Development of a recombinase-aided amplification assay for rapid and sensitive detection of *Pneumocystis jirovecii* infection: A single-center molecular study

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Abstract. *Pneumocystis jirovecii* (PJ) is a major opportunistic pathogen that causes severe pneumonia in immunocompromised individuals, leading to significant morbidity and mortality. Early and accurate diagnosis is critical for effective clinical management. However, existing diagnostic methods have limitations such as low sensitivity, long turnaround time and high equipment requirements. Recombinase-aided amplification (RAA) is an isothermal nucleic acid amplification technology with advantages of rapidity, simplicity and high sensitivity. The aim of this study was to develop and validate an RAA assay for the rapid detection of PJ. Clinical bronchoalveolar lavage fluid samples were collected from 44 PJ-positive patients confirmed by targeted next-generation sequencing (tNGS) and extracted nucleic acid. A recombinant PJ plasmid (pUC57-PJ) was constructed as the standard positive template. Specific primers and probes for PJ were designed and the RAA reaction conditions were optimized. The analytical performance (sensitivity, specificity) of the RAA method was evaluated using pUC57-PJ and clinical sample nucleic acids. Additionally, the clinical performance of RAA was validated by comparing with tNGS and RAA results. In conclusion, a rapid, sensitive and specific RAA assay for PJ detection was successfully developed. This assay exhibits excellent analytical and clinical performance, and is suitable for rapid diagnosis of PJ infection, particularly in resource-limited settings. It provides a valuable new tool for the early diagnosis and management of PJ pneumonia.

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Introduction

Pneumocystis jirovecii (PJ), an opportunistic fungal pathogen, is a major cause of Pneumocystis pneumonia (PCP) in immunocompromised individuals, such as those with HIV/AIDS, organ transplant recipients, and patients receiving chemotherapy or long-term immunosuppressive therapy (1-4). PCP is characterized by severe respiratory symptoms, and if not diagnosed and treated promptly, it can lead to respiratory failure and death (3). Therefore, early and accurate diagnosis of PJ infection is crucial for improving patient outcomes.

Currently, the diagnostic methods commonly used for PJ detection include microscopic examination of bronchoalveolar lavage fluid (BALF) smears, PCR-based assays and targeted next-generation sequencing (tNGS) (5-9). Microscopy and conventional PCR are the traditional 'gold standards' for PJ detection. Microscopy exhibits low sensitivity, particularly in cases with low PJ burdens (10,11). PCR has higher sensitivity than microscopy, but it requires expensive thermal cyclers and trained personnel, and its detection process is relatively time-consuming (12-15). tNGS has the advantage of high-throughput detection but is costly and not widely available in clinical settings, especially in resource-limited areas (7,16-19), and entails a relatively long detection time (20). Thus, there is an urgent need for a rapid, simple, sensitive and cost-effective diagnostic method for PJ detection.

Nucleic acid detection technologies have revolutionized the field of clinical diagnosis, pathogen surveillance and genetic analysis, with continuous advancements aiming to address the limitations of traditional methods such as microscopy and PCR-based assays (21). Among the emerging alternatives, isothermal amplification techniques have garnered considerable attention due to their inherent advantages of simplicity, rapidity and independence from expensive thermal cyclers, making them particularly promising for applications in resource-limited settings (22,23). For instance, loop-mediated isothermal amplification (LAMP) has been extensively explored for the detection of a broad spectrum of

targets, including *Plasmodium knowlesi*, severe acute respiratory syndrome coronavirus (SARS-CoV)-2 and various bacteria, fungi and sexually transmitted infection-related pathogens (24-28). Advances in molecular diagnostics have also promoted the application of isothermal amplification techniques in specialized fields such as tuberculosis diagnosis, where they serve as valuable alternatives for the detection of *Mycobacterium tuberculosis* (29). Beyond pathogen detection, LAMP has also found utility in crop breeding, disease diagnosis and genetic screening - for instance, in identifying single-nucleotide polymorphisms and gene translocations associated with cancer, highlighting its potential as a versatile tool for mutation screening (25,30). Another notable isothermal amplification technique is recombinase polymerase amplification (RPA), which has been successfully applied in the detection of bacteria, fungi, viruses, parasites, drug-resistant genes, genetically modified foods and SARS-CoV-2 (31-33).

Recombinase-aided amplification (RAA) is a novel isothermal nucleic acid amplification technology that has emerged in recent years (31,34,35). It utilizes recombinase, single-stranded DNA-binding protein and DNA polymerase to achieve rapid amplification of target nucleic acids at a constant temperature (37-42˚C) (36-38). RAA utilizes recombinase sourced from bacteria or fungi, while RPA employs recombinase primarily derived from the T4 bacteriophage (39). RAA has the advantages of rapid amplification (40-42) (usually completed within 30 min), simple operation, no need for complex thermal cycling equipment, and high sensitivity and specificity (38). It has been successfully applied for the detection of various pathogens, such as bacteria, viruses and parasites (37,43-47). Its application scope further extends to the agricultural and livestock sectors, enabling effective detection of viruses that cause infections in livestock (42,48,49). The RAA-amplified products can be detected by agarose gel electrophoresis or a real-time fluorescence detection platform (46,50,51). Its compatibility with point-of-care testing (POCT) (52) scenarios is further enhanced by integration with CRISPR-Cas technology (53), a combination that enables more convenient and intuitive interpretation of detection results while significantly expanding the application potential of RAA in pathogen detection (35,54,55). However, no reports have documented the application of RAA for PJ detection.

In the present study, specific primers and probes targeting PJ were designed, the RAA reaction conditions were optimized and the analytical performance (sensitivity, specificity, repeatability) of the assay was evaluated. Additionally, the clinical performance of the RAA assay was validated using clinical BALF samples, aiming to provide a rapid and reliable diagnostic tool for PJ infection.

Materials and methods

Ethical approval and consent. This study was approved by the Ethics Committee of Weifang Second People's Hospital (Weifang, China; approval no. KY2024-023-01; approval date: March 15, 2024) and conducted in strict accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients prior to BALF sample collection and study inclusion; for patients unable to provide consent due to critical illness (e.g., severe respiratory failure requiring mechanical ventilation or altered consciousness), consent was obtained from their legal representatives. All participants were informed about the purpose of the study, the procedures involved, potential risks and benefits, and their right to withdraw at any time without affecting their clinical care. Patient data were anonymized and de-identified prior to analysis to ensure confidentiality.

Case definition. PJ infection was defined based on the following composite criteria: i) Clinical manifestations consistent with PCP, including progressive dyspnea, nonproductive cough, fever and hypoxemia; ii) characteristic chest imaging findings of diffuse bilateral ground-glass opacities, interstitial infiltrates or cystic lesions on computed tomography (CT); and iii) laboratory confirmation of PJ nucleic acid detection by tNGS from BALF samples. Patients meeting all three criteria were classified as confirmed PJ infection cases. Patients with negative tNGS results and no clinical or radiological evidence of PCP were classified as non-PJ controls.

Inclusion and exclusion criteria. Inclusion criteria were as follows: i) Patients aged ≥18 years who underwent bronchoscopy and BALF collection for suspected lower respiratory tract infection at Weifang Second People's Hospital between March 18, 2024 and December 31, 2024; ii) BALF samples with sufficient volume (≥3 ml) and quality for nucleic acid extraction and molecular testing; iii) complete clinical, laboratory and imaging data available for review; iv) for PJ-positive cases, confirmation by tNGS detection of PJ with consistent clinical and radiological findings. Exclusion criteria were as follows: i) BALF samples with insufficient volume (<3 ml) or visible blood contamination exceeding 50% of the sample volume; ii) patients with incomplete medical records or missing tNGS results; iii) samples stored for >72 h at 4˚C or subjected to repeated freeze-thaw cycles (>2 cycles) prior to testing; iv) patients receiving empirical anti-PCP therapy (trimethoprim-sulfamethoxazole, pentamidine or atovaquone) for >72 h before BALF collection; and v) concurrent diagnosis of other fungal infections (e.g., invasive aspergillosis, mucormycosis) that could confound the clinical presentation. The participant selection process is summarized in Fig. 1.

Experimental materials

Clinical sample collection. A total of 44 BALF samples positive for PJ by tNGS and 17 BALF samples negative for PJ by tNGS were collected. All samples were processed within 2 h of collection.

Pathogen controls. Genomic DNA of common clinically relevant pathogens, including *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Stenotrophomonas maltophilia*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Serratia marcescens*, *Candida albicans* and *Candida krusei*, was obtained from the Clinical Microbiology Laboratory of Weifang No. 2 People's Hospital (Weifang, China), having been isolated from the patient's clinical specimen during diagnostic examination.

Kits and instruments. The RAA Nucleic Acid Amplification kit (Conventional Method; cat. no. B00000A-96; lot no. BH7OA0) and RAA Nucleic Acid Amplification kit

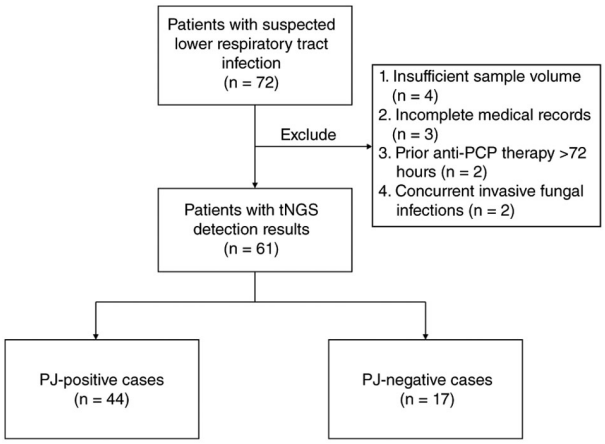


Figure 1. Flow chart showing the selection process of research participants. A total of 72 patients with suspected lower respiratory tract infection were assessed for eligibility. Of these, 11 were excluded (4 due to insufficient sample volume, 3 due to incomplete medical records, 2 due to prior anti-PCP therapy >72 h and 2 due to concurrent invasive fungal infection), leaving 61 participants included for analysis (44 PJ-positive cases confirmed by tNGS and 17 PJ-negative controls). All 61 samples were subjected to fluorescent RAA assay and the results were compared with tNGS as the reference standard. tNGS, targeted next-generation sequencing; PJ, *Pneumocystis jirovecii*; PCP, Pneumocystis pneumonia; RAA, recombinase-aided amplification.

(Fluorescent Method; cat. no. F00001A-96; lot no. FH9KD0) were purchased from QT Biotech Co., Ltd. Thermo Scientific™ PCR Premix (2X) (cat. no. K0172) was purchased from Thermo Fisher Scientific, Inc. The MGIEasy Nucleic Acid Extraction Kit (lot no. 111942507003; cat. no. RM0766) was purchased from BGI Genomics Co., Ltd. The Equalbit 1X dsDNA HS Assay Kit (cat. no. EQ121-02; lot no. 7E1352F5) was purchased from Vazyme Biotech Co., Ltd. DL1500 Ladder (100 bp to 1,500 bp; cat. no. MD1019) was purchased from Simgen Biotechnology Co., Ltd. Instruments included a Qubit 4 Fluorometer (Thermo Fisher Scientific, Inc.), CFX96™ Real-Time PCR System (Bio-Rad Laboratories, Inc.), T100™ Thermal Cycler (Bio-Rad Laboratories, Inc.) and ChemiDoc™ Imaging System (Bio-Rad Laboratories, Inc.).

Nucleic acid extraction. Nucleic acid extraction was performed using the MGIEasy Nucleic Acid Extraction Kit, with steps as follows: i) Reagent and sample preparation: Lysis buffer: Antifoaming agent was added to lysis buffer; Wash Buffer A/B: Anhydrous ethanol was added to Wash Buffer A/B, respectively. For BALF samples: Transferred 350 μl of BALF to a clean 1.5 ml centrifuge tube. ii) Preparation and addition of extraction reaction mix for each sample. iii) Magnetic bead extraction. Viscous residues were homogenized and the mixture was then incubated at 56˚C for 10 min. Isopropanol and magnetic beads were added and after vortexing, the mixture was left to stand at room temperature for 10 min (invert every 3 min). Samples were centrifuged, placed on a magnetic rack and the supernatant was discarded. Beads were washed with Wash Buffer A/B sequentially and then air-dried at room temperature, followed by addition of nuclease-free water and elution of nucleic acid, which was stored at -20˚C. iv) Quality control. The DNA concentration was measured with the Equalbit 1X dsDNA HS Assay Kit (Vazyme Biotech Co., Ltd.) and Qubit 4 Fluorometer (Thermo Fisher Scientific, Inc.).

Samples were considered to meet the requirements for subsequent library preparation if their DNA concentration, as determined by NanoDrop DS-11, was ≥1 ng/μl (total amount ≥10 ng), and if the A260/A280 ratio and A260/A230 ratio, as measured by spectrophotometry (NanoDrop DS-11; Thermo Fisher Scientific, Inc.), were 1.8-2.0 and ≥1.8, respectively.

Construction of recombinant plasmid standard. A 149 bp conserved region of the PJ major surface glycoprotein gene (GenBank ID: KP168716.1) was synthesized and cloned into the pUC57 vector by Sangon Biotech, generating a recombinant plasmid. The plasmid was serially diluted to concentrations ranging from 10^4 to 10 copies/μl with nuclease-free water. The number of copies/μl was calculated using the following formula:

$$Copies/\mu l = \frac{6.02 \times 10^{23} \times Concentration\ (ng/\mu l) \times 10^{-9}}{Plasmid\ length\ (bp) \times 660}$$

Primer and probe design. The 149 bp conserved region of the PJ major surface glycoprotein gene was selected as the target for RAA development. Specific primers and an exo probe for RAA were designed using Oligo 7 software (Molecular Biology Insights, Inc.). The probe was labeled with a FAM fluorophore at the 5' end and a BHQ-1 quencher at the 3' end, and a tetrahydrofuran spacer was included to facilitate cleavage by the exonuclease. All oligonucleotides were synthesized by Sangon Biotech (Shanghai, China). Sequences are listed in Table I.

Specificity of primers and RAA assay. To evaluate the specificity of PJ-RAA-forward(F)/reverse (R) primers and confirm the amplification capability of the fluorescent RAA assay for PJ, conventional PCR was performed. Conventional PCR used genomic DNA templates including 8 common respiratory bacteria, 2 opportunistic fungi and a PJ-positive BALF sample. Nuclease-free water served as the negative control (NC) for both assays.

Workflow of tNGS. tNGS was performed using the PTseq Respiratory Pathogen Targeted High-Throughput Genetic Detection Kit (lot no. 513392407006; cat. no. RM1261; MGI Tech Co., Ltd.) on the MGI DNBSEQ-G99 platform according to the manufacturer's instructions. The tNGS workflow encompasses sample collection, nucleic acid extraction, multiplex targeted enrichment, library construction and bioinformatics analysis, covering a total of 274 common respiratory pathogens, including 72 bacteria, 95 fungi, 50 viruses and 15 other pathogens. Bioinformatics analysis was performed using the MGI platform to align reads against the reference genome. A positive result was defined as detection of 13 or more unique reads with >95% coverage of the target region.

Evaluation of clinical diagnostic efficacy of RAA for PJ PCR amplification system. The total volume of the PCR reaction system was 25 μl. Each reaction contained 12.5 μl of Thermo Scientific™ PCR Premix (2X) (containing Taq DNA polymerase, dNTPs and reaction buffer), 2 μl of 10 μM PJ-F primer, 2 μl of 10 μM PJ-R primer, 2 μl of template DNA (genomic DNA extracted from PJ-positive samples, non-PJ pathogens or NC) and 6.5 μl of nuclease-free water to bring the volume to 25 μl.

PCR amplification program. Thermal cycling was carried out on a PCR instrument with the following parameters: First, pre-denaturation was performed at 94˚C for 10 min to fully

Table I. Sequences of primers and probe for PJ RAA.

Primer name	Sequence (5'-3')	Target region	Length, bp
PJ-RAA-F	CGACTCATACCAGCACATCCACAACCACAT	2858-2887	30
PJ-RAA-R	ACTCATCCTCAACCCTTCACTCGGCTTCAC	2977-3006	30
PJ-RAA-Probe	TCAAGAATAACACTCACTTCGACAAGAAGA [i6FAMdT][idsp][iBHQ1dt]AAACCGACCAAGTGT [C3Spser]	2899-2947	49

F, forward; R, reverse; PJ, *Pneumocystis jirovecii*; RAA, recombinase-aided amplification.

denature genomic DNA. This was followed by 30 amplification cycles, each consisting of denaturation at 94˚C for 30 sec (to separate double-stranded DNA), annealing at 60˚C for 30 sec (to enable specific binding of primers to target sequences) and extension at 72˚C for 30 sec (to synthesize new DNA strands via Taq polymerase). After the cycling process, a final extension step was conducted at 72˚C for 6 min to ensure complete extension of all amplified products, and the reaction was then held at 4˚C indefinitely to preserve the amplified products.

Agarose gel electrophoresis. PCR products were analyzed by agarose gel electrophoresis to confirm amplification specificity. A 1% agarose gel was prepared using 1X Tris-acetate-EDTA (TAE) buffer, with ethidium bromide added for subsequent nucleic acid visualization. Electrophoresis was run at a constant voltage of 90 V; the running time was adjusted based on gel separation efficiency, typically 20-30 min, until DNA bands were clearly distinguishable. The PCR products were mixed with 6X DNA loading buffer. For sample loading, 1.3 μl of DNA Marker (GeneRuler 100 bp DNA Ladder; cat. no. SM0241; lot no. 3498729; Thermo Fisher Scientific, Inc.) and 4 μl of PCR product from each reaction were loaded into separate wells of the gel. After electrophoresis, gel images were captured using the ChemiDoc™ Imaging System (cat. no. 12003153; Bio-Rad Laboratories, Inc.), and the presence or absence of target bands was used to evaluate the specificity of the PJ primers.

Establishment and optimization of RAA for PJ

RAA reaction system. The RAA assay was performed in a 50 μl reaction volume using an RAA Nucleic Acid Amplification kit according to the manufacturer's instructions. For fluorescent RAA amplification, the system included 25 μl of RAA reaction buffer, 2.6 μl of the forward primer PJ-RAA-F (10 μM) and 2.6 μl of the reverse primer PJ-RAA-R (10 μM), 0.8 μl of PJ-RAA-Probe (10 μM), 12 μl of nuclease-free water, 5 μl magnesium acetate and 2 μl of template DNA (25 ng/μl). For conventional (electrophoresis-based) RAA amplification, the system excluded the 0.8 μl of PJ-RAA-Probe (10 μM), and the volume of nuclease-free water was adjusted to 12.8 μl to maintain a total reaction volume of 50 μl. Fluorescent reactions were incubated at 40˚C for 20 min on the CFX96™ Real-Time PCR System, with fluorescence signals recorded every 20 sec (corresponding to 1 cycle per 20 sec).

Optimization of reaction conditions. Key parameters were optimized using DNA extracted from clinical samples as the template, with tests performed across a series of conditions: Temperatures of 37, 38, 39, 40, 41 and 42˚C (each with a 20 min incubation). For fluorescent RAA, samples that gave rise to an exponential amplification curve above the NC threshold within 20 min were judged as positive. Optimal reaction conditions were defined as the combination that yielded the earliest amplification initiation and the fastest plateau phase arrival.

Evaluation of clinical diagnostic efficacy of RAA for PJ

Sensitivity. For sensitivity evaluation, the recombinant pUC57-PJ plasmid was serially diluted to generate a concentration gradient ranging from 10^4 to 10^1 copies/μl (10^4, 10^3, 10^2, 10) for fluorescent RAA assays. Fluorescent RAA was performed under the optimized reaction conditions. All dilutions were tested in triplicate to ensure reproducibility.

Clinical validation. The optimized fluorescent RAA assay was used to detect 44 PJ-positive and 17 PJ-negative BALF samples. Diagnostic performance indicators, including sensitivity, specificity, positive predictive value, negative predictive value, total agreement and κ value (κ: 0.61-0.80=substantial agreement; 0.41-0.60=moderate agreement) were calculated using SPSS 26.0 (IBM Corp.).

Statistical analysis. Data were analyzed using ImageJ (version 1.52a; National Institutes of Health) and SPSS 26.0. The clinical diagnostic performance of the RAA assays was evaluated, with tNGS used as the reference standard. Analyses were based on a 2x2 contingency matrix using the chi-square test. A two-tailed $P<0.05$ was considered to indicate statistical significance.

Results

Primer/probe validation and plasmid construction. Conventional PCR using PJ-RAA-F/R primers amplified a 149 bp fragment from PJ-positive BALF DNA (Fig. 2A), confirming the validity of the target region within the PJ major surface glycoprotein gene. A specific amplification band was only visible in the PJ-positive sample, while no bands were detected in the NC and all non-PJ pathogens. This result confirmed the high specificity of the primers for PJ detection. NGS of pUC57-PJ showed 100% homology with the reference PJ major surface glycoprotein sequence gene (GenBank ID: KP168716.1), confirming successful plasmid construction (Fig. 2B).

Optimization of RAA reaction conditions. Fluorescence amplification was successfully detected across all tested temperatures (37-42˚C) in the detection of 3 PJ-positive BALF

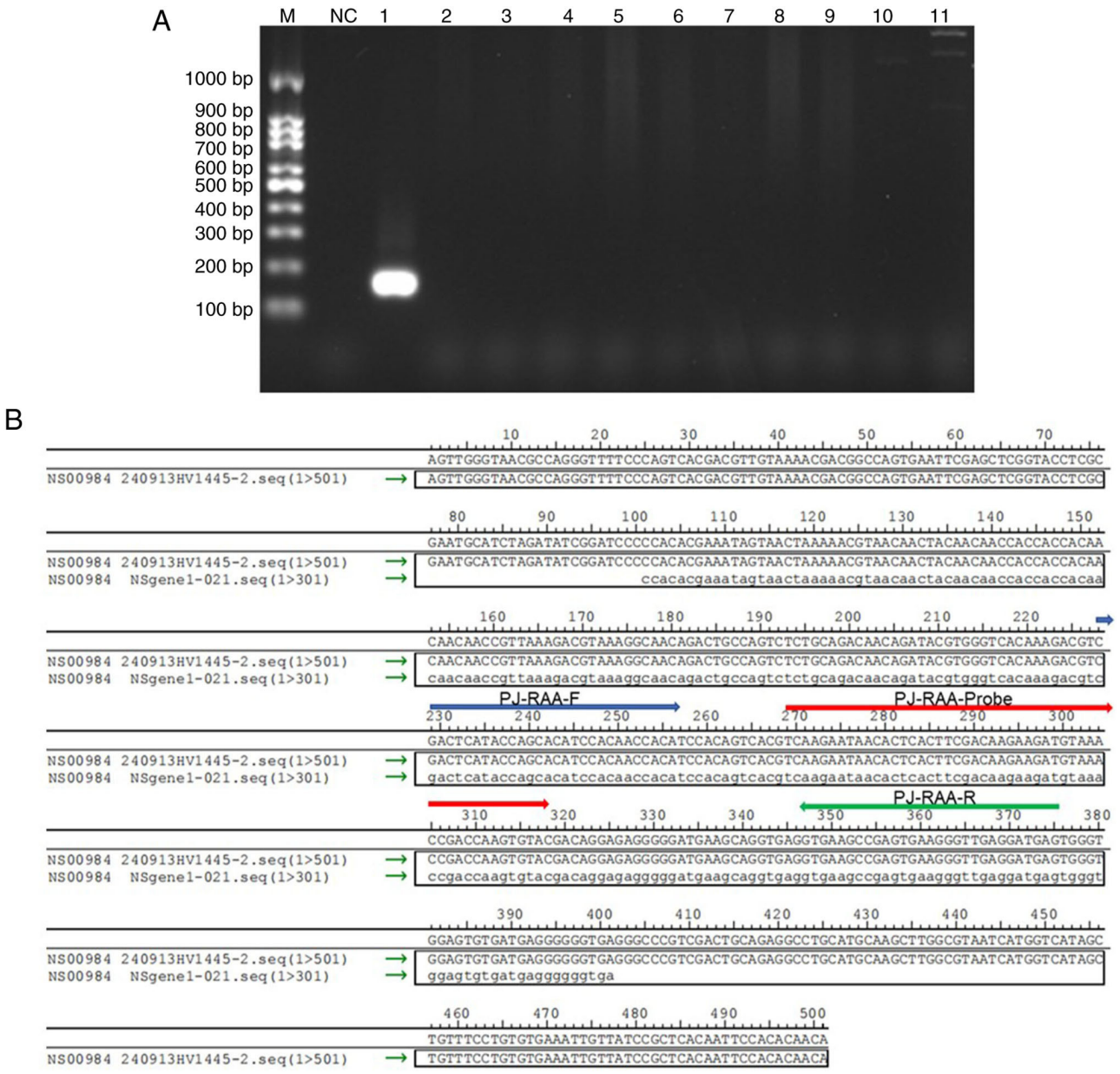


Figure 2. Detection of the specificity of primers and RAA assay. (A) Agarose gel electrophoresis profile of amplification products from the PJ-targeted PCR assay. Lanes: 1: PJ-positive bronchoalveolar lavage fluid sample; 2: *Acinetobacter baumannii*; 3: *Klebsiella pneumoniae*; 4: *Pseudomonas aeruginosa*; 5: *Proteus mirabilis*; 6: *Stenotrophomonas maltophilia*; 7: *Enterobacter aerogenes*; 8: *Enterobacter cloacae*; 9: *Serratia marcescens*; 10: *Candida albicans*; 11: *Candida krusei*. (B) The consensus sequence (top) was generated from the alignment of the reference sequence NSgene1-021.seq (bottom) and the confirming next-generation sequencing read 240913HV1445-2.seq (middle). Numbers indicate nucleotide positions. M, DL1500 ladder; NC, negative control; PJ, *Pneumocystis jirovecii*; RAA, recombinase-aided amplification.

samples. At temperatures <40˚C (37, 38 and 39˚C), amplification initiation was relatively delayed, and the arrival time to the plateau phase was prolonged. By contrast, the system exhibited the earliest amplification initiation (mean: 20 cycles) and the fastest arrival at the plateau phase at 40˚C (Fig. 3), demonstrating the most robust and efficient amplification kinetics. This superior performance could be attributed to the highest and most stable recombinase activity at 40˚C in the fluorescent RAA system, which ensured consistent and rapid nucleic acid amplification. When the temperature exceeded 40˚C (41 and 42˚C), the amplification curves showed suboptimal morphological characteristics, with slower overall kinetics compared to those at 40˚C. Collectively, these results indicated that 40˚C was the optimal amplification temperature, as it balanced both the speed of amplification initiation and the efficiency of plateau phase arrival.

Analytical performance of RAA assay

Sensitivity. To evaluate the sensitivity of the fluorescent RAA assay for PJ detection, serial dilutions of the PJ-positive plasmid (10^4, 10^3, 10^2 and 10 copies/μl) were used as templates for amplification under the optimized reaction conditions, and the corresponding curves are shown in Fig. 4. The amplification curves exhibited a concentration-dependent trend: The template with the highest concentration (10^4 copies/μl) initiated amplification earliest (within 5-10 cycles) and produced

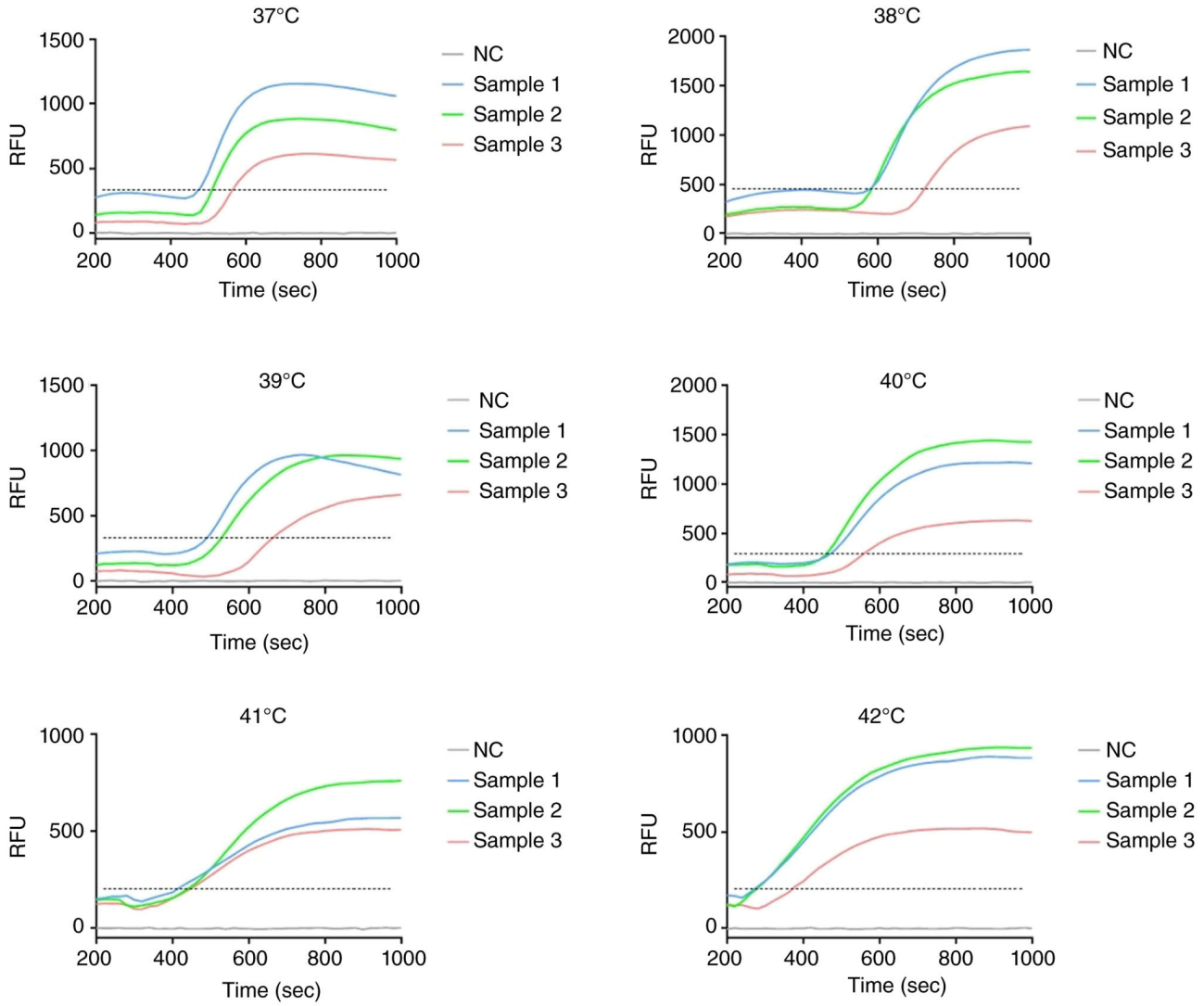


Figure 3. Optimization of reaction conditions for RAA assays. Fluorescent RAA amplification curves of 3 PJ-positive bronchoalveolar lavage fluid samples (1, 2, 3) at different temperatures (37-42˚C). PJ, *Pneumocystis jirovecii*; RAA, recombinase-aided amplification; RFU, relative fluorescence units.

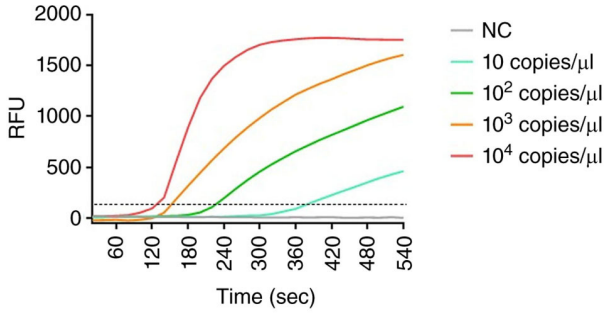


Figure 4. Sensitivity analysis of PJ detection assays. Sensitivity analysis of fluorescent RAA assay for PJ detection using serial dilutions of pUC57-PJ recombinant plasmid templates (10^4-10 copies/μl) at 40˚C for 60 cycles. NC, negative control; PJ, *Pneumocystis jirovecii*; RAA, recombinase-aided amplification; RFU, relative fluorescence units.

the strongest final relative fluorescence unit (RFU) signal. As the plasmid concentration decreased (10^3, 10^2 and 10 copies/μl), the initiation cycle of amplification was gradually delayed, and the final RFU signal was sequentially reduced. Notably, even the lowest concentration template (10 copies/μl) still generated a detectable amplification signal (crossing the baseline within 25 cycles), indicating that the fluorescent RAA assay has good sensitivity and can effectively amplify PJ target sequences at low copy numbers.

Clinical validation. To evaluate the diagnostic performance of fluorescent RAA, 44 BALF samples from patients suspected of PCP were tested with tNGS as the reference standard. Among 44 PJ-positive samples confirmed by tNGS, 36 were detected by fluorescent RAA, and no false-positive results were observed in 17 PJ-negative samples (100% specificity for all assays). Diagnostic efficacy metrics (Table II) showed that fluorescent RAA achieved 81.82% sensitivity, 86.89% overall agreement and a κ value of 0.715 ($P<0.001$). The fluorescent RAA assay exhibited good rule-out diagnostic value, coupled with excellent clinical diagnostic performance. Among the 61 patients, 48 (78.7%) were male and 13 (21.3%) were female, with a mean age of 70.2±9.8 years. In the PJ-positive group, 36 (81.8%) were male and 8 (18.2%) were female, with a mean age of 70.4±10.8 years; in the PJ-negative group, 12 (70.6%) were male and 5 (29.4%) were

Table II. Diagnostic performance of fluorescent RAA in clinical bronchoalveolar lavage fluid samples.

Parameter	N fluorescence on RAA	P fluorescence on RAA	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Overall agreement, %	κ value	P-value
tNGS P/ tNGS N	36/8	0/17	81.82	100	100	68	86.89	0.715	0.001

Cross-tabulation of detection results and diagnostic efficacy metrics of fluorescent RAA, relative to tNGS. P, positive; N, negative; PPV, positive predictive value; NPV, negative predictive value; RAA, recombinase-aided amplification; tNGS, targeted next-generation sequencing.

Table III. Characteristics of patients of the two groups at baseline.

Characteristic	All patients (n=61)	PJ-positive (n=44)	PJ-negative (n=17)	P-value
Sex, n (%)				0.486
Male	48 (78.7)	36 (81.8)	12 (70.6)	
Female	13 (21.3)	8 (18.2)	5 (29.4)	
Age, mean ± SD	70.2±9.8	70.4±10.8	69.5±6.9	0.444

PJ, *Pneumocystis jirovecii*.

female, with a mean age of 69.5±6.9 years. The characteristics of the cohort are summarized in Table III. There were no significant differences between the two groups in terms of gender or age (P>0.05).

Discussion

In this study, a novel RAA assay for the rapid and sensitive detection of PJ was successfully developed and optimized. The establishment of a robust molecular diagnostic tool for PCP is of paramount clinical importance, particularly for immunocompromised patients, where timely diagnosis can significantly impact outcomes.

The RAA assay demonstrated several key advantages over microscopy and PCR. It exhibited superior analytical sensitivity, detecting down to 10 copies/μl of the target plasmid. The RAA sensitivity was comparable to, or even exceeded, the sensitivity of numerous previously reported molecular assays for PJ (56-60). The ability to detect low pathogen loads is critical for PCP diagnosis, as patients can present with subclinical or early-stage infections.

The isothermal nature of the RAA technology is a major strength. The optimized reaction at 40˚C eliminates the need for sophisticated and expensive thermal cyclers, which are required for qPCR. This feature, combined with the rapid turnaround time (results within 20-30 min depending on the template concentration), makes the RAA assay a promising candidate for POCT and use in resource-limited settings (61-63). The entire process, from DNA extraction to result interpretation, can be streamlined and potentially performed in decentralized laboratories.

In terms of cost-effectiveness, the RAA assay offers significant economic advantages over tNGS. The estimated reagent cost per RAA test is ~50-80 RMB (~7-12 USD), which is substantially lower than the tNGS cost of 400-600 RMB (~59-88 USD) per sample on the MGI DNBSEQ-G99 platform. Furthermore, the RAA assay does not require expensive thermal cyclers or high-throughput sequencing platforms, reducing both capital investment and maintenance costs. The total turnaround time from sample processing to result reporting is ~40-50 min for RAA, compared with 24-48 h for tNGS, which may translate into reduced hospitalization costs and earlier initiation of targeted therapy. These features make RAA particularly suitable for resource-limited clinical settings where budget constraints limit access to advanced molecular diagnostics.

The clinical performance of the RAA assay was highly encouraging. When tested on 44 tNGS-positive BALF samples, the RAA assay detected PJ in 36 samples, demonstrating a high sensitivity that supports its utility as a rapid screening tool. Notably, the assay achieved perfect specificity, with no false-positive results, which is clinically valuable for avoiding unnecessary antifungal therapy and reducing patient risk. The substantial agreement between the RAA assay and the tNGS reference standard further validates its reliability, while the observed false-negative cases highlight the need for complementary testing in high-risk settings. Overall, these findings position the RAA assay as a promising diagnostic option for PJ, particularly in resource-constrained environments where timely detection is critical.

The systematic optimization process was crucial for maximizing the assay's performance. Of note, 40˚C was identified as the optimal temperature, which provided a balance between amplification efficiency and enzyme stability.

Despite these promising results, the present study has certain limitations. The sample size was relatively small. Future studies should include a larger, more diverse cohort, including clinically suspected samples, to comprehensively

evaluate the clinical sensitivity and specificity. Additionally, formal specificity testing against a broader panel of respiratory pathogens is warranted to conclusively demonstrate the assay's exclusivity for PJ.

In addition to these study limitations, several practical challenges should be considered when implementing the RAA assay in clinical practice. The high analytical sensitivity of RAA, while enabling detection of low pathogen loads, concomitantly increases the risk of false-positive results due to cross-contamination and nonspecific amplification arising from short target amplicons or primer dimers (35). Furthermore, on-site nucleic acid extraction remains a practical bottleneck for point-of-care deployment, and achieving multiplexed target detection under single-tube closed-tube conditions represents an unresolved technical challenge that may limit the assay's broader application (35). In the present clinical evaluation, the observed sensitivity of 81.82% indicates that a subset of PJ-positive cases may be missed, necessitating complementary testing with tNGS or qPCR in high-risk patients with strong clinical suspicion. Additionally, the assay has been validated only with BALF samples, and its performance with less invasive specimens (such as expectorated sputum, induced sputum, nasopharyngeal swabs and oropharyngeal swabs), along with the long-term stability of lyophilized reagents under decentralized storage conditions, requires further investigation. Addressing these operational and technical hurdles is essential to maximize the clinical utility of RAA in resource-limited settings.

In conclusion, in the present study, a rapid, sensitive and specific RAA assay for the detection of PJ was developed. Its isothermal nature, speed and compatibility with portable fluorescence detectors position it as a valuable tool for the rapid diagnosis of PCP, potentially facilitating earlier treatment and improved patient management.

The current study established and optimized a RAA assay for the detection of PJ. The assay demonstrates high sensitivity, with a detection limit of 10 copies/μl, and can deliver results within 20 min under an isothermal incubation at 40˚C. The clinical evaluation revealed excellent concordance with tNGS results, underscoring the superior diagnostic performance of the RAA assay for PJ detection. With its simplicity, rapidity and potential for point-of-care application, the RAA assay represents a significant advancement in the molecular diagnosis of PCP.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

HZ and BY performed the conceptual design, designed the primers and probes, and drafted the original manuscript. BY was responsible for methodology and performed the RAA experiments. KC collected the clinical BALF samples and clinical data, participated in the nucleic acid extraction experiments and critically revised the manuscript. FX performed the analytical performance evaluation of the RAA assay, analyzed the clinical validation data and contributed to writing the original manuscript. MQ quantified and analyzed the data, prepared the figures and tables, and critically revised the manuscript. YT conceived and designed the study, supervised the project, was responsible for project administration and funding acquisition, and reviewed and edited the manuscript. MQ and YT checked and confirmed the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Weifang No. 2 People's Hospital (approval no. KY2024-023-01; Weifang, China) and was performed in accordance with the Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, artificial intelligence tools were used to improve the readability and language of the manuscript, and subsequently, the authors revised and edited the content produced by the artificial intelligence tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

References

1. Arsić Arsenijevic V, Vyzantiadis TA, Mares M, Otasevic S, Tragiannidis A and Janic D: Diagnosis of pneumocystis jirovecii pneumonia in pediatric patients in serbia, greece, and romania. Current status and challenges for collaboration. J Fungi (Basel) 6: 49, 2020.
2. Lemiale V, Resche-Rigon M, Zerbib Y, Mokart D, De Prost N, Wallet F, Perez P, Kouatchet A, Argaud L, Decavèle M, *et al*: Adjunctive corticosteroids in non-AIDS patients with severe Pneumocystis jirovecii pneumonia (PIC): A multicentre, double-blind, randomised controlled trial. Lancet Respir Med 13: 800-808, 2025.
3. Lécuyer R, Issa N, Camou F, Lavergne RA, Gabriel F, Morio F, Canet E, Raffi F, Boutoille D, Cady A, *et al*: Characteristics and prognosis factors of pneumocystis jirovecii pneumonia according to underlying disease: A retrospective multicenter study. Chest 165: 1319-1329, 2024.
4. Chen YH, Fang XY, Li YT, Liu YL, Hang YP, Xiao YP, Cao XW, Zhong QS and Hu LH: Characterization of Pneumocystis jirovecii pneumonia at three tertiary comprehensive hospitals in southern China. Braz J Microbiol 51: 1061-1069, 2020.

5. Alshahrani MY, Alfaifi M, Ahmad I, Alkhathami AG, Hakami AR, Ahmad H, Alshehri OM and Dhakad MS: Pneumocystis Jirovecii detection and comparison of multiple diagnostic methods with quantitative real-time PCR in patients with respiratory symptoms. Saudi J Biol Sci 27: 1423-1427, 2020.
6. Bateman M, Oladele R and Kolls JK: Diagnosing Pneumocystis jirovecii pneumonia: A review of current methods and novel approaches. Med Mycol 58: 1015-1028, 2020.
7. Yin Y, Zhu P, Guo Y, Li Y, Chen H, Liu J, Sun L, Ma S, Hu C and Wang H: Enhancing lower respiratory tract infection diagnosis: implementation and clinical assessment of multiplex PCR-based and hybrid capture-based targeted next-generation sequencing. EBioMedicine 107: 105307, 2024.
8. Almeida-Siva F, Almeida-Paes R, Serra-Damasceno L, Motta-Santos E, Ferreira LC, Pereira-Quintella L, De Faria Ferreira M, De Medeiros-Muniz M and Zancopé-Oliveira RM: The conventional diagnosis challenge: Real-time PCR and nested PCR correlation with the scoring system for individuals at high-risk of Pneumocystis jirovecii pneumonia. Biomedica 43(Sp. 1): 255-266, 2023 (In English, Spanish).
9. Lagrou K, Chen S, Masur H, Viscoli C, Decker CF, Pagano L and Groll AH: Pneumocystis jirovecii disease: Basis for the revised EORTC/MSGERC invasive fungal disease definitions in individuals without human immunodeficiency virus. Clin Infect Dis 72 (Suppl 2): S114-S120, 2021.
10. White PL, Backx M and Barnes RA: Diagnosis and management of Pneumocystis jirovecii infection. Expert Rev Anti Infect Ther 15: 435-447, 2017.
11. Wills NK, Lawrence DS, Botsile E, Tenforde MW and Jarvis JN: The prevalence of laboratory-confirmed Pneumocystis jirovecii in HIV-infected adults in Africa: A systematic review and meta-analysis. Med Mycol 59: 802-812, 2021.
12. Ruiz-Ruiz S, Ponce CA, Pesantes N, Bustamante R, Gatti G, San Martin V, Gutierrez M, Bórquez P, Vargas SL, Magne F, *et al*: A real-time PCR assay for detection of low pneumocystis jirovecii levels. Front Microbiol 12: 787554, 2022.
13. Liu W, Li M, Xu Y, Wang F, Wang J, Wang H, Xu X, Wang Y and Sun H: Evaluation of the performance of a multiplex real-time PCR assay for the identification of aspergillus, cryptococcus neoformans, and pneumocystis jirovecii simultaneously from sputum in multicenter. Infect Drug Resist 15: 6009-6017, 2022.
14. Belanger CR, Locher K, Velapatino B, Dufresne PJ, Eckbo E and Charles M: Quick versus quantitative: Evaluation of two commercial real-time PCR assays for the detection of pneumocystis jirovecii from bronchoalveolar lavage fluids. Microbiol Spectr 11: e0102123, 2023.
15. Alsayed AR, Talib W, Al-Dulaimi A, Daoud S and Al Maqbali M: The first detection of Pneumocystis jirovecii in asthmatic patients post-COVID-19 in Jordan. Bosn J Basic Med Sci 22: 784-790, 2022.
16. Shrestha S, Addae A, Miller C, Ismail N and Zwerling A: Cost-effectiveness of targeted next-generation sequencing (tNGS) for detection of tuberculosis drug resistance in India, South Africa and Georgia: A modeling analysis. EClinicalMedicine 79: 103003, 2024.
17. Liu Y, Wu W, Xiao Y, Zou H, Hao S and Jiang Y: Application of metagenomic next-generation sequencing and targeted metagenomic next-generation sequencing in diagnosing pulmonary infections in immunocompetent and immunocompromised patients. Front Cell Infect Microbiol 14: 1439472, 2024.
18. Chen Z, Liu X, Tan L, Lyu X, Long Q, Wu W, Guo Z, Liu Z, Li Z and Hu M: Comparative of metagenomic and targeted next-generation sequencing in lower respiratory tract fungal infections. Front Cell Infect Microbiol 15: 1534519, 2025.
19. Wang H, Chen X, Wu X, Cao Q, Wu Y, Wang F, Wang Y, Zhou Y, Tang Y, Ren T and Wang M: Combined multiplex polymerase chain reaction-based targeted next-generation sequencing and serum 1, 3-β-D-glucan for differential diagnosis of Pneumocystis pneumonia and Pneumocystis colonization. Front Cell Infect Microbiol 15: 1611391, 2025.
20. Chen Q, Yi J, Liu Y, Yang C, Sun Y, Du J, Liu Y, Gu D, Liu H, Xu Y and Chen Y: Clinical diagnostic value of targeted next-generation sequencing for infectious diseases (Review). Mol Med Rep 30: 153, 2024.
21. Zhu H, Zhang H, Xu Y, Laššáková S, Korabečná M and Neužil P: PCR past, present and future. Biotechniques 69: 317-325, 2020.
22. Soroka M, Wasowicz B and Rymaszewska A: Loop-mediated isothermal amplification (LAMP): The better sibling of PCR? Cells 10: 1931, 2021.
23. Ngoc LTN and Lee YC: Current trends in RNA virus detection via nucleic acid isothermal amplification-based platforms. Biosensors (Basel) 14: 97, 2024.
24. Lai MY, Abdul Hamid MH, Jelip J, Mudin RN and Lau YL: Recombinase-aided loop-mediated isothermal amplification on human plasmodium knowlesi. Am J Trop Med Hyg 110: 648-652, 2024.
25. Park JW: Principles and applications of loop-mediated isothermal amplification to point-of-care tests. Biosensors (Basel) 12: 857, 2022.
26. Ahmadi Y, Yu Y, Cui Z, Huang WE and Andersson MI: Loop-mediated isothermal amplification (LAMP) for the diagnosis of sexually transmitted infections: A review. Microb Biotechnol 18: e70153, 2025.
27. Owoicho O, Olwal CO, Tettevi EJ, Atu BO and Durugbo EU: Loop-mediated isothermal amplification for Candida species surveillance in under-resourced setting: A review of evidence. Expert Rev Mol Diagn 22: 643-653, 2022.
28. Gieroń M, Żarnowiec P, Zegadło K, Gmiter D, Czerwonka G, Kaca W and Kręcisz B: Loop-mediated isothermal amplification of DNA (LAMP) as an alternative method for determining bacteria in wound infections. Int J Mol Sci 25: 411, 2023.
29. Acharya B, Acharya A, Gautam S, Ghimire SP, Mishra G, Parajuli N and Sapkota B: Advances in diagnosis of Tuberculosis: An update into molecular diagnosis of Mycobacterium tuberculosis. Mol Biol Rep 47: 4065-4075, 2020.
30. Srividya A, Maiti B, Chakraborty A and Chakraborty G: Loop mediated isothermal amplification: A promising tool for screening genetic mutations. Mol Diagn Ther 23: 723-733, 2019.
31. Munawar MA: Critical insight into recombinase polymerase amplification technology. Expert Rev Mol Diagn 22: 725-737, 2022.
32. Tan M, Liao C, Liang L, Yi X, Zhou Z and Wei G: Recent advances in recombinase polymerase amplification: Principle, advantages, disadvantages and applications. Front Cell Infect Microbiol 12: 1019071, 2022.
33. Feng X, Liu Y, Zhao Y, Sun Z, Xu N, Zhao C and Xia W: Recombinase polymerase amplification-based biosensors for rapid zoonoses screening. Int J Nanomedicine 18: 6311-6331, 2023.
34. Lu X, Cao Y, Zhang P, Chen X, Irwin DM and Shen Y: Visual recombinase aided amplification technology for detecting feline coronavirus. Vet J 312: 106356, 2025.
35. Li X, Zhu S, Zhang X, Ren Y, He J, Zhou J, Yin L, Wang G, Zhong T, Wang L, *et al*: Advances in the application of recombinase-aided amplification combined with CRISPR-Cas technology in quick detection of pathogenic microbes. Front Bioeng Biotechnol 11: 1215466, 2023.
36. Trinh TND and Nam NN: Isothermal amplification-based microfluidic devices for detecting foodborne pathogens: A review. Anal Methods 16: 1150-1157, 2024.
37. Yan C, Zhou Y, Du S, Du B, Zhao H, Feng Y, Xue G, Cui J, Gan L, Feng J, *et al*: Recombinase-aided amplification assay for rapid detection of hypervirulent klebsiella pneumoniae (hvKp) and Characterization of the hvKp pathotype. Microbiol Spectr 11: e0398422, 2023.
38. Duan X, Ma W, Jiao Z, Tian Y, Ismail RG, Zhou T and Fan Z: Reverse transcription-recombinase-aided amplification and CRISPR/Cas12a-based visual detection of maize chlorotic mottle virus. Phytopathol Res 4: 23, 2022.
39. Pang F and Long Q: Recent advances in diagnostic approaches for orf virus. Appl Microbiol Biotechnol 107: 1515-1523, 2023.
40. Zhang X, Guo L, Ma R, Cong L, Wu Z, Wei Y, Xue S, Zheng W and Tang S: Rapid detection of salmonella with recombinase aided amplification. J Microbiol Methods 139: 202-204, 2017.
41. Xiao X, Yang X, Xu K, Huang F, Zhang Y, Jiang Y, Shi Y, Zhou Q, Wang L, Lu J, *et al*: DNA fragment fusion and nucleic acid detection by fusion recombinase-aided amplification. Anal Chem 97: 6538-6547, 2025.
42. He Y, Chen W, Fan J, Fan S, Ding H, Chen J and Yi L: Recombinase-aided amplification coupled with lateral flow dipstick for efficient and accurate detection of porcine parvovirus. Life (Basel) 11: 762, 2021.
43. Wang S, Zhuang Q, Jiang N, Zhang F, Chen Q, Zhao R, Li Y, Yu X, Li J, Hou G, *et al*: Reverse transcription recombinase-aided amplification assay for avian influenza virus. Virus Genes 59: 410-416, 2023.
44. Ceruti A, Faye M, Diagne MM, Kobialka RM, Makiala-Mandanda S, Faye O, Faye O, El Wahed AA and Weidmann M: Rapid detection of Ebolavirus using isothermal recombinase-aided amplification. J Med Virol 96: e29744, 2024.

45. Wang K, Huang R, Zhang L, Liu D and Diao Y: Recombinase-aided amplification combined with lateral flow (LF-RAA) assay for Rapid AAV genome detection. ACS Omega 7: 47832-47839, 2022.
46. Lin H, Zhao S, Ye Y, Shao L, Jiang N and Yang K: A fluorescent recombinase aided amplification assay for detection of babesia microti. Korean J Parasitol 60: 201-205, 2022.
47. Liu N, Ning X, Zhang X, Zhou Z, Fu M, Wang Y and Wu T: Gradient galectin-1 coating technology: Bionic multichannel nerve guidance conduits promote neural cell migration. Adv Technol. Neurosci 1: 276-289, 2024.
48. Wang S, Li Y, Zhang F, Jiang N, Zhuang Q, Hou G, Jiang L, Yu J, Yu X, Liu H, *et al*: Reverse transcription recombinase-aided amplification assay for H5 subtype avian influenza virus. Virol J 19: 129, 2022.
49. Liu J, Qian W, Wang J, Bai Y, Gui Y, Xia L, Gong G, Ge F, Shen H, Chang X and Zhao H: A recombinase-aided amplification assay for the detection of chlamydia felis. Pol J Microbiol 72: 339-343, 2023.
50. Wang ZH, Zhang W, Zhang XZ, Yao XR, Huang W, Jia H, Liu XL, Hou SH and Wang XJ: Development of a real-time recombinase-aided amplification (RT-RAA) molecular diagnosis assay for sensitive and rapid detection of Toxoplasma gondii. Vet Parasitol 298: 109489, 2021.
51. Li Q, Wang N, Pang M, Miao H, Dai X, Li B, Yang X, Li C and Liu Y: Rapid and highly sensitive detection of mycobacterium tuberculosis utilizing the recombinase aided amplification-based CRISPR-Cas13a system. Microorganisms 12: 1507, 2024.
52. Samuel L: Point-of-care testing in microbiology. Clin Lab Med 40: 483-494, 2020.
53. Wang Y, Du P, Shao Y, Wang W, Liu Y, Ma Y, Hu P, Cao J, Wang X and Abd El-Aty AM: An innovative and efficient fluorescent detection technique for salmonella in animal-derived foods Using the CRISPR/Cas12a-HCR system combined with PCR/RAA. J Agric Food Chem 72: 8831-8839, 2024.
54. Zhang X, Ge X, Shen F, Qiao J, Zhang Y and Li H: Diagnostic efficiency of RPA/RAA integrated CRISPR-Cas technique for COVID-19: A systematic review and meta-analysis. PLoS One 17: e0276728, 2022.
55. Xiao X, Zhang Q, Wu S, Li Y, Zhong Z, Guo Q, Li J, Meng Q, Cheng Z, Duan J, *et al*: Detection of clostridium perfringens using novel methods based on recombinase-aided amplification assay-assisted CRISPR/Cas12a System. Transbound Emerg Dis 2023: 6667618, 2023.
56. Brown L, Alanio A, Cruciani M, Barnes R, Donnelly JP, Loeffler J, Rautemaa-Richardson R and White PL: Strengths and limitations of molecular diagnostics for Pneumocystis jirovecii pneumonia. Expert Rev Mol Diagn 24: 899-911, 2024.
57. Boldiš V, Ondriska F, Kováč Ľ, Steinhübel J and Bastlová M: High incidence of Pneumocystis jirovecii pneumonia in oncological patients: A 19-year study. Epidemiol Mikrobiol Imunol 72: 93-98, 2023.
58. Wang Y, Zhang X, Zhang Z, Xu R, Yang P, Yuan P, Zhang L, Cui Y, Xie Z and Li J: Reverse transcription recombinase-aided amplification assay combined with a lateral flow dipstick for detection of duck Tembusu virus. J Virol Methods 322: 114810, 2023.
59. Wu X, Liu Y, Gao L, Yan Z, Zhao Q, Chen F, Xie Q and Zhang X: Development and application of a reverse-transcription recombinase-aided amplification assay for porcine epidemic diarrhea virus. Viruses 14: 591, 2022.
60. Mu D, Zhou D, Xie G, Liu J, Xiong Q, Feng X and Xu H: The fluorescent probe-based recombinase-aided amplification for rapid detection of Escherichia coli O157:H7. Mol Cell Probes 60: 101777, 2021.
61. Plebani M, Nichols JH, Luppa PB, Greene D, Sciacovelli L, Shaw J, Khan AI, Carraro P, Freckmann G, Dimech W, *et al*: Point-of-care testing: State-of-the art and perspectives. Clin Chem Lab Med 63: 35-51, 2024.
62. Hansen GT: Point-of-care testing in microbiology: A mechanism for improving patient outcomes. Clin Chem 66: 124-137, 2020.
63. Gentilotti E, De Nardo P, Cremonini E, Górska A, Mazzaferri F, Canziani LM, Hellou MM, Olchowski Y, Poran I, Leeflang M, *et al*: Diagnostic accuracy of point-of-care tests in acute community-acquired lower respiratory tract infections. A systematic review and meta-analysis. Clin Microbiol Infect 28: 13-22, 2022.

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