

Received: 2019.10.22

Accepted: 2020.01.07

Available online: 2020.02.18

Published: 2020.04.16

# Progress and Perspectives in Point of Care Testing for Urogenital *Chlamydia trachomatis* Infection: A Review

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Study Design A

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Data Interpretation D

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**Source of support:** This study was supported by the Natural Science Foundation of China (Grant No. 31670178) and the Natural Science Foundation of Hunan Province, China (Grant No. 2017JJ2350)

Worldwide, genital infection with *Chlamydia trachomatis* (*C. trachomatis*) is one of the most common sexually transmitted infections. Most infections are asymptomatic. However, particularly in women, untreated infection with *C. trachomatis* can lead to complications that include pelvic inflammatory disease, infertility, and tubal ectopic pregnancy. Rapid methods for early and accurate diagnosis for infection with *C. trachomatis* that can be performed in the clinic would allow for earlier treatment to prevent complications. Traditional laboratory-based tests for *C. trachomatis* infection include culture, enzyme immunoassay, direct immunofluorescence, nucleic acid hybridization, and nucleic acid amplification tests, which take time but have high diagnostic sensitivity. Novel and rapid diagnostic tests include extraordinary optical transmission (EOT), loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), and microwave-accelerated metal-enhanced fluorescence (MAMEF). Although these new tests offer the promise of rapid screening and diagnosis, they may have lower diagnostic sensitivity. This review aims to provide an overview of traditional methods for the diagnosis of urogenital infection with *C. trachomatis*, the current status of POC testing for urogenital *C. trachomatis* infection and discusses recent progress and perspectives.

**MeSH Keywords:** **Chlamydophila Infections • Diagnosis • Point-of-Care Systems**

**Full-text PDF:** <https://www.medscimonit.com/abstract/index/idArt/920873>

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## Background

Worldwide, urogenital infection with *Chlamydia trachomatis* (*C. trachomatis*) is one of the most common sexually transmitted infections (STIs). According to data from the World Health Organisation (WHO), there are approximately 127.2 million new cases of urogenital infection with *C. trachomatis* each year [1]. In 2018, approximately 1.76 million chlamydial infections were reported to the Centers for Disease Control and Prevention (CDC) in the US, and the rates of reported cases increased by 2.9% between 2017 and 2018 [2].

*C. trachomatis* is an obligate, intracellular, Gram-negative bacterium. A distinctive feature of urogenital *C. trachomatis* infection is that the majority of patients are asymptomatic and usually do not seek medical attention [3]. The lack of symptoms makes early diagnosis difficult, and treatment is often delayed or patients are never treated, particularly in developing countries. Untreated *C. trachomatis* infections result in the continued transmission of infection with *C. trachomatis* and also augment the venereal transmission and acquisition of human immunodeficiency virus (HIV) [4]. In some women, untreated *C. trachomatis* infections involve the upper genital tract and become chronic, which may lead to severe complications that include pelvic inflammatory disease (PID), ectopic pregnancy, and tubal factor infertility (TFI) [5]. The presence of mild and nonspecific symptoms in most patients with *C. trachomatis* supports the need for routine screening for infection, which is particularly recommended for sexually active women younger than 25 years of age, and older women at risk [6]. The implementation of screening and early diagnostic testing for urogenital *C. trachomatis* infections would require rapid, cost-effective, and accurate testing using point of care (POC) tests that can be performed in the clinic and provide results within minutes, rather than hours or days. This review aims to provide an overview of traditional methods for the diagnosis of urogenital infection with *C. trachomatis*, the current status of POC testing for urogenital *C. trachomatis* infection and discusses recent progress and perspectives.

## The Limitations of Traditional Diagnostic Methods

Traditional detection methods used to diagnose urogenital *C. trachomatis* infection include cell culture, the direct fluorescence assay, enzyme immunoassay, serology, and nucleic acid amplification tests. Unfortunately, all of these methods cannot fully meet the requirement of early diagnosis and treatment in the clinic. Cell culture was the initial gold standard method for the diagnosis of *C. trachomatis*, but is now seldom performed as a diagnostic procedure [7]. The main problem with cell culture is that the sample requires a rapid cold transport

system to preserve the sample and the procedure of cell culture is demanding and time-consuming.

Compared with cell culture, antigen detection methods, including the direct fluorescence assay and enzyme immunoassay, are relatively rapid and simple. Although still performed in some low-resource settings, antigen detection methods are not recommended as routine screening and diagnostic tests for *C. trachomatis* infection due to the suboptimal diagnostic accuracy [8–10]. Serology can be useful in the diagnosis of chronic infection and the prediction of complications [11]. However, since antibodies are detectable with a delay of several weeks or even absent following *C. trachomatis* infection in some individuals, serological testing has little value in screening for uncomplicated urogenital *C. trachomatis* infection and is not recommended as a screening tool [12].

Nucleic acid amplification tests are the most sensitive tests for the diagnosis of urogenital *C. trachomatis* infection and also have an excellent specificity comparable to culture. Also, nucleic acid amplification tests do not rely on viable pathogens and permit the use of noninvasive clinical samples such as first-catch urine specimens or self-collected vaginal swabs [13]. The simplified sampling procedures also facilitate specimen transport and preservation. Therefore, nucleic acid amplification tests have replaced cell culture as the new diagnostic gold standard and became the primarily recommended tests for the diagnosis of *C. trachomatis* infection [9,13,14].

Although many highly accurate nucleic acid amplification tests are available in the developed world, they are generally more expensive than other tests. Nucleic acid amplification tests require trained technicians and complicated laboratory equipment to run the tests. Therefore, nucleic acid amplification tests as screening tools are unaffordable and inaccessible to patients in developing countries. Also, the results from nucleic acid amplification testing are not available at the time of testing. The delay between patients receiving the test results and receiving treatment increases the risk of complications and reduces the rate of patients returning for treatment and follow-up [15]. Some clinicians will provide empiric treatment according to symptoms and epidemiologic exposure of patients. Unfortunately, a large number of patients treated empirically are negative for *C. trachomatis* [16], and are over-treated, which contributes to antibiotic resistance. Therefore, there is a clinical need for a cheap, rapid, and simple assay that would allow clinicians to make an early diagnosis and guide antibiotic use during the initial patient visit.

## Current Standard Point of Care (POC) Tests

A point of care (POC) diagnostic test is performed near the patient or in the clinic with results that are reported rapidly, within minutes, so that patients can be managed optimally [17].

**Table 1.** The ASSURED criteria from the World Health Organisation (WHO) [18].

<b>A.</b> Affordable, by those at risk for infection
<b>S.</b> Sensitive, with very few false-negatives
<b>S.</b> Specific, with very few false-positives
<b>U.</b> User-friendly, and simple to perform in a few steps with minimal training
<b>R.</b> Robust and rapid, and not requiring refrigerated storage with results in <30 minutes
<b>E.</b> Equipment-free (or simple), non-invasive, with easily collected specimens
<b>D.</b> Deliverable, to those who need them

In 2006, the Sexually Transmitted Disease Diagnostics Initiative of the World Health Organisation (WHO) developed the ASSURED criteria for use in a resource-limited setting for a POC test [18]. According to the ASSURED criteria, an ideal POC test should be affordable, sensitive, specific, user-friendly, rapid, equipment-free, and deliverable for the end-user (Table 1) [18].

Compared with traditional nucleic acid amplification tests, the POC test can provide test results in a short time. Rapid test results enable clinicians to make rapid clinical decisions and prescribe appropriate antibiotic therapy immediately if the test results are positive. POC testing prevents delays in treatment, increases treatment compliance, and reduces onward transmission of *C. trachomatis* infection [12,19]. Gift et al. [20] used mathematical models to show that even a POC test with 63% sensitivity will treat more patients than nucleic acid amplification tests when the treatment return rate <65%. Other mathematical models demonstrated that the POC test with high sensitivity could be a more cost-effective diagnostic test compared with traditional nucleic acid amplification tests and has the potential to improve the impact of infection screening [21,22].

## The Performance of Current Commercial Antigen-Based POC Tests

There are several commercially available POC tests for the diagnosis of *C. trachomatis* infection that are based on antigen and antibody interactions, and the manufacturers claimed that these kits have high sensitivity and specificity. However, most of the commercial POC tests showed disappointing test performance compared with nucleic acid amplification tests, especially in terms of sensitivity (17.1–66.7%) in some non-manufacturer-sponsored clinical studies (Table 2) [23–28]. The poor sensitivity of antigen-based POC tests for *C. trachomatis* infection may be due to inadequate antigen exposure in the specimen and low bacterial load in asymptomatic patients.

*Chlamydia sp.* are strict intracellular pathogens that are able to inhibit host cell apoptosis defense functions and enter and survive in neutrophil and macrophage phagocytes [29,30]. Insufficient and inappropriate pre-analytical stages in some antigen-based POC tests do not make the antigen fully exposed, which limits their sensitivity. Also, *C. trachomatis* becomes persistent form and ceases dividing under stress situations, enabling long-term survival in the host. The bacterial load in urogenital specimens of these patients may not reach the limit of detection of antigen-based POC tests. However, the specificity of antigen-based POC tests is limited by cross-reactivity. Some of these POC tests detect chlamydial lipopolysaccharide (LPS) antigen that is considered to be a genus-specific antigen that is common to all *Chlamydia* species, but cross-reactivity with LPS of other Gram-negative bacteria has also been reported [31]. Due to the inadequate detection accuracy, these tests have little benefits in the management of *C. trachomatis* infections and were not recommend for routine screening for *C. trachomatis* infection (Table 2).

In contrast to antigen-based POC tests, molecular tests based on nucleic acid amplification tests have a greater diagnostic performance, which is comparable to traditional nucleic acid amplification tests (Table 2). In 2012, the Cepheid GeneXpert CT/NG assay (Cepheid, Sunnyvale, CA, USA), a newly developed real-time polymerase chain reaction (PCR)-based assay for the detection of both *C. trachomatis* and *Neisseria gonorrhoea*, was approved by the US Food and Drug Administration (FDA) [26]. The assay has high sensitivity (97.4–98.7%) and specificity (99.4–99.9%) using urine specimens, vaginal swabs, and endocervical swabs. The sensitivity (85.7–86.0%) and specificity (99.2–99.4%) from rectal swabs are acceptable. Also, this assay enables automated sample preparation, extraction, amplification, and detection in a closed system that minimizes the process and opportunity of contamination with results available in approximately 87 minutes. Although the assay has extraordinary testing performance, it still needs the expensive experimental device, and the detection cycle of 87 min does not meet the ASSURED criteria of less than 30 minutes (Table 1) [18]. Therefore, there are remaining needs for POC tests that can meet the ASSURED criteria for POC testing, which should be affordable, sensitive, specific, user-friendly, rapid, equipment-free, and deliverable to the end-user (Table 1) [18].

## Recently Developed POC Tests for *C. trachomatis* Infection

### Multiplex nanoplasmmonic biosensor test

In 2017, Soler et al. [32] developed a novel nanoplasmmonic biosensor for multiplexed detection of *C. trachomatis* and *Neisseria gonorrhoeae* in urine samples. This POC test is based on direct

**Table 2.** The performance of commercial *Chlamydia trachomatis* point of care (POC) tests.

Name of test	Type of test	Test length	Specimen	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	Study
ACON EIA test	ICT	30 min	Urine (M)	43.8% (19.8–70.1%)	98.3% (93.9–99.8%)	77.8%	92.7%	Hurly et al. [23]
			Vaginal swab	66.7% (22.3–95.7%)	91.3% (82.0–96.7%)	40.0%	96.9%	Hurly et al. [23]
Chlamydia Rapid Test	ICT	30 min	Urine (M)	41.4% (23.5–51.1%)	89.0% (82.2–93.8%)	46.2%	86.9%	Hurly et al. [23]
			Vaginal swab	74.2% (61.5–84.5%)	95.7% (91.3–98.2%)	86.8%	90.6%	Hurly et al. [23]
			Vaginal swab	41.2% (31.9–50.9%)	96.4% (95.0–97.5%)	59.2%	92.9%	van der Helm et al. [24]
HandiLab-C test	Enzyme detection	15 min	Vaginal swab	22.5% (13.3–31.7%)	88.9% (86.4–91.3%)	19.8%	90.4%	van Dommelen et al. [25]
Biorapid Chlamydia Ag test	ICT	20 min	Vaginal swab	17.1% (8.9–25.2%)	93.7% (91.9–95.5%)	24.6%	90.4%	van Dommelen et al. [25]
QuickVue Chlamydia Test	ICT	15 min	Vaginal swab	25.0% (15.7–34.3%)	99.7% (99.3–100%)	91.3%	91.5%	van Dommelen et al. [25]
Cepheid GeneXpert	RT-PCR	87 min	Rectal swab	86.0% (72.1–94.7%)	99.2% (97.6–99.8%)	92.5%	98.4%	Goldenberg et al. [27]
			Rectal swab	85.7% (72.8–94.1%)	99.4% (97.9–99.9%)	–	–	Bristow et al. [28]
			Urine (M)	97.5% (91.4–99.7%)	99.9% (99.6–100%)	98.7%	99.8%	Gaydos et al. [26]
			Urine (F)	97.6% (91.5–99.7%)	99.8% (99.5–100%)	96.4%	99.9%	Gaydos et al. [26]
			Endocervical swab	97.4% (91.0–99.7%)	99.6% (99.1–99.8%)	91.6%	99.9%	Gaydos et al. [26]
			Vaginal swab	98.7% (93.1–100%)	99.4% (98.9–99.7%)	88.6%	99.9%	Gaydos et al. [26]

ICT – immunochromatographic test; NAAT – nucleic acid amplification test; POC – point of care; RT-PCR – real-time polymerase chain reaction; PPV – positive-predictive value; NPV – negative predictive value; CI – confidence interval. ACON enzyme immunoassay (EIA) test (ACON, Scottsdale, AZ); Chlamydia Rapid Test (Becton-Dickinson, Sparks, MD, USA); HandiLab-C test (Zonda, Dallas TX, USA); Biorapid Chlamydia Ag test (Biokit SA, Barcelona, Spain); QuickVue Chlamydia Test (Quidel, San Diego, CA, USA); Cepheid GeneXpert PCR assay (Cepheid, Sunnyvale, CA, USA).

immunoassay and selectively functionalize the nano-arrays with specific antibodies against the major outer membrane protein (MOMP) to capture and identify the bacteria [32]. Soler et al. applied a functionalization strategy based on protein A/G, a recombinant fusion protein that has a high affinity for the Fc region of the antibodies to maximize the sensitivity of the test [32]. Spectroscopic imaging was based on the extraordinary optical transmission (EOT) phenomenon to obtain real-time quantitative bioanalytical information from antigen and antibody interactions when urine samples flowed over the surface of the sensors [32]. The biosensor had a limit of detection of 300 colony forming units (CFUs)/mL for *C. trachomatis* [32]. Even though direct immunoassays are less sensitive

than nucleic acid amplification tests, sandwich immunoassays, or the use of magnetic beads for signal enhancement increases the sensitivity [33–35]. Also, this testing method takes advantage of novel biosensors to improve the sensitivity, and has the unique advantage that there is no need for sample pretreatment or post-amplification steps. Therefore, the test result is available in a few minutes, and the biosensor provides a highly sensitive test in one step and at a low cost. However, the sensitivity, specificity, and cost-effectiveness of the novel nanoplasmonic biosensor for multiplexed detection of *C. trachomatis* require validation for use in POC testing by controlled clinical trials.

### Loop-mediated isothermal amplification (LAMP) test

Loop-mediated isothermal amplification (LAMP), an isothermal amplification technique, which has the potential to replace PCR for the amplification and detection of specific gene sequences [36]. Compared with conventional PCR, LAMP is easy to perform because it does not require a sophisticated thermal cycler and is used under isothermal conditions (60–65°C), but only requires a DNA polymerase, a set of four oligonucleotide primers, and a simple water bath or using a heat block [37]. The designed primers recognize six distinct sequences on the target DNA to make sure the high specificity of LAMP. Also, LAMP remained sensitive and specific when using untreated samples such as urine or stool that commonly inhibit PCR amplification and a large amount of DNA product can be amplified within 30–60 minutes [38], which indicates that LAMP is not only robust, but is also a time-saving method. Due to these advantages, LAMP has not only been of interest to clinical researchers but has become a promising platform for POC testing.

Jevtusevskaia et al. [39] combined LAMP with antimicrobial peptide lysis and developed a novel POC test for *C. trachomatis* infection using first-void urine samples. Typically, samples require prior extraction and purification of genetic material, and most of lysate preparation techniques are expensive and time-consuming. In contrast, antimicrobial peptides can efficiently lyse many kinds of bacteria, including *C. trachomatis*, and release the DNA, which provides a simple way to finish the sample preparation before LAMP. Due to the tolerance shown by LAMP to various inhibitors from the crude samples, the clinical analysis showed that this assay had 73% sensitivity and 100% specificity compared with conventional techniques based on nucleic acid amplification tests. Also, the assay takes 21 minutes, and the result can be visualized using lateral flow strips. Overall, this assay almost meets the ASSURED criteria [18], except for the sensitivity of the test, which could be increased by further optimizing the sample pretreatment process so that the test may compare well with other POC tests.

There are other feasible methods of sample pretreatment, and the evaluation of the result can be combined with LAMP to establish a POC test. Choopara et al. [40] heated endocervical swab samples at 95°C for 5 minutes, and the released DNA products were successfully used as the DNA template for LAMP. However, hydroxy naphthol blue (HNB) was used, which is a metal indicator, to show the result [40]. During the LAMP reaction, pyrophosphate ions combine with  $Mg^{2+}$  and form the white precipitates of magnesium pyrophosphate, so that the concentration of  $Mg^{2+}$  in the solution will decrease and HNB can be used for colorimetric detection of LAMP products by a change in  $Mg^{2+}$  concentration. A color change indicates a positive reaction from violet to sky blue that can be measured with naked eyes or a microplate reader at 650 nm. The simple and

easy methods of sample pretreatment and reading of the result shortens the total assay time to 45 minutes and reduces the cost to \$3 USD per reaction. Also, the effectiveness of the test is comparable to a commercial kit based on the PCR method with the 90–100% sensitivity and 95% specificity. However, this assay still had the limitation that the results were affected by poor visual read-out, which required the use of more enhanced methods to determine the results. For example, the visual distance-based paper analytical device (dPAD) for LAMP proposed by Hongwarittorn et al. [41] is a promising solution to obtain semi-quantitative results.

### Recombinase polymerase amplification (RPA) test

Recombinase polymerase amplification (RPA) is also an isothermal amplification technique. Compared with LAMP, the main advantage of RPA is that the design of primers is simpler, only using two primers, and the operating temperature is lower [42]. Since the reaction runs at temperatures between 37–42°C, the assay has a low power requirement that can be satisfied even by using portable battery [43]. Due to its simplicity, RPA is an ideal platform for POC testing. Krolov et al. [44] developed a POC test based on RPA for the detection of genital *C. trachomatis* infection. CDS2 of the *C. trachomatis* cryptic plasmid is targeted and requires only 10 minutes to produce a detectable amount of CSD2-specific product by using 5  $\mu$ L of heat-treated urine sample and product was visualized using lateral flow strips in a few minutes [44]. The test takes less than 20 minutes, including sample pretreatment, target sequence amplification, and result evaluation. Clinical findings showed that this assay had a sensitivity of 83% and a specificity of 100% compared with the Roche Cobas Amplicor *C. trachomatis* test (Roche, Basel, Switzerland), which is based on the PCR. The amount of the urine sample limited the sensitivity, and more than 5  $\mu$ L of urine could significantly inhibit the amplification efficiency. Therefore, optimizing sample collection, such as using the novel first-void urine collection device, could further increase the sensitivity of this assay because there is 6.3 times the organism load of *C. trachomatis* in first-void urine compared with using a routine urine container [45].

### Microwave-accelerated metal-enhanced fluorescence (MAMEF)

Microwave-accelerated metal-enhanced fluorescence (MAMEF) is a highly sensitive direct DNA detection technique that combines the benefits of the two technologies of low-power microwave heating and metal-enhanced fluorescence (MEF) [46]. Low-power microwave heating kinetically accelerates the biological reaction so that the bioassay run time is reduced to a few seconds. MEF increases the DNA detectability and the sensitivity of the assays by amplifying the fluorescence signatures [47]. Therefore, MAMEF is an innovative and promising method for POC testing.

**Table 3.** The performance of point of care (POC) tests under development for *Chlamydia trachomatis*.

Study	Type of test	Time to result	Specimen	Sensitivity (95% CI)	Specificity (95% CI)	LOD	Cost per test
Soler et al. [32]	EOT	Real-time	Urine	–	–	300 CFU/ml	–
Jevtuševskaja et al. [39]	LAMP	21 min	Urine	73% (39.0–94.0%)	100% (95–100%)	70 copies	–
Choopara et al. [40]	LAMP	45 min	Endocervical swab	91%	95%	4,500 copies	\$3 USD
Krolov et al. [44]	RPA	20 min	Urine	83% (51–97%)	100% (92–100%)	50 copies	–
Zhang et al. [48]	MAMEF	1 min	–	–	–	100 CFU/ml	–
Melendez et al. [49]	MAMEF (plasmid-based)	9 min	Vaginal swab	82.2% (71.0–93.4%)	92.9% (89.8–96.0%)	10 IFU/ml	\$2 USD
	MAMEF (16S rRNA-based)	9 min	Vaginal swab	75.5% (60.4–86.2%)	92.9% (89.8–96.0%)	–	\$2 USD

EOT – extraordinary optical transmission; LAMP – loop-mediated isothermal amplification; RPA – recombinase polymerase amplification; POC – point of care; MAMEF – microwave-accelerated metal-enhanced fluorescence; LOD – limit of detection; CFU – colony-forming unit; IFU – inclusion forming unit; CI – confidence interval.

Zhang et al. [48] demonstrated an inexpensive technology based on MAMEF to both lyse *C. trachomatis* and detect DNA released from *C. trachomatis*, taking 10 seconds using gold and focused microwaves. The released target DNA sequence was detected with a fluorescence probe and an anchor probe attached to a silvered glass slide, which formed a three-component assay construct. This special construct closed the fluorophore label to metallic nanoparticles, thereby inducing metal-enhanced fluorescence-based optical enhancement. The silvered surfaces preferentially focus the microwave energy and accelerate the DNA hybridization kinetics. This approach had a limit of detection of 100 CFU/mL for *C. trachomatis*, and the whole assay was performed within one minute.

In 2013, Melendez et al. [49] optimized MAMEF technology and developed a POC test for *C. trachomatis* detection. In a blind comparative study, they evaluated two distinct MAMEF assays targeting the 16S rRNA gene and cryptic plasmid, respectively [49]. The results showed that the plasmid-based assay had a sensitivity of 82.2% and a specificity of 92.9%, and the 16S rRNA assay had a sensitivity of 75.5% and a specificity of 92.9% when compared with nucleic acid amplification tests [49]. The testing cycle was less than 9 minutes, and the cost of each assay was \$2 USD [49]. The size of the detection device was about the size of a shoebox, which makes this assay possible to perform in a low-resource setting and in the clinic. Although this assay had the limitation of a large number of false-positive samples [49], it still has the potential to meet the ASSURED criteria (affordable, sensitive, specific, user-friendly, rapid, equipment-free, and deliverable) after the further improvements [18,49].

## Discussion

This review has shown that the main obstacles to the development of a point of care (POC) test for the diagnosis of urogenital infection with *Chlamydia trachomatis* (*C. trachomatis*) is the balance between sensitivity, or detection efficiency, and speed of testing. Even though some commercially available POC tests for *C. trachomatis* infection based on immune chromatographic tests can be reported in a short time, their lack of sensitivity is unsuitable for clinical needs. Currently, ensuring the adequate sensitivity and specificity is the priority for developing POC tests for the diagnosis of *C. trachomatis* infection.

Most of the novel POC tests under development are molecular tests that are based on nucleic acid detection, and they show better detection performance when compared with commercially available immune chromatographic POC tests (Table 3). With the integration of isothermal amplification techniques, such as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA), novel molecular POC tests have become less restrained by the long duration of laboratory testing and by large, complex, and expensive laboratory equipment. The molecular POC tests based on microwave-accelerated metal-enhanced fluorescence (MAMEF) also show adequate detection efficiency and do not require nucleic acid amplification steps. Also, the selection of specific target sequences greatly reduces the cross-reactivity between different species [39,49]. Due to the optimal balance between detection efficiency and testing time, the molecular POC tests may become the future diagnostic POC methods for the diagnosis of urogenital *C. trachomatis* infection.

Utility and cost-effectiveness are the most important factors to consider in novel POC testing, after diagnostic sensitivity and specificity. The acceptability of the test to patients directly affects the utility of the POC tests, as patients may be unwilling to wait in the clinic for several hours for their results. Harding-Esch et al. [50] reported that patients were unwilling to wait more than two hours for GeneXpert Cepheid nucleic acid amplification (Cepheid, Sunnyvale, CA, USA) test results, with only 21.4% of patients receiving their results before leaving the clinic. Another study showed that 75% of women were prepared to wait between 30 minutes and two hours, and only 18% were prepared to wait more than two hours [51]. Since most of these novel POC tests can be completed within 30 minutes, the shorter turnaround time is expected to be more acceptable for patients.

Also, the type of specimen is also related to patient acceptance and is an important consideration in the development of POC tests. Although cervical swabs may have higher sensitivity than vaginal swabs, it is preferable for patients to use specimens that are easy to collect or obtain themselves for POC tests. A recent systematic review showed that the sensitivity and specificity of vaginal self-collected swabs was increased when compared with the swabs collected by clinicians [52]. For male patients, using first-void urine and urethral swabs have equivalent performance in nucleic acid amplification tests [9]. Urine and vaginal swabs are the preferred types of specimens in the development of POC tests, and self-collected specimens would increase patient acceptance of their use [53].

Although novel POC tests for *C. trachomatis* infection have not yet been made commercially available and the cost-effectiveness of these tests remain to be determined, some have been shown to have a low cost. The cost of \$2–3 USD for each test is

a notable feature, especially in resource-limited settings where the prevalence of sexually transmitted diseases (STDs) and the burden of complications associated with STDs is greatest. However, the sensitivity, specificity, utility, and cost-effectiveness of these novel POC tests require evaluation using high-quality, large-scale, randomized controlled trials to provide evidence-based support for their clinical use.

## Conclusions

This review has presented an overview of traditional methods for the diagnosis of urogenital infection with *Chlamydia trachomatis* (*C. trachomatis*), and has reviewed the current status of point of care (POC) testing and recent progress in the development of rapid commercial tests. Current laboratory tests for *C. trachomatis* infection are regarded as the gold standard in diagnosis as they have high diagnostic sensitivity and specificity. However, traditional laboratory tests can take hours to obtain a result and require expensive equipment that can only be used by trained technicians. Most commercially available point of care (POC) tests for *C. trachomatis* have disappointing performance, with poor sensitivity. Fortunately, with the integration of novel techniques, recently developed POC tests have met most requirements of the ASSURED criteria (affordable, sensitive, specific, user-friendly, rapid, equipment-free, and deliverable). Since the novel POC tests are more simple, rapid, sensitive, and cost-saving, they hold promise to improve patient outcomes and reduce the prevalence of urogenital *C. trachomatis* and its long-term complications.

## Conflict of interest

None.

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