Practical Laboratory Medicine 40 (2024) e00412

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm

Registered Report Stage II

Evaluation of the diagnostic performance of an immunochromatographic test for *Chlamydia trachomatis*

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ARTICLE INFO

Keywords:
Chlamydia trachomatis
Immunochromatographic test
Diagnostic performance
Clinical application
Quality control

ABSTRACT

Objectives: To evaluate the diagnostic performance of different brands of immunochromatographic test (ICT) reagents for *Chlamydia trachomatis* using homogenized samples to provide a reference for reagent quality control.

Methods: Eight commercially available ICT reagents were evaluated, of which three used the latex method and five used the colloidal gold method. Analytical performance evaluation using a pure culture broth of *C. trachomatis*, as well as clinical application validation using cervical epithelial cell samples acquired from the research subjects, were conducted. The concentration of *C. trachomatis* was quantified using a nucleic acid amplification test.

Results: The limit of detection (LOD) of different ICT reagents in the analytical performance evaluation varied from 9.5×10^3 to 1×10^5 IFU/mL, and only one reagent met the LOD specified in the manufacturer's instructions. Likewise, only one reagent in the clinical application validation achieved the analytical LOD, four reagents were 2.1–4.2-fold of the analytical LODs, and three reagents failed to detect positive results in clinical samples.

Conclusions: The diagnostic performance of different methods and different brands of ICT reagents in clinical practice was different from the manufacturer's instructions and the results of laboratory evaluation. The diagnostic performance of reagents should be evaluated before they are actually used in clinical practice.

1. Introduction

Chlamydia trachomatis infection is one of the most common sexually transmitted infections that may cause urethritis and epididymitis in males, as well as cervicitis and pelvic inflammatory disease in females [1]. *C. trachomatis* infections in pregnant women can

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https://doi.org/10.1016/j.plabm.2024.e00412
Received 1 March 2024; Received in revised form 7 May 2024; Accepted 25 May 2024
Available online 27 May 2024
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Table 1
List of elements of reagent instructions of immunochromatographic tests for *Chlamydia trachomatis*.

Brand	A	B	C	D	E	F	G	H
Methodology	Latex	Latex	Latex	Colloidal gold	Colloidal gold	Colloidal gold	Colloidal gold	Colloidal gold
Effective duration	24 months	24 months	24 months	24 months	18 months	24 months	24 months	12 months
Coated antibody	Monoclonal antibody against *C. trachomatis*	*C. trachomatis* specific antibodies	*C. trachomatis* specific antibodies	Lipopolysaccharide monoclonal antibody	*C. trachomatis* monoclonal antibody	*C. trachomatis* specific antibodies	Lipopolysaccharide monoclonal antibody	*C. trachomatis* specific antibodies
Applicable sample scope	Female cervix epithelial cells	Cervical/male urinary tract: epithelial cells	Cervical/male urinary tract: epithelial cells	Cervical/male urinary tract: epithelial cells	Female cervical epithelial cells	Cervical/male urinary tract: epithelial cells	Female cervical epithelial cells	Cervical/male urinary tract: epithelial cells
Antigen cleavage method	Acid-base	Acid-base	Acid-base	Acid-base	Acid-base	Alkali	n.d.	Alkali
Observation time	15 min	Within 10–20 min	15 min	Within 10–15 min	Within 15 min	Within 15 min	Within 10–15 min	Within 20 min
Limit of detection	4×10^3 IFU/mL	n.d.	5×10^5 IFU/mL	4×10^3 IFU/mL	n.d.	4×10^3 IFU/mL	5×10^4 IFU/mL	2×10^3 IFU/mL

Abbreviations: IFU, inclusion-forming units; n.d., not described.

also lead to neonatal ophthalmia and pneumonia [2]. It was reported by the World Health Organization (WHO) that the global new cases of chlamydia in women and men aged 15–49 years increased to 128.5 million (95 % uncertainty interval: 90.0 m–173.8 m) in 2020 [3]. According to the data drive from 105 national sexually transmitted diseases surveillance sites in China, a total of 50874 cases of genital chlamydial infection were reported in 2019 [4]. This case count corresponds to an incidence of 55.32 cases per 100,000 people, an increase of 9.98 % compared with the incidence in 2018 (50.30 cases per 100,000) and an increase of 48.79 % from 2015 (37.18 cases per 100,000). *C. trachomatis* infection has become one of the most important public health issues worldwide.

There is an extended incubation period after *C. trachomatis* infection, and most women and some men show subclinical manifestations. The clinical diagnosis predominantly depends on laboratory test results [1]. *C. trachomatis* detection methods are mainly classified into three categories [5]: cell culture of *C. trachomatis*, molecular biological testing of *C. trachomatis* nucleic acids, and immunological detection of *C. trachomatis* antigens. Cell culture is the traditional gold standard for the detection of *C. trachomatis* infections. However, the experimental process is complicated and is currently only used for scientific research in specific institutes. The *C. trachomatis* nucleic acid amplification test (NAAT) surpasses cell culture and immunological methods in sensitivity and specificity and is considered an alternative gold standard. However, implementation of NAAT requires professional technical personnel and equipment, which cannot be carried out in primary medical institutions. A nationwide survey reported that only 15.5 % (721/4640) of laboratories were capable of NAAT in China, and there were regional differences such that the proportion was as low as 2.6 % (23/872) in some regions [6]. The *C. trachomatis* immunochromatographic test (ICT), because of its advantages, including its simple operation steps and fast results, which are suitable for point-of-care testing, primary medical institutions, and home self-testing, has become one of the preferred methods to screen for *C. trachomatis* infection [7]. Moreover, the specialists at WHO recommend rapid testing and the provision of appropriate interventions to reduce the risk of disease transmission in high-risk *C. trachomatis* groups [8].

However, due to different antibody materials, experimental reaction principles, and production processes, the quality of different brands of ICT reagent kits can vary. Differences pertaining to the characteristics can directly affect the sensitivity and specificity of clinical tests. A systematic review including eight reports evaluated ten point-of-care tests for *C. trachomatis*, showing that the sensitivity varied from 17.1 % to 98.7 % and the specificity varied from 53.1 % to 100 % [9]. Additionally, the prevalence rates were different in different regions and varied from 4 % to 16 % [10], which may have an impact on the diagnostic accuracy. So, evaluation of the detection performance of ICT reagents has become a key part of laboratory quality control strategies.

The previous studies were not able to determine whether the detection performance of the ICT reagents or the differential morbidity prevalence of *C. trachomatis* in a different report caused the inconsistencies in the diagnostic results. Currently, more than 10 brands of ICT reagent kits are commercially available in the Chinese market. According to the manufacturer's instructions, ICT directly uses the undiluted cervical or urethral swabs or cell brushes for testing, so that a smaller sample volume is obtained. Multiple sampling is required for parallel comparison of multiple reagents simultaneously. At present, there is no mature method to systematically evaluate the diagnostic performance of multiple reagents at the same time. This study aimed to compare the diagnostic accuracy of different brands of ICT reagents using homogenized samples to provide a reference for quality control of reagents before clinical application.

2. Methods

2.1. Evaluation procedures and sample preparation

This study was conducted at Zhongshan Hospital, School of Medicine, Xiamen University on March 28, 2023. The evaluation consisted of an analytical performance evaluation using a pure culture broth of *C. trachomatis* and interferential microorganisms, and clinical application validation using cervical epithelial cell samples collected from the study subject. All the study samples were collected using commercial dacron swabs with poles made of PVC materials and were dipped into 600 μL of physiological saline. The samples were eluted by vortexing, and the swabs were squeezed, leaving approximately 500 μL of eluent as homogenized samples and packed into a 50-μL aliquot. One of the packaged 50-μL eluents was used for NAAT. The other eight packaged 50-μL eluents were used for parallel comparisons of eight ICT reagents.

2.2. Instructions for ICT reagents

Eight commercially available ICT antigen detection kits that had previously attained product certification from the China State Administration for Market Regulation were tested. To avoid commercial competition, the names of reagent companies were replaced by the letters A to H. Kits A–C require a latex method for detection, while kits D–H utilize a colloidal gold method. Reagents were valid for a maximum of 24 months and a minimum of 12 months. The reagent storage temperature range was 2 ~ 30 °C. All reagents were extracted from *C. trachomatis* lipopolysaccharide antigen by acid-base lysis. The immune response partners were *C. trachomatis* lipopolysaccharide antibodies or specific antibodies. The scope of test samples for kits A, E, and G was limited to cervical epithelial cells. The remaining kits required cervical and urethral epithelial cells for detection. There was no description of the limit of detection (LOD) for *C. trachomatis* in the instructions of kits B and E, and the remaining kits gave LODs of between 2×10^3 IFU/mL and 5×10^5 IFU/mL (Table 1).

2.3. CT-NAAT

The concentration of *C. trachomatis* in each specimen was quantified by NAAT. The results of NAAT for identification of

C. trachomatis were used as the gold standard in this study. The NAAT was performed through the fluorescent polymerase chain reaction method using the *C. trachomatis* nucleic acid detection kit (PCR-fluorescence method, Suzhou BACME Biotech Co., Ltd., Suzhou, China), automatic nucleic acid extractor SLA-D14800 (Suzhou BACME Biotech Co., Ltd., Suzhou, China), and Applied Biosystems 7500 Real Time PCR System (ABI7500, ThermoFisher Scientific, Waltham, MA) according to the manufacturer's instructions.

2.4. Analytical performance evaluation

The analytical sensitivity was evaluated using gradient concentrations of *C. trachomatis* pure culture broth. The intermittent ultrasonic lysis of *C. trachomatis* bacterial suspensions was performed using an ice bath. The *C. trachomatis* pure culture broth was diluted using normal saline to a range of samples of different concentrations (500 μL each) and was stored for future analyses. Samples of different concentrations (50 μL) were detected by eight kinds of ICT reagents. The LOD was defined as the lowest concentration that yielded a positive reaction.

The analytical specificity was evaluated using other common genital tract pathogens as interference factors, including *Escherichia coli*, *Candida albicans*, *Streptococcus agalactiae*, *Gardnerella vaginalis*, *Lactobacillus*, *Staphylococcus aureus*, *Coagulase-negative bacteria*, *Shower cocci*, of which the pure culture on solid medium were all adjusted to a concentration of 10^7 with physiological saline; *Trichomonas*, of which was cultured overnight in diamond medium and adjusted to 50 worm bodies in a high-magnification field; *Mycoplasma humanis* and *Ureaplasma urealyticum*, of which used the suspension cultured in liquid medium for 24 h was used.

2.5. Clinical application validation and study subjects

Clinical samples were collected from ten cases diagnosed with *C. trachomatis* infection by NAAT and ten controls diagnosed with other genitourinary infections but not *C. trachomatis*. Among the ten controls, one was detected with *Escherichia coli*, one with *Candida albicans*, one with *Streptococcus agalactiae*, one with *Gardnerella vaginalis*, three with ureaplasma urealyticum, and three were detected with both ureaplasma urealyticum and mycoplasma hominis. All the study subjects included were female. The project was approved by the Ethics Committee of School of Medicine, Xiamen University, and it was conducted in compliance with the national legislation of China and the Declaration of Helsinki guidelines. Written informed consent was obtained from all study participants.

2.6. Statistical analyses

The true positive, false positive, true negative, and false negative results of ICT were computed using the results of NAAT as the gold standard for the identification of *C. trachomatis* in this study. The diagnostic performance of the ICT assay was determined using sensitivity and specificity according to the Yerushalmy model. The strength of agreement between the paired groups was defined according to the kappa values, respectively, and was classified as almost perfect (κ, 0.81–1.00), substantial (κ, 0.61–0.80), moderate (κ, 0.41–0.60), fair (κ, 0.21–0.40), slight (κ, 0.00–0.20), and poor (κ < 0.00) [11]. All statistical analyses were performed using SPSS 25.0 for Windows (SPSS Inc., Chicago, IL, USA). A two-sided *P* value < 0.05 was considered statistically significant.

Table 2
Analytical performance evaluation of immunochromatographic tests for *Chlamydia trachomatis*.

Testing samples	ICT Reagent							
	A	B	C	D	E	F	G	H
C. trachomatis pure culture broth (IFU/mL)								
1.0×10^5	+	+	+	+	+	±	±	+
5.0×10^4	+	+	–	+	–	–	–	+
2.5×10^4	+	+	–	+	–	–	–	+
9.5×10^3	+	+	–	+	–	–	–	+
4.0×10^3	–	–	–	–	–	–	–	–
1.0×10^3	–	–	–	–	–	–	–	–
1.0×10^2	–	–	–	–	–	–	–	–
Interference factor								
Escherichia coli	–	–	–	–	–	–	–	–
Candida albicans	–	–	–	–	–	–	–	–
Streptococcus agalactiae	–	–	–	–	–	–	–	–
Gardnerella vaginalis	–	–	–	–	–	–	–	±
Lactobacillus	–	–	–	–	–	–	–	–
Staphylococcus aureus	–	–	–	–	–	–	–	–
Coagulase-negative bacteria	–	–	–	–	–	–	–	–
Shower cocci	–	–	–	–	–	–	–	–
Trichomonas	–	–	–	–	–	–	–	–
ureaplasma urealyticum + mycoplasma hominis	–	–	–	–	–	–	–	–

Abbreviations: ICT, immunochromatographic tests; IFU, inclusion-forming units.

3. Results

3.1. Analytical performance evaluation of the ICT

The analytical sensitivity and specificity of the ICT were evaluated (Table 2). The analytical sensitivity results showed that the LODs of reagents A, B, D, and H reached 9.5×10^3 IFU/mL of *C. trachomatis* pure culture broth; the LODs of reagents C, E, F, and G reached 1.0×10^5 IFU/mL of *C. trachomatis* pure culture broth. The analytical specificity results showed that reagents A, B, C, D, E, F, and G exhibited no cross-reactivity with the interferential pathogens. The reagent H exhibited cross-reactivity with *Gardnerella vaginalis*.

3.2. Results of ICT on clinical samples

A total of ten cases diagnosed with *C. trachomatis* infection by NAAT were tested using ICT. The results showed that, when the concentration of *C. trachomatis* was $\geq 7.5 \times 10^4$ IFU/mL, reagents A, B, C, D, and H presented a positive result; when the concentration of *C. trachomatis* was 4.0×10^4 IFU/mL, reagents A, B, D, and H presented a positive result; when the concentration of *C. trachomatis* was 3.0×10^4 IFU/mL, reagents A, B, and D presented a positive result; when the concentration of *C. trachomatis* was 2.0×10^4 IFU/mL, only reagents A and D presented a positive result; when the concentration of *C. trachomatis* was $\leq 1.2 \times 10^4$ IFU/mL, none of the reagents presented a positive result. In the same way, ten controls excluded from *C. trachomatis* infection by NAAT were tested using ICT. All tests were negative except for reagent H, which showed a weak positive in one control that was detected with *Gardnerella vaginalis* (Table 3).

3.3. Clinical diagnostic accuracy of different reagents of ICT

The clinical diagnostic accuracy of ICT was evaluated using the results of NAAT as gold standard. Among the latex ICT, the sensitivities of reagents A, B, and C were 60.0 %, 50.0 %, and 30.0 %, respectively, with an overall sensitivity of 46.7 %, and the specificity was all 100.0 %. The Kappa values of reagents A, B, and C were 0.600, 0.500, and 0.300, respectively, with an overall Kappa value of 0.467, indicating a moderate agreement with the result of NAAT.

Among the colloidal gold ICT, the sensitivities of reagents D, E, F, G, and H were 60.0 %, 0.0 %, 0.0 %, 0.0 %, and 40.0 %, respectively, with an overall sensitivity of 20.0 %. The specificities were 100.0 % for reagents D, E, F, and G, and 90.0 % for reagent H, with an overall specificity of 98.0 %. The Kappa values of reagents D, E, F, G, and H were 0.600, 0.000, 0.000, 0.000, and 0.300, respectively, with an overall Kappa value of 0.180, indicating a slight agreement with the result of NAAT (Table 4).

4. Discussion

In recent years, ICT has become widely used for *C. trachomatis* screening due to its advantages of being rapid, low-cost, user-friendly, requiring no highly skilled personnel, intensive labor, or specialized devices, and being suitable for the majority of primary medical institutions. According to a national survey from 2013 to 2018, ICT had been used and accounted for 76.7 % of all participating laboratories in China [12]. However, the sensitivity of ICT varies greatly in areas with different incidences of *C. trachomatis*.

Table 3
Results of immunochromatographic tests for *Chlamydia trachomatis* on clinical samples.

Clinical samples	NAAT (IFU/mL)	ICT Reagent							
		A	B	C	D	E	F	G	H
Case 1	1.5×10^5	+	+	+	+	–	–	–	+
Case 2	8.0×10^4	+	+	+	+	–	–	–	+
Case 3	7.5×10^4	+	+	+	+	–	–	–	+
Case 4	4.0×10^4	+	+	–	+	–	–	–	+
Case 5	3.0×10^4	+	+	–	+	–	–	–	–
Case 6	2.0×10^4	+	–	–	+	–	–	–	–
Case 7	1.2×10^4	–	–	–	–	–	–	–	–
Case 8	1.2×10^4	–	–	–	–	–	–	–	–
Case 9	1.0×10^4	–	–	–	–	–	–	–	–
Case 10	7.0×10^3	–	–	–	–	–	–	–	–
Control 1	Negative	–	–	–	–	–	–	–	–
Control 2	Negative	–	–	–	–	–	–	–	–
Control 3	Negative	–	–	–	–	–	–	–	–
Control 4	Negative	–	–	–	–	–	–	–	±
Control 5	Negative	–	–	–	–	–	–	–	–
Control 6	Negative	–	–	–	–	–	–	–	–
Control 7	Negative	–	–	–	–	–	–	–	–
Control 8	Negative	–	–	–	–	–	–	–	–
Control 9	Negative	–	–	–	–	–	–	–	–
Control 10	Negative	–	–	–	–	–	–	–	–

Abbreviations: NAAT, nucleic acid amplification test; ICT, immunochromatographic tests; IFU, inclusion-forming units.

Table 4
Clinical diagnostic accuracy of different reagents and methods of immunochromatographic tests for *Chlamydia trachomatis*.

Reagents	True positive	False positive	False negative	True negative	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Kappa (95%CI)	*P* for Kappa
Latex ICT								
A	6	0	4	10	60.0 (27.4–86.3)	100.0 (65.5–100.0)	0.600 (0.279–0.921)	0.003
B	5	0	5	10	50.0 (20.1–79.9)	100.0 (65.5–100.0)	0.500 (0.171–0.829)	0.010
C	3	0	7	10	30.0 (8.1–64.6)	100.0 (65.5–100.0)	0.300 (0.002–0.598)	0.060
Total	14	0	16	30	46.7 (28.8–65.4)	100.0 (85.9–100.0)	0.467 (0.277–0.657)	<0.001
Colloidal gold ICT								
D	6	0	4	10	60.0 (27.4–86.3)	100.0 (65.5–100.0)	0.600 (0.279–0.921)	0.003
E	0	0	10	10	0.0 (0.0–34.5)	100.0 (65.5–100.0)	0.000 (0.000–0.000)	n.a.
F	0	0	10	10	0.0 (0.0–34.5)	100.0 (65.5–100.0)	0.000 (0.000–0.000)	n.a.
G	0	0	10	10	0.0 (0.0–34.5)	100.0 (65.5–100.0)	0.000 (0.000–0.000)	n.a.
H	4	1	6	9	40.0 (13.7–72.6)	90.0 (54.1–99.5)	0.300 (0.000–0.663)	0.121
Total	10	1	40	49	20.0 (10.5–34.1)	98.0 (88.0–99.9)	0.180 (0.058–0.302)	0.004

Abbreviations: ICT, immunochromatographic test; CI, confidence interval; n.a., not applicable.

Moreover, there was a lack of comparison among reagents considering different methods of ICT. In this study, the undiluted testing samples were eluted by adding normal saline and configured into homogenized samples to achieve a parallel comparison of the diagnostic performance of multiple reagents. The diagnostic accuracy of eight brands of ICT reagents, including latex and colloidal gold ICT, was evaluated compared to the results of NAAT to provide a reference for quality control of reagents before clinical application.

In this study, the concentration of *C. trachomatis* in each specimen was quantified by NAAT, which could measure the gap in detection capacity between NAAT and ICT. The diagnostic performance of the ICT reagents was evaluated using gradient-diluted *C. trachomatis* pure culture broth. Compared with NAAT, the sensitivity of ICT was obviously insufficient. The LOD of NAAT could reach 1×10^2 IFU/mL, while the best LOD of the eight evaluated ICT reagents was only 9.5×10^3 IFU/mL, which was 95 times that of NAAT. A national survey showed that, even for low (5×10^2 copies/swab) concentration samples of *C. trachomatis*, the detection sensitivity of NAAT could reach 91.5 % (95 % confidential intervals [CIs]: 87.9%–94.1 %), while the detection sensitivity of ICT was only 21.2 % (95 % CIs: 18.9%–23.6 %) [12]. But for medium (5×10^3 copies/swab) and high (5×10^4 copies/swab) concentration samples of *C. trachomatis*, the detection sensitivity of ICT could reach 93.1 % (95 % CIs: 91.5%–94.4 %) and 94.2 % (95 % CIs: 92.7%–95.5 %), respectively, close to the level of NAAT. Therefore, strengthening the standardization of cervical swab sampling and obtaining more *C. trachomatis* specimens could help improve the sensitivity of ICT. In areas where NAAT conditions are lacking, ICT could still act as an alternative to NAAT for medium and high concentration samples of *C. trachomatis*.

According to the manufacturer's instructions, the LODs of the eight evaluated ICT reagents varied from 2×10^3 to 5×10^5 IFU/mL, of which the difference could be up to 250 times. The results of analytical sensitivity showed that the LODs of different reagents varied from 9.5×10^3 to 1×10^5 IFU/mL, of which the difference was 10.5 times. Only reagent C met the LOD specified in the product description. The LODs of reagents A, D, G, and H, were close but higher than the data provided by the instruction, which were 2.4-fold, 2.4-fold, 2-fold, and 4.8-fold of the LODs specified in the product description, respectively. Particularly, the LOD of reagent F was 25-fold of the LOD provided by instruction. That is, only *C. trachomatis* concentrations $\geq 1 \times 10^5$ IFU/mL could be detected by the ICT reagents evaluated in this study.

However, the re-evaluation using clinical samples showed that only the sensitivity of reagent C could achieve the LOD specified in the analytical performance evaluation. In clinical practice, the LODs of reagents A, B, D, and H were higher than those measured by using *C. trachomatis* pure culture broth in the laboratory, which were 2.1-fold, 3.2-fold, 2.1-fold, and 4.2-fold of the LODs specified in the analytical performance evaluation. In addition, reagents E, F, and G failed to detect positive results in clinical samples. A similar situation was reported by a study that evaluated the diagnostic performance of an assay combining droplet digital PCR with propidium monoazide treatment for *Vibrio vulnificus* and showed that the LOD in simulated clinical samples was 2.2-fold that in pure culture samples [13]. Since the dead microbial DNA also remained in the clinical samples for a long time, routine PCR assays cannot distinguish the target DNA coming from live or dead bacteria in the actual samples, which results in an overestimation of the concentration of pathogens. This may be the reason for the difference in the LODs between clinical samples and pure culture samples.

In addition, we found that the diagnostic performance varied greatly between different manufacturers who either use latex ICT or colloidal gold ICT for *C. trachomatis*. Although previous studies have shown that ICT has generally lower sensitivities compared to NAAT [14,15]. In China, the sensitivity of ICT varied from 21.2 % to 94.2 % in different regions [12]. However, the diagnostic performance of immunochromatographic assays with different methods may be different. In a previous study, the sensitivity of colored

latex-ICT was compared to colloidal gold-IC for the detection of influenza virus strains. The results showed that the LOD of colored latex-IC was lower than colloidal gold-ICT in the detection of influenza A virus/duck/Hokkaido/Vac-3/2007, but the same as colloidal gold-ICT in the detection of other influenza virus strains [16]. The performance of the diagnostic reagent will be impacted by the differences in the antibody used, the diluent used, and the reagent's manufacturing method. Therefore, it is crucial to conduct inter-laboratory quality control in order to address the inadequacies of various products with varying diagnostic performance and enhance the quality of diagnostic reagents. It is suggested that a clinical evaluation be performed before selecting a certain reagent, and a traceability system be established.

There were some limitations to this study that needed to be addressed. First, the homogenized sample was diluted and eluted by adding normal saline, which may result in a reduction in the ICT positive rate. Therefore, it is recommended that the number of reagents to be evaluated at the same time not be too large. Second, the clinical sample included in this study was insufficient. The spectrum of pathogens detected in the clinical samples did not cover the interference factors used in the analytical specificity evaluation. The types of specimens were also relatively simple; only female cervical swabs were included in this study to account for the consistency of samples applicable to the reagent to be evaluated. Future studies should expand the sample size and include more different types of samples, such as male urine and semen samples, to further evaluate the diagnostic performance of each reagent.

In conclusion, due to the different manufacturing processes of reagents, the diagnostic performance of different methods and different brands of reagents in clinical practice was different from the manufacturer's instructions and even different from the results of laboratory evaluation. The diagnostic performance of reagents should be evaluated before they are actually used in clinical practice. To improve the consistency of different ICT products, it is important to carry out external quality control led by health authorities.

Funding

This work was supported by the National Natural Science Foundation of China [grant numbers 82003512] and Xiamen Science and Technology Plan Project [grant numbers 3502Z20214ZD1069]. The funders played no role in the study design, data collection, or analyses, the decision to publish, or manuscript preparation.

CRediT authorship contribution statement

Shu-Lian Li: Methodology. **Hui-Ling Lin:** Methodology. **Hong-Fei Mi:** Writing – original draft, Software, Funding acquisition. **Qing-Qi Meng:** Data curation. **Ya Yan:** Data curation. **Xiao-Luo Zhang:** Writing – review & editing, Validation. **Wei-Ming Gu:** Conceptualization. **Yao Xiao:** Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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