% with HBV	HIV/HCV/HBsAG Combo Rapid Test Cassette (ITHD- C43)	Capillary blood	NA	NA
Menzato, 2018[25]	Inhabitants of rural Guinea Bissau, West Africa	6.8%	NA	Abbott Determine	Vaginal	NA	NA
Pant Pai, 2014[30]	Injection drug users	3.7%	NA	Miriad rapid TP/HBV/HIV/HCV antibody test (MedMira)	Fingerstick blood	NA	NA
Pant Pai, 2014[30]	STD clinic attendees	14.9%	NA			NA	NA

CI, confidence interval; HBV, hepatitis B virus; HIV, human immunodeficiency virus; NA, not available; STD, sexually-transmitted disease.

D. Hepatitis B virus (HBV)

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections	Test Name	Specimen Type	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Kalla, 2019[15]	Volunteers	8.6%	0.1% with HIV	HIV/HCV/HBs Ag (Triplex, Biosynex, France)	Blood	100.0 (95.6-100.0)	100.0 (99.6-100.0)

CI, confidence interval; HIV, human immunodeficiency virus; NA, not available.

Supplementary Table 4. Additional STI Case Positivity Results

A. Hepatitis B virus (HBV)

Reference	Population at Risk	Case Positivity	Prevalence of Co-Infections
Longo, 2018[19]	Patients with unknown HIV status	23.9%	1.4% with HIV
Mboumba Bouassa, 2018[23]	Childbearing aged females in resource limited settings	3.0%	0.4% with HCV
Pant Pai, 2014[30]	STD clinic attendees	20.0%	NA
Pant Pai, 2019[31]	Pregnant females	1.1%	NA

HCV, hepatitis C virus; HIV, human immunodeficiency virus; NA, not available; STD, sexually-transmitted disease.

B. Human papillomavirus (HPV)

Reference	Strain	Population at Risk	Case Positivity	Prevalence of Co-Infections
Ho, 2015[13]	Any HPV	Females with no cervical abnormalities referred to undergo a cervical exam	92.8%	NA
Ho, 2015[13]	Any HPV	Females with cervical dysplasia (<CIN1)	94.0%	NA
Ho, 2015[13]	Any HPV	Females with CIN1	90.7%	NA
Ho, 2015[13]	Any HPV	Females with CIN2	92.6%	NA
Ho, 2015[13]	Any HPV	Females with CIN3	98.2%	NA
Ho, 2015[13]	Any HPV	Females with cervical cancer	96.1%	NA
Gimenes, 2014[11]	Any HPV	Infertile males	38.0%	NA
Roberts, 2011[33]	Any HPV	Females aged 16-23 years	69.2%	51.3% with multiple HPV co-infections
Zhao, 2012[42]	HPV-16	Patients with suspected HPV and HSV infection	22.5%	1.6% with HPV-18; 1.1% with HSV-1; 2.7% with HSV-2; 1.1% with HPV-18 and HSV-2
Zhao, 2012[42]	HPV-18	Patients with suspected	12.3%	1.6% with HPV-16;

Reference	Strain	Population at Risk	Case Positivity	Prevalence of Co-Infections
		HPV and HSV infection		2.1% with HSV-2; 1.1% with HPV-16 and HSV-2

CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HSV, herpes simplex virus; NA, not available.

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