Registered Report Stage II

Performance verification and clinical evaluation of the NAP-Fluo Cycler system for detecting five genital tract pathogens based on microfluidic technology

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ABSTRACT

Introduction: Sexually transmitted infections (STIs) are among the most common infectious diseases worldwide, often leading to coinfections. Timely detection of genital tract pathogens in at-risk populations is crucial for preventing STIs. We evaluated the NAP-Fluo Cycler System, an innovative microfluidic nucleic acid detection platform, for its ability to simultaneously identify *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Ureaplasma urealyticum* (UU), *Mycoplasma genitalium* (MG), and *Mycoplasma hominis* (MH) in urethral or cervical secretions.

Materials and methods: The limits of detection (LODs), repeatability, specificity, and interference resistance of the system were evaluated using standard strains, a panel of 24 pathogens, and seven interferents. We used the system to analyze 302 clinical samples and compared the results with those of five approved commercial reference kits.

Results: The system achieved LODs of 500 IFU/mL, 500 CFU/mL, and 500 CCU/mL for CT, NG, and UU/MG/MH, respectively, demonstrating high stability (coefficient of variation <1.1 %), specificity, and resistance to interference. Among 302 clinical samples, 237 tested positive with single, dual, and triple infection rates of 35.6 %, 16.2 %, and 3.0 %, respectively. The reference kits detected 138 positive samples. The concordance rates with commercial reference kits were 100 % for UU, NG, and MH; 94.85 % for CT; and 80.00 % for MG.

Conclusions: This system offers a streamlined, rapid, and multiplex detection method that reduces testing time and complexity. Although it performs well with pure strains, it has limitations when using clinical samples of CT and MG, suggesting the need for further refinement before its widespread use in the clinic.

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1. Introduction

Sexually transmitted infections (STIs) are common conditions prevalent among both women and men and are caused by various pathogens, including bacteria, viruses, fungi, and mycoplasma, individually or simultaneously. The primary etiological agents of STIs include *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Ureaplasma urealyticum* (UU), *Mycoplasma genitalium* (MG), and *Mycoplasma hominis* (MH). Although the clinical manifestations of these diseases vary, they can lead to urethral inflammation, epididymoorchitis, and prostatitis in men [1,2]; and cervicitis, endometritis, pelvic inflammatory disease, infertility [3], and miscarriage [4] in women. Some individuals are asymptomatic, leading to overlooked infections, ascending infections, and complications [5]. Traditional diagnostic approaches, such as antigen assays, are often limited by their relatively low sensitivity and specificity [6,7]. The culture method, while a standard in diagnostic microbiology, is time-consuming and not optimal for rapid diagnosis. Nucleic acid amplification tests (NAATs) are currently popular. However, these techniques require laboratory conditions and a high level of technical expertise. Furthermore, NAATs often detect just a single pathogen, leading to potential diagnostic deficiencies, and undiagnosed patients are potential sources of further transmission. Multiplex polymerase chain reaction (MPCR) can detect multiple pathogens simultaneously but is still subject to factors that affect amplification and detection efficiency, such as primer dimer formation. Microfluidics-based MPCR amplifies each pathogen in relatively independent spaces, reducing primer dimer formation and ensuring amplification efficiency. This method offers high sensitivity, high specificity, rapid results, ease of use, reduced experimental waste, and improved biosafety [8].

Recently, Merlin Biomedical (Xiamen) Co., Ltd. developed a microfluidics-based MPCR detection method, the NAP-Fluo Cycler System, consisting of a disposable microfluidic chip and an automated nucleic acid analyzer. This system eliminates the need for complex operator interventions and expensive molecular biology laboratories, enabling the detection of CT, NG, UU, MH, and MG in a single step within 90 min. Leveraging the advantages of point-of-care testing (POCT) platforms, we anticipate that this represents a major step forward in the field of STIs towards efficient and reliable immediate detection, thereby improving disease prevention and control in communities with limited laboratory and technical personnel capabilities. In this study, we validated the performance of the NAP-Fluo Cycler System and evaluated its effectiveness in screening clinical samples for STIs.

2. Materials and Methods

2.1. Reagents and materials

The CT strain (1×10^6 inclusion-forming units [IFU]/mL) was purchased from Shanghai Beinuo Biology Co., Ltd (Shanghai, China). NG (1×10^6 colony-forming units [CFU]/mL) and UU (1×10^6 color-changing units [CCU]/mL) strains were obtained from the National Institute for Food and Drug Control. MH (1×10^6 CCU/mL) and MG (1×10^6 CCU/mL) were cultured at the 174th Hospital of the Chinese People's Liberation Army. The sequences of the primer set for CT/NG/UU/MH/MG, as well as the internal standards, are listed in Table S1. All primers and probes were synthesized by General Biology Co., Ltd. (Anhui, China) and purified using high-performance liquid chromatography.

To compare the limits of detection (LODs) by converting IFU/CFU/CCU/mL to copies/mL, we performed the following procedure: the target fragments of five specific genes were inserted into the pUC57 vector to create plasmids by General Biology Co., Ltd. Next, 5 μg of each plasmid was dissolved in 100 μL TE Buffer. The plasmid DNA concentration was determined using a microplate spectrophotometer. DNA copy numbers were calculated using the following formula:

$$6.02 \times 10^{23} \text{ copy numbers/mol} \times \text{plasmid concentration (g/mL) / L} \times \text{MW (g/mol)} = \text{copies/mL}$$

where MW represents average molecular weight.

The quantified plasmids of the five target genes were then diluted to a series of concentrations using TE buffer: 1.0×10^7, 1.0×10^6, 1.0×10^5, 1.0×10^4, and 1.0×10^3 copies/mL. These dilutions were used as templates for PCR amplification. Subsequently (Figs. S1–S2), a linear curve was plotted with the logarithm of the plasmid concentration as the X-axis and the corresponding cycle threshold (Ct) values as the Y-axis (Fig. S3). Ct was defined as the number of amplification cycles required to reach a specific fluorescence signal threshold.

2.2. NAP-fluo cycler system

The microfluidic chip used to detect CT/NG/UU/MH/MG was provided by Merlin Biomedical Co., Ltd (Xiamen, China). The NAP-Fluo Cycler, a fully automated nucleic acid analysis instrument, is equipped with a rod-shaped component capable of vertical movement and 360-degree rotation. It contains two heaters: a Peltier device for thermocycling and a component for lysis function. It also has a movable magnetic component for magnetic bead adsorption and a microplunger liquid pump. The fluorescence detector can simultaneously detect three different fluorescence signals. A custom structure on top of the rod-shaped component forms a reversible seal with the micropipetting arm in the cassette. Thus, the micropipetting arm can move liquid within the cassette for nucleic acid purification, PCR amplification, and fluorescence detection. Each NAP-Fluo Tube consists of tubes, a tube holder, and a micropipette. The micropipette can rotate 360° and move vertically. Reagents for the magnetic bead method of nucleic acid extraction were preloaded in the "C" type test tube and sealed with a pierceable heat-sealing film. The PCR reagents were preloaded into three PCR tubes, lyophilized, and sealed with a pierceable heat-sealing film. Nucleic acids from each sample were directed into three PCR tubes to detect

the CT/NG/internal, UU/MH/internal, and MG/internal standards (Fig. 1). A sample was considered positive only if both the target gene and internal standard were positive and the negative control was negative.

To prepare the clinical sample, 1 mL of sterile physiological saline was added to the clinical sample collection tube, and the swab was repeatedly squeezed. Following this, 200 μL of each sample was aliquoted into the system. The process from sample to result took 90 min.

2.3. Detection performance evaluation

The LODs, repeatability, specificity, and resistance to interference of this diagnostic system were evaluated. The LOD was determined by repeatedly testing low-concentration samples and selecting a concentration with a positivity rate >95 %. Standard strains were mixed and subjected to three gradient dilutions: CT concentrations of 1×10^3, 500, and 100 IFU/mL; NG concentrations of 1×10^3, 500, and 100 CFU/mL; and UU/MG/MH concentrations of 1×10^3, 500, and 100 CCU/mL. Each concentration was tested in duplicate 20 times.

To compare the LOD among the different assay kits, we constructed linear curves using the logarithmic values of different concentrations of plasmid copies and Ct values (each concentration was tested thrice) to convert the LOD to the corresponding copies for each pathogen (Fig. S3). After conversion, the approximate copies/mL values for the CT, NG, UU, MG, and MH groups were 200, 2400, 1560, 2400, and 1150.

We prepared medium positive precision reference samples by mixing equal parts (1:1:1:1:1) of CT, NG, UU, MG, and MH at 5.0×10^5 IFU/mL, 5.0×10^5 CFU/mL, 5.0×10^5 CCU/mL, 5.0×10^5 CCU/mL, and 5.0×10^5 CCU/mL, respectively. Weak positive precision reference samples were similarly prepared with each pathogen at 5.0×10^4 of their respective units. Both sets were tested ten times.

Repeatability was assessed by calculating the coefficient of variation (CV) of the Ct values in repeated testing of samples at various concentrations. Specificity was evaluated using 24 other pathogens that can cause similar or identical symptoms at approximately 1×10^6 copies/mL, including *Human papillomavirus* types 16, 18, 45, and 31; *Herpes simplex* virus type 2; *Treponema pallidum*; *Staphylococcus epidermidis*; *Staphylococcus saprophyticus*; *Streptococcus agalactiae*; *Escherichia coli*; *Gardnerella vaginalis*; *Candida albicans*; *Trichomonas vaginalis*; *Lactobacillus crispatus*; *Lactobacillus iners*; *Mobiluncus curtisii*; *Lactobacillus jensenii*; *Prevotella bivia*; *Leptotrichia*; *Adenovirus*; *Cytomegalovirus*; *Alpha streptococcus*; human immunodeficiency virus; and *Lactobacillus casei*. To assess the resistance of the system to interference, endogenous interferents (hemoglobin, cervical mucus, and mucin) and exogenous interferents (contraceptive dissolution, tampons, vaginal suppository dissolution liquid, and lubricants) were added separately to positive samples.

2.4. Clinical sample collection

A total of 302 clinical samples were collected from 302 patients visiting STI and infertility clinics at the Xiamen Maternal and Child Health Hospital from July 2022 to October 2023. The average age of the male and female patients was 32.8 and 31.7 years, respectively. The collection method was as follows: for males, a urethral swab was taken by inserting a cotton swab 2–4 cm into the urethra, rotating it for 3–5 s, and then removing it. For females, a cervical swab was taken by inserting a cotton swab 1–1.5 cm into the cervical os, slightly rotating it, and leaving it in place for 10–30 s to allow full absorption of the secretions. The swab containing the collected secretions was placed back into protective casing and sealed for transportation to the laboratory. In total, 47 male urethral secretion samples and 255 female cervical secretion samples were collected. The main clinical diagnoses included vaginitis, urethritis, threatened miscarriage, and infertility.

2.5. Reference kits and equipment

As current nucleic acid POCT products do not match the performance of traditional nucleic acid detection methods, the reference
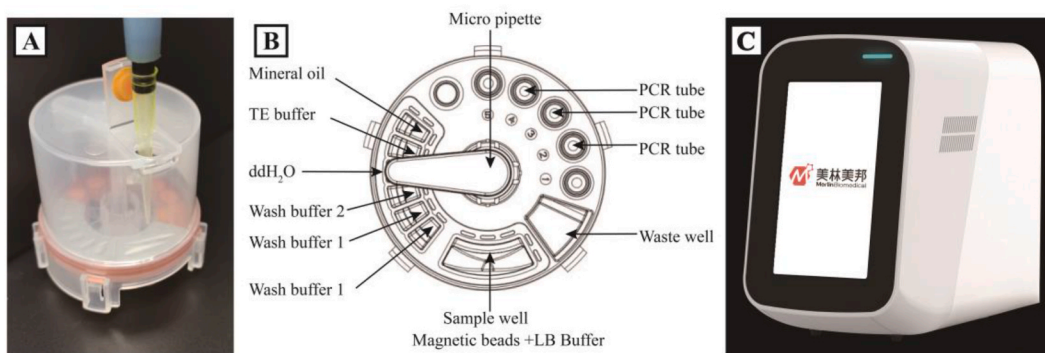

Fig. 1. The NAP-Fluo Cycle System
(A) Exterior view of the NAP-Fluo Tube; (B) Inner structure of the NAP-Fluo Tube; (C) The accompanying NAP-Fluo Cycler, a fully automated nucleic acid analyzer, which integrates with the NAP-Fluo Tube for sample processing and analysis.

kits for this study were selected from common high-performance single-target conventional nucleic acid detection kits. These kits were clinically validated, and their intended use and sample types are consistent with the system under evaluation. We selected PCR kits approved by the China Food and Drug Administration (CFDA) for clinical sample diagnosis: CT, Daan Gene Co., Ltd. (Guangzhou, China; CFDA approval No. 20213400572); NG, Daan Gene Co., Ltd (CFDA approval No. 20163400962); UU, Daan Gene Co., Ltd (CFDA approval No. 20153401995); MG, Suzhou Bacme Biotech (Suzhou City, China; CFDA approval No. 20163400753); and MH, Suzhou Bacme Biotech (CFDA approval No. 20163400756). The Smart32 Nucleic Acid Extraction Instrument (Daan Gene Co., Ltd.) was used as the extraction reference. The reference nucleic acid extraction reagent was the Nucleic Acid Isolation Mix from Daan Gene (Guangdong Medical Device Registration No. 20170583). A fully automated medical PCR analysis system, SLAN-96S (Shanghai Hongshi Medical Technology Co., Ltd., Shanghai, China) was the amplification reference. Each clinical sample was tested for only one type of pathogen using a reference kit at the physician's discretion (Table 1). After testing, the remaining samples were stored at −20 °C until use in the NAP-Fluo Cycler System.

2.6. Handling of inconsistent results

When there were inconsistent results, the samples were retested using the NAP-Fluo Cycler System. If the results remained inconclusive, further confirmation was performed using reference kits and a third-party kit, including the CT nucleic acid detection kit (Sansure Biotech Inc., Hunan, China; CFPA approval No. 20153400084) and the MH nucleic acid detection kit (Shanghai Rendu Biotech Co., Ltd., Shanghai, China; CFPA approval No. 20173404323).

2.7. Statistical analysis

Consistency analysis between the NAP-Fluo Cycler System and the reference kits was conducted using SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA). Analysis included calculating the positive agreement rate, negative agreement rate, overall agreement rate, and Kappa statistics.

3. Results

3.1. NAP-Fluo Cycler system performance

First, the LOD was determined. CT was detectable at 1×10^3 and 500 IFU/mL, NG at 1×10^3 and 500 CFU/mL, and UU/MG/MH at 1×10^3 and 500 CCU/mL. However, at a concentration of 100 IFU/mL, the CT positivity rate was 0 %. For NG, it was 80 % at 100 CFU/mL; at 100 CCU/mL, this was 45 % for UU, 85 % for MG, and 0 % for MH. Ultimately, the LODs were 500 IFU/mL for CT, 500 CFU/mL for NG, and 500 CCU/mL for UU/MG/MH (Tables S2–S4).

The CVs of the medium-positive samples were 0.98 %, 0.98 %, 0.82 %, 1.06 %, and 0.73 % for CT, NG, UU, MH, and MG, respectively. For weakly positive samples, the CVs were 0.90 %, 0.82 %, 1.09 %, 0.83 %, and 0.85 % for CT, NG, UU, MH, and MG, respectively. The exact Ct values for the precision tests are presented in Tables S5–S6. These results indicate that the detection system had good stability across samples with varying concentrations.

Subsequently, we tested the specificity of the detection system by evaluating its response to 24 other pathogens causing similar clinical symptoms. The results demonstrate that the detection system had good specificity with no cross-reactivity when targeting CT, UU, NG, MH, or MG (Table S7).

Finally, we assessed the resistance of the system to interference by adding endogenous and exogenous interferents. The results show no significant change in the target Ct values, indicating that interferents at these concentrations did not affect the detection outcomes (Table S8).

3.2. Clinical sample detection

Of 302 clinical samples, 138 tested positive using the reference assay kits: 57, 20, 39, 20, and 2 for CT, NG, UU, MG, and MH, respectively. In contrast, the NAP-Fluo Cycler System yielded 237 positive results, including 62, 21, 135, 14, and 5 for CT, NG, UU, MG, and MH, respectively. The detection results from the NAP-Fluo Cycler System are presented in Table 2.

Table 1
Number of samples tested with reference kits for each pathogen.

Pathogen	Number of samples tested
CT	136
NG	30
UU	99
MG	30
MH	7
Total	302

Table 2
Detection results from 302 clinical samples tested with the NAP-Fluo Cycler System according to specimen type.

Pathogen	Urethral Swabs (n = 47)	Cervical Swabs (n = 255)	Total (n = 302)
CT	6	56	62 (26.2 %)
NG	4	17	21 (8.9 %)
UU	16	119	135 (57.0 %)
MG	1	13	14 (5.9 %)
MH	0	5	5 (2.1 %)
Total	27	210	237 (100 %)

The distribution of the detected pathogens using the NAP-Fluo Cycler System is presented in Table 3. The single, double, and triple infection rates were 35.6 %, 16.2 %, and 3.0 %, respectively. The highest detection rate for a single pathogen was for UU (25.8 %). The most common combination of double infections was CT + UU (11.9 %). The most frequent combination of triple infections was CT + NG + UU at 2.0 %, and a quadruple infection was observed with CT + UU + MG + MH at 0.3 %.

3.3. Comparative detection performance for CT

The NAP-Fluo Cycler system yielded 50 positive and 86 negative results, whereas the reference assay kit yielded 57 positive and 79 negative results. The positive agreement rate was 87.72 % (with seven discrepancies), the negative agreement rate was 100 %, and the overall agreement rate was 94.85 %, with an overall Kappa value of 0.892 (Table 4). The seven discrepant results, which included four male urethral swabs and three female cervical swabs, were retested using a reference assay kit and a third-party assay kit, all of which resulted in weakly positive outcomes.

3.4. Comparative detection performance for MG

The NAP-Fluo Cycler System detected 14 positive and 16 negative results, whereas the reference assay kit detected 20 positive and 10 negative results. The positive agreement rate was 70.00 % (with six discrepancies, all from female cervical swabs), the negative agreement rate was 100.00 %, and the overall agreement rate was 80.00 %, with an overall Kappa value of 0.609 (Table 4). Retesting five discrepant results (one sample had insufficient remaining volume for retesting) using the reference assay kit and a third-party assay kit resulted in positive findings.

3.5. Comparative detection performance for NG/UU/MH

The NAP-Fluo Cycler System demonstrated excellent agreement with the reference assay kit for all three pathogens. The positive agreement rate was 100.00 %, negative agreement rate was 100.00 %, and overall agreement rate was 100.00 %, with an overall Kappa value of 1.000 (Table 4).

4. Discussion

We comprehensively evaluated an integrated microfluidic nucleic acid detection system. It demonstrated superior performance when detecting standard strains, including favorable sensitivity, stability, specificity, and interference resistance. However, this system

Table 3
Pathogens detected in 302 patients using the NAP-Fluo Cycler System.

Single and Multiple Infections	Number of Patients (n)	Percentage (%)
UU	78	25.8
CT	14	4.6
NG	11	3.6
MG	4	1.3
MH	1	0.3
CT + UU	36	11.9
UU + MG	5	1.7
NG + UU	3	1.0
UU + MH	3	1.0
CT + MG	1	0.3
CT + NG	1	0.3
CT + NG + UU	6	2.0
CT + UU + MG	3	1.0
CT + UU + MG + MH	1	0.3
Total positive patients	167	55.3
Total negative patients	135	44.7
Total	302	100

Table 4
Summary of the comparative performance of the NAP-Fluo Cycler System with reference kits according to pathogen type.

Pathogen	NAP-Fluo Cycler System	Reference Kit			Positive Agreement Rate	Negative Agreement Rate	Overall Agreement Rate	Kappa Statistic (95 % CIs)
		+	−	Total				
CT	+	50	0	50	87.72	100.00	94.85	0.89 (0.82–0.97)
	−	7	79	86				
	Total	57	79	136				
NG	+	20	0	20	100.00	100.00	100.00	1.00 (1.00–1.00)
	−	0	10	10				
	Total	20	10	30				
UU	+	39	0	39	100.00	100.00	100.00	1.00 (1.00–1.00)
	−	0	60	60				
	Total	39	60	99				
MG	+	14	0	14	70.00	100.00	80.00	0.61 (0.35–0.87)
	−	6	10	16				
	Total	20	10	30				
MH	+	2	0	2	100.00	100.00	100.00	1.00 (1.00–1.00)
	−	0	5	5				
	Total	2	5	7				

faces certain challenges when processing clinical samples for CT and MG.

Molecular diagnostic techniques, such as PCR, a thermocycling amplification method, and loop-mediated isothermal amplification (LAMP), an isothermal amplification method, have become cornerstones for pathogen detection [9]. However, these methods have limitations. LAMP can produce false positives due to methodological flaws [10], while PCR requires stringent laboratory conditions to prevent contamination. MPCR allows the detection of multiple pathogens in a single reaction, enhancing both diagnostic efficiency and cost-effectiveness. However, as the number of targets increases, challenges arise, such as decreased amplification efficiency and primer dimer formation, potentially leading to false-positive or false-negative results. To address these issues, a microfluidic chip-based MPCR technology has been developed. This technology preloads different primer pairs into microwells on a chip and employs an accurate fluidic control system to direct the template to the microwells for specific PCR amplification. This approach minimizes non-specific amplification, ensures amplification efficiency [11], and enables accurate differentiation and identification of multiple targets [12]. Despite their widespread application in respiratory pathogen detection, using microfluidic technologies for reproductive tract pathogen detection remains relatively rare [13]. A randomized clinical trial in the United States of America reported that screening women at high risk for CT infection reduced the incidence of pelvic inflammatory diseases from 2.8 % to 1.3 % per year [14]. STI screening is crucial to reduce treatment costs, minimize transmission risks among sexual partners, and control the development of antibiotic resistance.

In the future, nucleic acid testing is expected to develop toward closed, highly accurate, convenient, highly sensitive, fully automated, and integrated systems. Compared to traditional detection methods, the NAP-Fluo Cycler System simplifies operation, shortens detection time, and enables multiplex detection. In a previous study, we conducted 40 single or combined tests on standard strains of CT serotypes D–K, UU serotypes 1–14, and NG/MG/MH, achieving a 100 % positive concordance rate (data not shown). Performance verification for standard strains demonstrated a LOD of 500 IFU/mL, 500 CFU/mL, and 500 CCU/mL for CT, NG, and UU/MG/MH, respectively, exhibiting good stability (CV < 1.1 %), specificity, and anti-interference capabilities.

When validating the clinical samples, the reference kit, which could only detect one pathogen at a time and relied on a physician's judgment, produced only 138 positive results (Table 4), while the NAP-Fluo Cycler System produced 237 (Table 2), a 71.7 % increase over the reference kit. Notably, 55.3 % of patients tested positive for at least one pathogen, and 19.5 % had coinfections (Table 3). MG coinfections were often present in patients with CT infections. CT treatment with 1 g azithromycin can select macrolide-resistant strains of MG [15], which remain undetected without multiplex detection. The use of the NAP-Fluo Cycler System allowed for the concurrent diagnosis of such co-infections. In countries where doxycycline is a first-line treatment, this system could facilitate safer MG treatment without inducing resistance when CT + MG co-infection is confirmed [16]. This is one advantage of using MPCR for STI detection. In contrast, UU was detected at the highest rate in clinical samples, with many patients being asymptomatic. Therefore, this system may improve the detection and treatment of asymptomatic UU/MG/MH monoinfections, which contribute to increased antibiotic resistance [17,18]. Therefore, clinicians should consider these results carefully.

In terms of consistency with reference assay kits, the NAP-Fluo Cycler System performed satisfactorily when detecting NG/UU/MH; however, it has limitations when detecting CT and MG. The positive agreement rate for CT detection was 87.72 %, whereas that for MG was notably lower at 70.00 %, suggesting that the NAP-Fluo Cycler System missed clinically positive CT and MG samples. To facilitate comparison, we converted IFU to copy numbers and found that the system's LOD for CT was similar to that stated in the reference kit's instructions (200 copies/mL vs. 400 copies/mL); however, weakly positive samples were not detected. This indicates that when the template concentration is low, the probability of the microfluidics-based method diverting the template into multiple wells containing specific primers decreases, resulting in false-negative outcomes. Additionally, the median bacterial load of MG was 42 times lower than that of CT, which may have contributed to the lower sensitivity of current MG detection methods, increasing the likelihood of false-negative results [19]. Several other MPCR assay kits reportedly also miss the detection of MG-positive samples [20]. Upon comparing LODs, we discovered that the system's LOD for MG was higher than that indicated in the reference kit's instructions (500 CCU/mL vs. 0.1 CCU/mL), which is likely the primary reason for the missed detection of weakly positive samples. Therefore, we recommend

further evaluation of the detection system for MG, including reconsideration of the primer design sites and validation with different mutant strains to ensure the detection of known MG variants.

This study has certain limitations. First, due to regional differences, the natural positivity rate for MH is low, making the collection of positive samples challenging. Consequently, only a small number of clinically positive samples (n = 2) were available for validation, which was insufficient to draw definitive conclusions. Second, residual clinical samples were used, which may have resulted in reduced DNA integrity due to additional freeze-thaw cycles. To determine whether the missed positive samples not detected by the NAP-Fluo Cycler System were missed due to reduced pathogen concentrations or issues with the detection system, we retested the discordant results using a reference assay kit and a third-party assay kit. Both yielded positive results, essentially ruling out the possibility of false positives and confirming missed detections by the NAP-Fluo Cycler System. More rigorous validation should be conducted via further clinical trials. Additionally, the UU primer set used in this study did not differentiate between *U. urealyticum* and *Ureaplasma parvum*. The necessity of designing more specific primers in the future will depend on clinical needs. Ultimately, considering the stability of DNA and its relatively easier extraction compared to RNA, and the future deployment in medical facilities with limited laboratory conditions, this system was designed to detect pathogen DNA rather than RNA. Technically, this meant that it could not ascertain whether the pathogens in the samples were alive or dead. For monitoring therapeutic efficacy and distinguishing between pathogens that may have died post-treatment, an RNA-based detection kit would likely offer more reliable results.

5. Conclusions

The NAP-Fluo Cycler System, a novel POCT system, has great potential for detecting reproductive tract pathogens. It performs well with pure strain samples but has limitations when detecting CT and MG clinical samples. Future research should focus on improving detection sensitivity, expanding the range of clinical sample validation, and exploring the application of this system in various clinical settings. Moreover, when considering the issue of antibiotic resistance in relation to such test results, clinicians should interpret the test results cautiously and make comprehensive judgments in conjunction with the patient's clinical presentation and history.

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Informed consent

Informed consent was obtained from all individuals included in this study.

Ethical approval

This study was reviewed and approved by the Ethics Committee of the Xiamen Maternal and Child Health Hospital (Approval No.: KY-2021-014-H01). Informed consent was obtained from all participants (or their legal guardians or authorized representatives).

CRediT authorship contribution statement

Ye Wang: Writing – original draft, Methodology, Investigation, Funding acquisition. **Qunshan Xu:** Writing – original draft, Investigation, Data curation. **Jianguo Cai:** Validation, Investigation, Data curation. **Lijin Zheng:** Validation, Investigation. **Weilun Zuo:** Formal analysis, Data curation. **Jumei Liu:** Supervision, Investigation. **Jiali Cao:** Investigation, Formal analysis, Data curation. **Mingxin Lin:** Validation, Investigation. **Hongli Liu:** Writing – review & editing, Supervision, Resources, Conceptualization. **Huiming Ye:** Writing – review & editing, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing financial interests or personal relationships that could have influenced the work described in this article.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2024.e00417.

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