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# An improved procedure based on fluorescence immunochromatography for rapid detection of group B *streptococcus* from enrichment cultures

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## Abstract

**Background** Group B *streptococcus* (GBS) is a major cause of perinatal infectious morbidity and mortality. Although intrapartum antibiotic prophylaxis (IAP) administration for ≥ 4 h is effective in preventing neonatal early-onset GBS diseases, the conventional culture-based approach to identify GBS often takes 24–72 h. This study aimed to find a strategy to improve the efficacy of GBS screening.

**Methods** We developed a fluorescence immunochromatographic test (FICT) strip to detect GBS within 15 min. The detection limit, analytical sensitivity, cross-reactivity and performance of the strip were evaluated. The performance of the strip on vaginal-rectal swabs with or without enrichment culture was compared with real-time quantitative polymerase chain reaction (qPCR) and colloidal gold immunochromatography (GIC) assay with conventional enrichment culture method as the reference method.

**Results** The detection limit of the strip ranges from $10^4$ CFU/mL to $10^6$ CFU/mL. Additionally, the strip has detected all of the positives from 48 h enrichment cultures (175/175). and 30 GBS strains representing different serotypes at cell density of $10^6$ CFU/mL yielded positive results. Cross-reactivity test indicated no false-positive results. The sensitivity on direct samples was 34.48%, while 4 h enrichment in LIM broth prior to FICT has greatly increased the sensitivity to 90.91% with the specificity being 95.35%.

**Conclusions** The improved procedure based on the FICT for GBS detection from short-term LIM broth cultures was expected to guide IAP administration in obstetrical emergencies.

**Keywords** Group B *streptococcus*, Fluorescence immunochromatography, Performance evaluation, Rapid test, Methodological improvement

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## Background

Group B *streptococcus* (GBS), also referred as *Streptococcus agalactiae*, is an opportunistic pathogen asymptomatically colonizes in human rectovaginal tract that can lead to invasive diseases in adults under certain conditions [1–3]. Numerous studies have shown that the prevalence of GBS colonization in pregnant women ranging from 11 to 35%, with regional differences [4, 5]. Our previous report suggests that the overall GBS colonization rate in women at 35–37 gestational weeks was 13.47% in Xiamen, China [6]. Maternal GBS colonization is implicated in urinary tract infection, intra-amniotic infection, endometritis, preterm birth and still birth [7–9]. Furthermore, there were about 50% of GBS colonized pregnant women vertically transmitted the pathogen to their offspring during labor [10]. GBS infection was one of the leading causes of neonatal early-onset diseases (GBS-EOD) and late-onset diseases (GBS-LOD) such as bacteremia, pneumonia and meningitis [11, 12]. From 2000 to 2018 in China, the pooled estimated incidence of invasive GBS disease was 0.55 cases/1,000 live births, bring about heavy burden on medical and health care [13].

It is recommended by the US Centers for Disease Control and Prevention (CDC) and Chinese Medical Association that pregnant women at 35–37 gestational weeks should be screened for GBS colonization status and the GBS carriers receive intrapartum antibiotic prophylaxis (IAP) administration [14]. However, the antenatal GBS screening strategy carries some limitations such as low sensitivity of GBS detection and at least 10% of women diagnosed as antenatal GBS-negative turned out positive at delivery [15, 16]. Since no licensed GBS vaccine is available, adequate IAP is an effective method to prevent adverse maternal and neonatal outcomes, which is supported by our previous data [6, 17]. Although the exact duration of antibiotics needed to prevent vertical GBS transmission remains a controversy, optimal antibiotic application to the target population for ≥ 4 h before delivery has been proved to be highly effective in preventing vertical transmission of GBS and GBS-EOD, and ≥ 2 h might provide some protection [18, 19]. In obstetrical emergencies, IAP applies to parturient admit with signs and symptoms of preterm labor and rupture of membranes who enter labor with unknown GBS status could lead to overuse or inadequate use of antibiotics. Therefore, it is mandatory to implement a highly sensitive and rapid technique to identify GBS carriers especially with low bacteria loads.

According to CDC, the rectal/vaginal specimens are inoculated on blood agar plates and isolated presumptive GBS colonies are confirmed by CAMP test or serologic identification using latex agglutination with GBS antisera. It seems that the standard culture approach for GBS determination often takes 24–72 h before a result is achieved, which cannot meet the clinical demands in obstetrical emergencies. Despite chromogenic mediums employed to detect β-hemolytic strains of GBS, culture method still carries some limitations, for example inability to determine non-hemolytic strains and requirement of incubation overnight [20]. Reaction of optical immunoassays and enzyme immunoassays can reach a result within minutes and has been commercially available, while the inadequate sensitivity has restricted their wide use [21, 22]. qPCR-based techniques seem to be feasible for rapid and accurate identification of GBS in the intrapartum setting, Nevertheless, they are based on special laboratory instruments, designated areas as well as technically skilled personnel, which hampers their implementation in resource-limited settings.

In this work, we developed a GBS test strip based on fluorescence immunochromatography and conducted performance evaluation on the strip. We compared the current GBS screening strategies, aimed to find out a method achieves optimal sensitivity and time-to-result.

## Methods

### Participants and study design

This work was carried out at Women and Children's Hospital, School of Medicine, Xiamen University, China and was conducted strictly in accordance with the approval by the Human Research Ethics Committee of Women and Children' s Hospital of Xiamen University (KY-2023-074-H01). Pregnant women who were admitted to the antenatal clinic or attended the hospital for routine prenatal follow-up between January, 2022 and June, 2024 were eligible for this research. According to the algorithms released by CDC, women at 35–37 weeks of gestation and admitted with signs and symptoms of preterm labor and with rupture of membranes at < 37 weeks and 0 days' gestation were collected vaginal-rectal swabs for culture identification of GBS. An informed consent was obtained from the patients who were included in the study.

### Preparation of the labeling complex of GBS-IgG-fluorescence microspheres and DNP-fluorescence microspheres

To wash the fluorescence microspheres, the mixture of 100 μL of time-resolved fluorescent latex microspheres $Eu^{3+}$ (MadeNew, Changsha, China) and 900 μL of 2-morpholinoethanesulfonic acid (MES) (pH = 5.0) was centrifuged at 16,000 × g, 4℃ for 15 min and 800 μL of the centrifuged supernatant was removed. 690 μL of MES buffer, 10 μL of 1 mg/mL 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC) (Sigma-Aldrich, Saint Louis, America) and 100 μL of 100 mg/mL N-hydroxysuccinimide (NHS) (Sigma-Aldrich, Saint Louis, America) were then added into the resuspended fluorescence

microspheres and mixed at room temperature for 30 min in order to activate the carboxyl groups on the surface of fluorescence microspheres, followed by centrifuge at 16,000 × g, 4℃ for 15 min and 800 μL of the supernatant was discarded. After washing by MES buffer, 15 μg of rabbit anti-GBS polyclonal antibodies 2 (Innobiomax, Xiamen, China) and 40 μg of dinitrophenol (DNP) antigen (Innobiomax, Xiamen, China) were added into the fluorescent microspheres respectively. The complexes were mixed at room temperature for 4 h. Afterward, the fluorescent microspheres were washed by blocking buffer and mixed at room temperature for 1 h to remove unbound antibodies. The GBS-IgG-fluorescent microspheres labeling complex and DNP-fluorescent microspheres labeling complex were obtained and stored at 4℃ for use.

#### Synthesis of the conjugates of anti-GBS antibody with biotin

The rabbit anti-GBS monoclonal antibodies 1 and biotin were added into 500 μL of PBS buffer in a molar ratio of 1:20 and mixed at room temperature for 2 h. The solution was then transferred to a dialysis bag and was dialyzed against PBS buffer at 250 rpm, 4℃ for 20 h. Prior to storage at -20℃, glycerol was added into the GBS-IgG-biotin conjugates.

#### Preparation and assembly of the fluorescence immunochromatographic test(FICT) strip

The GBS IgG-biotin conjugates, the GBS-IgG-fluorescent microspheres and the freeze-dried solution were mixed in a ratio of 1:40:560 on a vortex, The mixture was then sprayed onto the conjugate pad made from a piece of glass microfiber membrane SB08 (Kinbio, Shanghai, China) and freeze-dried in a freeze-dryer overnight. Mouse anti-DNP antibody (1 mg/mL; Innobiomax, Xiamen, China) and streptavidin (2 mg/mL) were coated on a nitrocellulose (NC) membrane as a control (C) line and a test (T) line by an HGS510 3D membrane spraying instrument (Autokun, Hangzhou, China), respectively. The NC membrane was then dried in an oven at 32℃ for 16 h. Then, the NC membrane, conjugate pad and absorbent pad were glued on a plastic backing plate and cut to 4 cm in length (As shown in Supplemental Fig. 1).

#### Detection principle of the FICT strip

For sample collection, a cotton swab was taken from the lower vagina and rectum of the participants. 250 μL buffer A (hydrochloric acid) and 250 μL buffer B (sodium nitrite) were sequentially added to the swabs and stayed for 5 min. Then 100 μL lysate was dripped onto the sample pad. After 15 min, the strip was subjected to the RTR-FS100 immunofluorescence card reader (UMIC, Xiamen, China) and the T, C values were quantified. For LIM broth enrichment cultures, 250 μL buffer A and 250 μL buffer B were sequentially added into 70 μL of the liquid enrichment cultures and the subsequent steps were described above.

The target GBS capsular polysaccharide, if existed, moved to the conjugate pad and bound to the GBS IgG-biotin conjugates and the GBS-IgG-fluorescent microspheres. The biotin-GBS IgG-GBS antigen- GBS-IgG-fluorescent microspheres complex was then captured by the immobilizing streptavidin on the T line and the remaining DNP-fluorescent microspheres combined with the immobilizing anti-DNP antibodies on the C line (As shown in Supplemental Fig. 2). The concentration of the target GBS antigen was positively correlated to the fluorescence intensity.

#### Performance evaluation of the FICT strip

The performance evaluation tests were conducted according to China National Accreditation Service for Conformity Assessment (CNAS) guideline GL038. The detection limit of the FICT strip was tested using the β-hemolytic (ATCC 12386) and non-hemolytic standard strains of GBS (ATCC 13813), which were grown on Columbia blood agar at 35℃ with 5% $CO_2$ for 18 h and suspended in sterile saline to adjust the cell suspension to $1.0 \times 10^8$ CFU/mL as tested by PhoenixSpec, a McFarland turbidimeter (BD, Newjersey, USA). The cell suspensions were ten-fold serially diluted (1:10, 1:100, 1:1000, 1:10000, 1:100000) and subjected to the FICT, which were then plated onto blood agars and the bacterial colonies were counted after 24 h incubation. There were three replicates for each concentration.

For the analytical sensitivity assessment of the FICT strip, the cultures of 30 GBS strains representing 10 different serotypes (as shown in Table 1) on Columbia blood agar were suspended in sterile saline and diluted into $10^5$ CFU/mL. The cell suspensions were then subjected to the FICT. There were three replicates for each strain.

In order to assess the cross-reactivity of the FICT strip, the following 20 clinically isolated microorganisms were grown on Columbia blood agar at 35℃ with 5% $CO_2$ for 18 h: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus dysgalactiae*, *Streptococcus salivarius/Streptococcus mitis*, *Streptococcus sanguis*, *Streptococcus bovis* complex, *Streptococcus porcinus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* complex, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Candida albicans* and *Candida glabrata*. Cell suspensions containing $10^8$ CFU of bacteria/ml or $10^8$ CFU of fungi/ml were subjected to the FICT. There were three replicates for each microorganism.

**Table 1** GBS strains used for reactivity test of FICT strips

| Serotypes | GBS Strains | Source |
|---|---|---|
| Ia | GBS_5405<br>GBS_0028<br>GBS_0055 | Clinical isolate, laboratory stock |
| Ib | GBS_0007<br>GBS_0009<br>GBS_0013 | Clinical isolate, laboratory stock |
| II | GBS_0002<br>GBS_0071<br>GBS_0072 | Clinical isolate, laboratory stock |
| III | GBS_8524<br>GBS_8528<br>GBS_0005 | Clinical isolate, laboratory stock |
| IV | GBS_2515<br>GBS_0229<br>ATCC 49,446 | Clinical isolate, laboratory stock<br>ATCC |
| V | GBS_0004<br>GBS_0006<br>GBS_0011 | Clinical isolate, laboratory stock |
| VI | GBS_0043<br>GBS_5814<br>GBS_5409 | Clinical isolate, laboratory stock |
| VII | GBS_7421<br>GBS_5602<br>BAA 2670 | Clinical isolate, laboratory stock<br>ATCC |
| VIII | GBS_0031<br>GBS_0037<br>BAA 2669 | Clinical isolate, laboratory stock<br>ATCC |
| IX | BAA 2668 | ATCC |

The study design is shown in Fig. 1. All the performance evaluation tests were conducted in the same manner as the LIM broth enrichment cultures procedure described above.

#### Culture identification of GBS

GBS culture was described previously [6]. Briefly, the vaginal/rectal cotton-tipped GBS TranSwabs (Creative Lifesciences, Taiwan, China) of the participants were subjected to selective enrichment incubation in the semi-solid transport medium (Creative Lifesciences, Taiwan, China; the patent number: CN 107287275 A) at 35 ℃ with 5% $CO_2$ for 24 h. The color change or red-orange pigment after enrichment was specific for the presence of β-hemolytic GBS strains. While the negative samples were further subcultured onto GBS Carrot Agar and β-γ Detection Agar (Creative Lifesciences, Taiwan, China) and were cultivated for another 24 h. The red-orange pigmented colonies were indicative of GBS strains, and the presumed GBS colonies (nonpigmented β-hemolytic colonies) were confirmed by CAMP test or matrix assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS; SKYRay MicroTyper MS, Hangzhou, China; Database: EX-Accuspec v.2.0).

#### GBS detection by qPCR technique

Vaginal/rectal swabs or enrichment cultures of the pregnant women were washed by sterile saline and

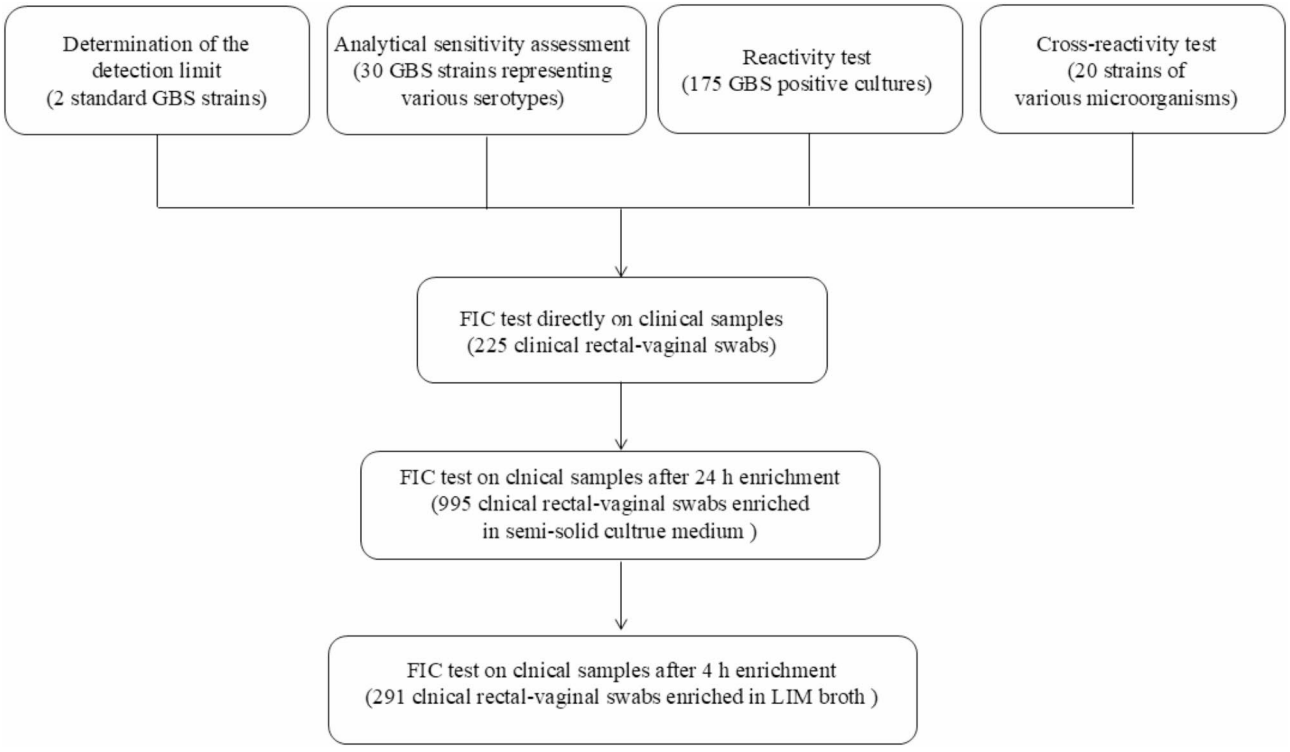


**Fig. 1** The study design of the current study

centrifuged at 12, 000 rpm for 5 min. To extract GBS nucleic acid, the precipitation was resuspended in 200 μL sterile saline and subjected to the nucleic acid extraction or purification kit (Daan, Guangzhou, China) according to the manufacturer's instruction. The eluted products were amplified using a GBS nucleic acid detection kit (Daan gene, Guangzhou, China) targeting the GBS *cfb* gene according to the manufacturer's protocol on the ABI Prism7500 system (Thermofisher, Massachusetts, America). The program was set at 50 °C for 2 min and 95 °C for 15 min for one cycle, and then at 94 °C for 15 s and 55 °C for 45 s for a total of 40 cycles. Positive and negative controls were included in each run. A result was considered GBS positive if the CT value of *cfb* gene < 38 no matter what the CT value of the inner control was. Amplification curve of the inner control and the CT value of *cfb* gene ≥ 38 indicated a negative result. The lack of amplification of the inner control was interpreted as an invalid result.

### Colloidal gold immunochromatography (GIC) assay of GBS colonization

GBS detection by commercially available GIC kit A (Huao, Anhui, China) and GIC kit B (Weimi, Guangzhou, China) was carried out according to the manufacturer's instructions. The reaction products at the test line and the control line were observed macroscopically. The appearance of a red band at both the T line and the C line within 15 min indicates a positive reaction, whereas the absence of a red band at the T line and appearance of a red band at the C line indicates a negative reaction.

### GBS identification from enrichment cultures

As shown in Fig. 2, rectal/vaginal specimens were collected by GBS TranSwabs and the latter were inoculated in the semisolid medium. After 24 h of enrichment, the negative samples (no color change or red-orange pigment) were subcultured onto the GBS Carrot Agar and β-γ Detection Agar. The pigment negative GBS TranSwabs and the bi-plates were incubated for another 24 h, and the color change or red-orange pigment on the bi-plates was considered GBS culture positive. GBS TranSwabs that didn't undergo color change after 24 h but produced red-orange pigment on the bi-plates after another 24 h of incubation were subjected to the FICT.

As shown in Fig. 3, rectal/vaginal specimens were taken from pregnant women using the GBS Transwabs and the latter were inoculated into the semisolid medium for 24 h of enrichment. Prior to streaking onto the GBS Carrot Agar and β-γ Detection Agar, dipped a new cotton swab in the nonpigmented enriched cultures for the FICT.

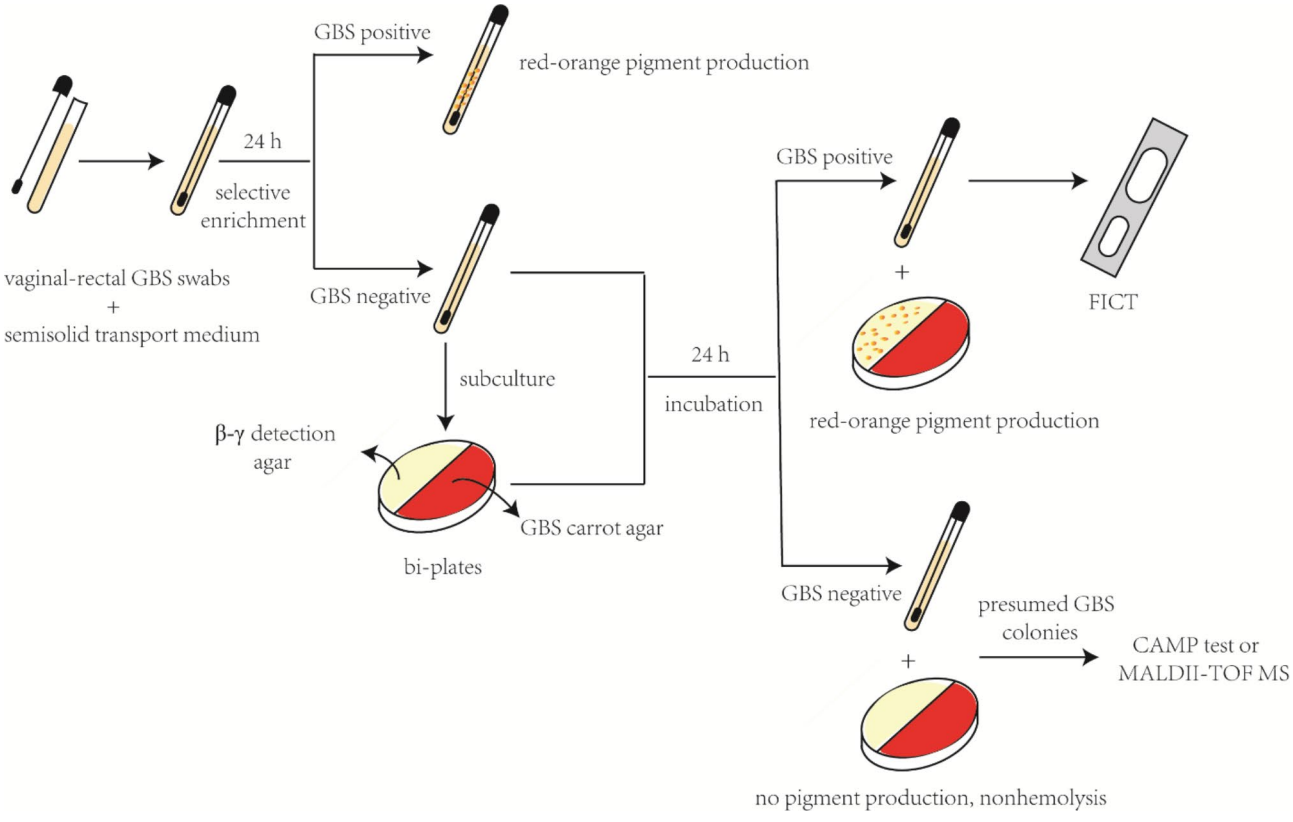


**Fig. 2** Reactivity test of FICT strip using GBS positive cultures after 48 h enrichment

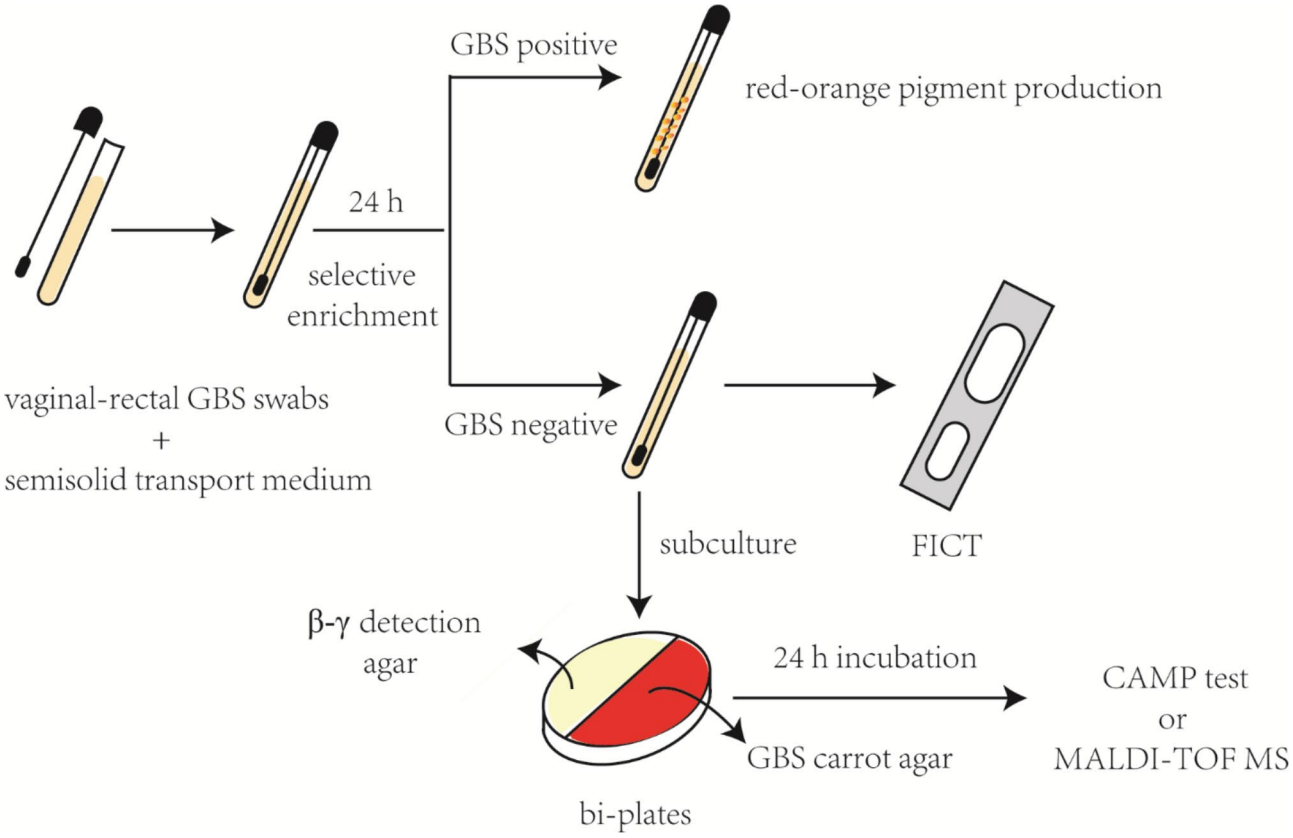


**Fig. 3** FICT detecting GBS colonization status from 24 h enriched samples

The tests using enriched samples were conducted in the same manner as described above.

#### Preparation of the LIM broth and GBS detection from LIM broth enrichment cultures

10 g of beef powder, 20 g of tryptone, 2 g of glucose, 2 g of sodium hydrogen carbonate, 2 g of sodium chloride and 0.4 g of sodium phosphate dibasic (all purchased from Solarbio, Beijing, China) were mixed and diluted with deionized water to 1 L. The broth was then sterilized by autoclaving at 121 °C for at least 15 min. When it cooled down to 45–50 °C, 10 mg of polymyxin (Solarbio, Beijing, China) and 15 mg of nalidixic acid (Solarbio, Beijing, China) were added into the broth and the LIM broth was obtained. The LIM broth was stored at 4 °C before use. As shown in Fig. 4, clinical rectovaginal swabs were collected and subjected to enrichment culture in LIM broth with 10 mg/L of polymyxin and 15 mg/L of nalidixic acid.

#### Statistical analysis

For clinical performance evaluation based on patient samples, we took culture method as the gold standard and calculated sensitivity [true positives/(true positives + false negatives)], specificity [true negatives/(true negatives + false positives)], positive predictive value [true positives/(true positives + false positives)], negative predictive value [true negatives/(true negatives + false negatives)] and concordance rate [(true positives + true negatives)/(true positives + false positives + false negatives + false positives)] of each method as shown in Tables 2, 3 and 4. We also conducted Cohen's Kappa coefficient analysis between the reference method and other diagnostic methods using SPSS statistical software v.23.0.

## Results

#### Performance of the FICT strip

The assembly and the detection principle of the FICT strip were shown in Supplemental Fig. 1 and in Supplemental Fig. 2, respectively.

For evaluation of the detection limit of the strip, $10^3$, $10^4$, $10^5$, $10^6$ and $10^7$ CFU/mL of ATCC 13,813 and ATCC 12,386 GBS strains were subjected to the FICT. We observed that the strip was able to detect $1.23 \times 10^3$ CFU/mL of ATCC 12,386 GBS cells and $1.23 \times 10^4$ CFU/mL of ATCC 13,813 GBS cells. We next used 30 GBS strains representing different serotypes listed in Table 1 to test the reactivity of the strip and 29 GBS strains could be determined by the strip at level of $1.23 \times 10^4$ CFU/mL. However, one of the tested strains (GBS_5409, serotype VI) showed positive result at cell density of $1.23 \times 10^6$ CFU/mL.

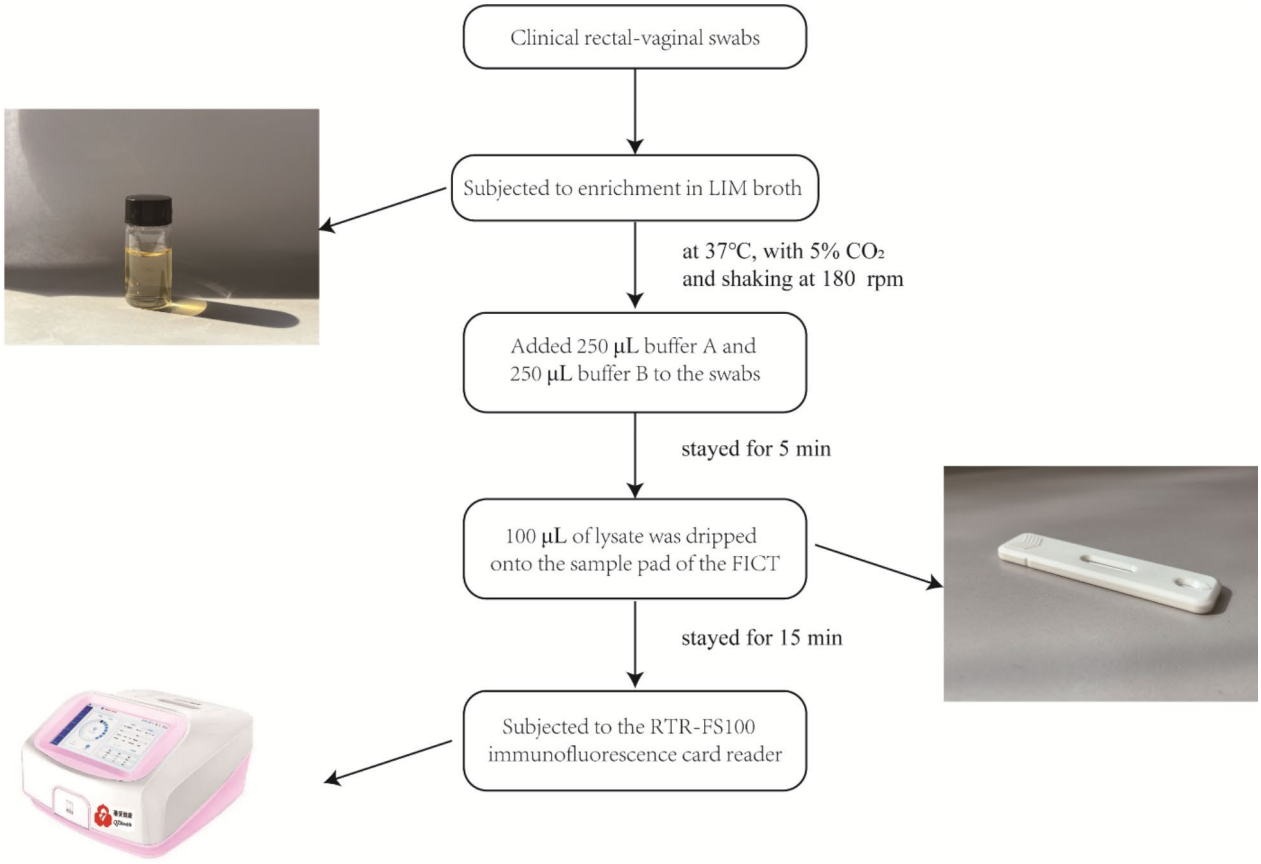


**Fig. 4** Diagram of detection GBS from LIM broth enrichment culture by FICT strip

**Table 2** Comparison between the FICT strip and GIC kit A with culture method as the gold standard

| | | FICT strip | | Total | GIC Kit A | | Total |
|---|---|---|---|---|---|---|---|
| | | positive | negative | | positive | negative | |
| Culture | positive | 10 | 19 | 29 | 8 | 21 | 29 |
| Method | negative | 0 | 196 | 196 | 0 | 196 | 196 |
| Total | | 10 | 215 | 225 | 8 | 217 | 225 |
| Sensitivity (%) | | 34.48 | | | 27.59 | | |
| Specificity (%) | | 100 | | | 100 | | |
| PPV[a] (%) | | 100 | | | 100 | | |
| NPV[b] (%) | | 91.16 | | | 90.32 | | |
| Concordance rate (%) | | 91.56 | | | 90.67 | | |

[a] Positive predictive value

[b] Negative predictive value

Subsequently, GBS positive cultures identified clinically by the culture method were also employed to test the analytical sensitivity of the strip (as shown in Fig. 2). The strip determined all of the positives from the 48 h enrichment cultures (175/175, data not shown).

For assessment of the cross-reactivity, 18 strains of bacteria species and 2 strains of *Candida* species that commonly colonized at women rectovaginal areas were examined using the strips. The results showed that none of them yielded false-positive results (data not shown).

### Comparison on the practical application between the FICT strip and other methods

We next assessed the practical application of the strip. Taken culture method as the gold standard, the detection efficacy of the FICT strip was evaluated using clinical rectovaginal swabs and was compared with that of GIC kit A and kit B. 225 samples were directly tested by the four assays. Gestational weeks of the participants range between 35 and 38 weeks. The overall mean age of them was 31.63 ± 4.72 years old, ranging from 20 to 48 years

**Table 3** Comparison between the FICT strip and qPCR technique with culture method as the gold standard

| | | FICT strip | | Total | PCR technique | | Total |
|---|---|---|---|---|---|---|---|
| | | positive | negative | | positive | negative | |
| Culture | positive | 8 | 13 | 21 | 17 | 4 | 21 |
| Method | negative | 0 | 165 | 165 | 6 | 159 | 165 |
| Total | | 8 | 178 | 186 | 23 | 163 | 186 |
| Sensitivity (%) | | 38.10 | | | 80.95 | | |
| Specificity (%) | | 100.00 | | | 96.36 | | |
| PPV[a] (%) | | 100.00 | | | 73.91 | | |
| NPV[b] (%) | | 92.70 | | | 97.55 | | |
| Concordance rate (%) | | 93.01 | | | 94.62 | | |

**Table 4** Time between admission and delivery from 2020 to 2023

| Years | Enrolled numbers | The time from admission to delivery | | |
|---|---|---|---|---|
| | | ≤ 2 h | ≤ 4 h | ≤ 6 h |
| 2020 | 13,000 | 988 (7.60%) | 2,419 (18.61%) | 3,517 (27.05%) |
| 2021 | 13,000 | 850 (6.54%) | 2,013 (15.48%) | 3,040 (23.38%) |
| 2022 | 12,000 | 699 (5.83%) | 1,699 (14.16%) | 2,553 (21.28%) |
| 2023 | 11,000 | 587 (5.34%) | 1,372 (12.47%) | 2,086 (18.96%) |
| Total | 49,000 | 3124 (6.38%) | 7,503 (15.31%) | 11,196 (22.85%) |

old. Neither the FICT strip nor the GIC kit A reported false-positive results. However, about 65.52% and 72.41% GBS culture isolates were not detectable by the FICT strip and the GIC kit A, respectively. Taken together, we found that the specificity of both FICT strip and GIC kit A for identifying GBS could reach 100%. Whereas the sensitivity of the FICT was slightly higher than that of the GIC kit A (Table 2). The same result was also obtained when compared FICT to the GIC kit B (data not shown). The κ values of the FIC test and GIC kits were 0.478 and 0.399 as estimated by Cohen's Kappa coefficient analysis, respectively, revealed low agreement of FIC test and GIC kits with the reference method in detecting GBS colonization status when employed directly on samples.

qPCR technique was then compared to the FICT strip, with the culture method as the gold standard. The 186 patients age between 20 and 48 years old, with the mean age being 31.93 ± 4.97 years old. The gestational weeks of them range from 35 to 38 weeks. Among the samples tested, culture method identified 21 positive samples (11.29%), the FICT strip determined eight (4.03%), and qPCR technique detected twenty-three (12.37%). As estimated in Table 3, the sensitivity and specificity of qPCR technique in detecting GBS colonization status were 80.95% and 96.36%, respectively. The sensitivity of qPCR technique was higher than that of FICT strip. Additionally, qPCR technology showed higher agreement with culture method compared to FIC test ($\kappa = 0.742$ vs. $\kappa = 0.522$).

Taken together, these data showed that the FICT strip and the GIC kits were not sufficient enough when applying to detecting GBS colonization status directly on vaginal-rectal samples.

#### Assessment of the FICT strip using enriched samples

In order to improve the sensitivity for identifying GBS, enriched cultures were examined by the FICT strip as shown in Fig. 3.

Among the 995 tested samples, 75 (7.54%) were GBS antigen positive by the FICT strip and the remaining 920 specimens (92.46%) were GBS antigen negative. By comparison, the culture method identified 5.63% (56/995) positives and 94.37% negatives. All of the 920 samples found to be GBS antigen negative by the FICT strip were also confirmed negative by culture method and considered true-negative. While of the 75 GBS antigen positive samples, only 56 were confirmed true-positive by culture method (including pigmented GBS isolates and hemolytic nonpigmented isolates), the remaining 19 GBS antigen positive samples were culture negative. These discrepant results were repeat examined by qPCR technique. We performed qPCR assays on the 19 enriched samples after 48 h of incubation and observed that 4 GBS antigen positive specimens were also positive by the qPCR technique, which were considered as true-positive. In contrast, the remaining 15 specimens were false-positive by the FICT strip.

Collectively, the FICT strip detected all the positives from the enriched cultures after 24 h of cultivation. Moreover, the strip enabled the detection of 4 additional samples and provided an identification of GBS 24 h sooner than the culture method. However, the other 20% positive results (15/75) reported by the FICT strip were proved to be falsely positive.

#### Estimate of the detection efficacy of the FICT strip on LIM broth enriched cultures

GBS identification by culture method often requires incubation overnight, while CDC recommends antibiotic application ≥ 4 h before delivery. As listed in Table 4, we retrospectively analyzed the time between admission and delivery of whom gave birth at this hospital during 2020 to 2023. In the 49,000 enrolled pregnant women, people

**Table 5** The results of FICT test on LIM broth enriched samples after 4 h of incubation and comparison with culture method

| | | FICT strip | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| Culture method | Positive | 30 | 3 | 33 |
| | Negative | 12 | 246 | 258 |
| Total | | 42 | 249 | 291 |
| Sensitivity (%) | | 90.91 | | |
| Specificity (%) | | 95.35 | | |
| PPV (%) | | 71.43 | | |
| NPV (%) | | 98.80 | | |
| Concordance rate (%) | | 94.85 | | |

who went into labor ≤ 2 h, ≤ 4 h and ≤ 6 h account for 6.38% (3,124/49,000), 15.31% (7,503/49000) and 22.85% (11,196/49,000) of the participants, respectively, which indicated insufficient time for culture method to be completed. Since Chinese experts consensus recommends outpatients visits for GBS screening after 35 gestational weeks, we have included the proportion of the emergent deliveries whose gestational weeks < 35 weeks that went into labor without GBS screening results (Supplemental Table 1). The proportion of the emergent deliveries before 35 gestational weeks were 3.94%, 2.71%, and 2.19% for 2 h, 4 h and 6 h, respectively. Therefore, we prepared LIM broth to maximum the efficacy for enrichment as much as possible in order to improve the specificity of FICT.

As shown in Fig. 4, clinical rectovaginal swabs were examined by the FICT strip after four hours incubation in LIM broth. For the 291 pregnant women in Table 5, the overall mean age of them was 32.35 ± 4.47 years old, ranging between 17 and 47 years old. The gestational weeks of them range from 35 to 38 weeks. Taken culture method as the gold standard, the sensitivity and specificity of the FICT strip were 90.91% and 95.35%, respectively (as shown in Table 5). The discrepant analysis was carried out using qPCR technique. For the 3 negative samples by the FICT strip while positive by culture, the recovery of GBS have been confirmed by qPCR technique using 48 h enriched cultures, which were considered false-negative results. Among the 12 positive samples by the FICT while negative by culture method, 7 were proved to be truly positive while 5 were falsely positive. Altogether, the FICT strip enabled the detection of 5 additional samples than the culture method whereas about 11.90% positive results (5/42) reported by the FICT were proved to be false-positive. Compared to the sensitivity of the FICT strip on samples without enrichment, short time enrichment in LIM broth has greatly increased the sensitivity of the FICT strip for GBS identification. The κ value of the FIC test has been increased to 0.771 through the modified procedure, indicating substantial agreement with the gold standard.

All in all, the improved procedures based on the FICT strip for GBS identification from 4 h enrichment cultures were presented showing excellent sensitivity and specificity, decreasing the time to results.

## Discussion

In the current study, we have developed a FICT strip of which the detection limit ranges between $10^4$ CFU/mL and $10^6$ CFU/mL GBS cells in samples. The reactivity tests didn't yield false-negative results. And the cross-reactivity tests indicated no false-positive results. The modified procedure that short-term LIM broth selective enrichment before the FICT enhanced the sensitivity for GBS identification, showed great potential in practical application.

Our previous study demonstrated that IAP applied to GBS carriers is effective in preventing severe GBS invasive diseases in newborns [6]. However, even in the era of IAP, the incidence rate of GBS-EOD was 0.37 of 1000 live births [23]. The majority of the mothers had cultured GBS negative in their prenatal screening and did not receive IAP treatment. The false-negative results might due to the low sensitivity or the poor relationship between the antenatal GBS screening and intrapartum GBS colonization status [24, 25]. A negative GBS screen was considered valid for 5 weeks before delivery [14]. However, the colonization status of GBS might change at the time of labor. In addition, our retrospective analysis showed there were about 15.31% pregnant women whose time from admission to delivery were no more than 4 h (Table 4) might go into labor with unknown GBS colonization status. Our data have previously shown that among the 43,822 pregnant women who were admitted to hospital and delivered from 2016 to 2018, about 7.81% delivered before 34 gestational weeks who might go into labor without outpatient visits [6]. Even though CDC recommends women with unknown GBS status be managed based on the risk factors, there were 65% neonatal cases of GBS-EOD didn't have any risk factors [26]. These factors underlined the need for a highly sensitive and specific rapid-screening test to detect GBS colonization with minimized time-to-result. The ideal test should be sufficiently rapid to allow at least 4 h IAP administration prior to delivery.

The culture-based strategy for GBS identification often requires selective enrichment on chromogenic agars overnight and subculture on blood agar plates or additional tests such as the CAMP test [20, 27]. In the current research, the culture method takes 24 h to 72 h. This has prompted us to seek out an improved procedure. Many researchers devoted themselves to find out an optimal method to determine GBS for intrapartum testing instead of prenatal screening. The optional assays were qPCR technique and immunological assays that could

obtain a result within minutes or up to several hours. Most of them observed that qPCR techniques were more sensitive than the immunological assays [21, 28]. In consisted with our data, the sensitivity of the two GIC kits were 27.59% compared with the standard culture method when applied to the direct specimens, lower than that of the qPCR technique (Tables 2 and 3, 27.59% vs. 80.95%). The sensitivity of the strip developed by us based on fluorescence immunochromatography was 34.48%, still failed to meet the 90% sensitivity threshold recommended by the CDC guidelines. In some comparative studies, qPCR techniques even outperformed culture methods [29, 30]. However, Kristin L. Atkins et al. demonstrated that PCR assay without enrichment exhibited a false-negative rate of 13.2% and a standard culture result was still needed [31]. In the present study, the false-negative rate of the PCR technique was 19.05% (4/21) when used on direct samples (Table 3). Meanwhile the PCR technique has determined 7 additional positive samples than the culture method, which might result from the presence of the fragments of unviable GBS cells. Fabien Rallu et al. reported an enhanced qPCR that performed with LIM broth enrichment cultures showed higher positive rates than the culture method [32]. In our opinion, the higher requirements of equipment and designated area as well as the much higher costs will hinder the utilization of nucleic acid amplification technologies in the universal screening of GBS. As reported by Deirdre L et al., the costs of qPCR were four times as much as the culture method [33]. On the other hand, many of the laboratories do not provide 24-h service and nucleic acid amplification technologies are batch-based, which means the rapid test result would not be available to all of the pregnant women. As indicated by Michelle J. Alfa et al., there were 75.51% women went into labor outside the laboratory hours [34].

We believe the immunological assays on enrichment cultures are the optimum method to replace the standard culture method for antennal GBS screening and the most potential strategy for intrapartum testing. Based on our data, the false-positive rate of the FICT strip detecting GBS from 24 h enrichment culture was 20%, which might be caused by the cross-reaction with other species of *Streptococcus*. It is reported *S. porcinus* and *S. pseudoporcinus* can be recovered from human (mostly from the female genitourinary tract) and have biochemical characteristics similar to those of GBS. Some *S. porcinus* and *S. pseudoporcinus* isolates were proved to cross-reacted with commercial GBS antigen-testing kits [35, 36]. Hidehito Matsui et al. developed a rapid immunochromatographic test for GBS detection targeting GBS-specific surface immunogenic protein and claimed the cross-reactivity of the antibody with the group-specific C carbohydrate [37]. In the current study, *S. porcinus* didn't cross-react with our FICT strip, more details are needed to elucidate the exact reason for the false-positive results of the FICT.

Through a 4 h enrichment step in LIM broth prior to the FICT, we were able to identify GBS carriers with low levels of colonization and inhibit the overgrowth of other species. Compared to the sensitivity of 34.48% or 38.10% on direct specimens, the sensitivity of the FICT has been elevated to 90.91% after short-term enrichment (Table 5). The LIM broth for selective enrichment was supplemented with 10 mg/L of polymyxin and 15 mg/L of nalidixic acid, which suppressed the growth of undesired microorganisms such as *Enterobacteriaceae* bacteria. Sousan Sayahtaheri Altaie et al. suggested that preincubation of cervical swabs in Lim broth improved the sensitivity of an enzyme immunoassay test for GBS determination. The enzyme immunoassay performed on the enriched samples detected 4% of the positives at 2 h, 21% at 4 h, 58% at 6 h, and 100% at 8 h post-incubation [38]. However, prolonged enrichment period might lead to the overgrowth of other undesired microorganisms like *S. aureus* and *E. faecalis*, which were suggested to interfere the growth of GBS [39, 40]. Notably, the use of enrichment broths will also allow for susceptibility testing for penicillin allergic patients, when necessary.

Our results indicated the improved procedure shown in Fig. 4 based on the FICT strip for GBS identification from LIM broth enrichment cultures increased the sensitivity of GBS screening while reduced the turnaround time, sufficiently rapid to allow adequate IAP administration prior to delivery. The FICT strip has the potential to aid in the diagnostic of GBS carriers both in antepartum screening and in intrapartum testing and guide IAP administration efficiently in the future.

**Abbreviations**

| | |
|---|---|
| GBS | Group B streptococcus |
| IAP | Intrapartum antibiotic prophylaxis |
| FICT | Fluorescence immunochromatographic test |
| GIC | Colloidal gold immunochromatography |
| qPCR | Real-time quantitative polymerase chain reaction |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12884-025-07544-w.

Supplementary Material 1

Supplementary Material 2

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Not applicable.

**Author contributions**
All authors have accepted responsibility for the entire content of this manuscript and approved its submission. Conceptualization: H. M. Ye and X. L. Chen; Methodology: H. M. Ye, X. L. Chen, Z. M. Tang and L. J. Zheng; Investigation: X. L. Chen, S. J. Cao and X. Y. Chen; Validation: X. L. Chen;

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**Data availability**
The original data presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

## Declarations

**Consent for publication**
Not applicable since the manuscript does not include information or images that could lead to the identification of a study participant.

**Competing interests**
The authors declare no competing interests.

**Ethics Statement**
The study was performed in accordance with the principles of the Declaration of Helsinki. The current study was approved by the Human Research Ethics Committee of Women and Children's Hospital of Xiamen University (KY-2023-074-H01) and performed in accordance with its guidelines.

**Informed consent**
Informed consent was obtained from all individuals tested in this study.

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