



# Recombinase Polymerase Amplification Assay with Lateral Flow Strips for Rapid Detection of Candidiasis Due to *Candida parapsilosis*

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## Abstract

*Candida parapsilosis* is a common cause of candidiasis among hospitalized patients, often surpassing *Candida albicans*. Due to the recent increase in *C. parapsilosis* infections, there is an urgent need for rapid, sensitive, and real-time on-site detection of nucleic acids for timely diagnosis of candidiasis. We developed an assay for detection of *C. parapsilosis* by combining recombinase polymerase amplification (RPA) with a lateral flow strip (LFS). The RPA-LFS assay was used to amplify the beta-1,3-glucan synthase catalytic subunit 2 (*FKS2*) gene of *C. parapsilosis* with a primer–probe set optimized by introducing base mismatches (four bases modified by the probe and one by the reverse primer) to achieve specific and sensitive detection of clinical samples. The RPA assays can rapidly amplify and visualize a target gene within 30 min, while the entire process can be completed within 40 min by pre-processing the sample. The product of RPA has two chemical labels, FITC and Biotin, of the amplification product can be carefully on the strip. The sensitivity and specificity of the RPA-LFS assay were determined by analysis of 35 common clinical pathogens and 281 clinical samples against quantitative PCR. The results confirmed that the proposed RPA-LFS assay is a reliable molecular diagnostic method for the detection of *C. parapsilosis* to meet the urgent need for rapid, specific, sensitive, and portable field testing.

## Introduction

*Candida parapsilosis* is a common cause of candidiasis in low birth weight neonates and critically ill patients who are mainly infected during invasive procedures, such as central venous catheter placement, surgery, and parenteral nutrition [1, 2]. The incidence of invasive candidiasis has increased in

recent decades and the detection rate of candidiasis caused by *C. parapsilosis* exceeds that of *Candida albicans* in some hospitals in Europe, Asia, and South America [3]. Incidences of candidemia increased from 1.97 episodes/10,000 patient-days in 2008 to 4.59/10,000 patient-days in 2016, 30 days mortality was 32.4% [4].

Traditional fungal culture methods are the gold standard for the diagnosis of fungal infectious diseases. The continued development of molecular diagnostic techniques has been applied in the clinical detection of *C. parapsilosis* [5]. Among these techniques include polymerase chain reaction (PCR)-restriction fragment length polymorphism based on DNA amplification [6], random amplified polymorphic DNA [7], real-time PCR [8], PCR for intron length analysis of polymorphisms [9], matrix-assisted laser desorption ionization time-of-flight mass spectrometry [10], and sequencing analysis of pan-fungal markers. Meanwhile, isothermal amplification is more frequently utilized in field testing, as this method is not limited by instrumentation and laboratory settings. The main isothermal amplification techniques currently available include loop-mediated isothermal amplification (LAMP) [11], nuclear acid sequence-based amplification (NASBA) [12], rolling circle amplification (RCA) [13], single primer isothermal amplification [14] (SPIA),

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helicase-dependent isothermal DNA amplification (HDA) [15], and strand displacement amplification (SDA) [16]. Recombinase polymerase amplification (RPA) is a recently developed technique that merges the advantages of thermostatic amplification and compensating for the shortcomings as a rapid, specific, sensitive, and portable diagnostic assay.

RPA uses recombinase junctions that bind specific primers to open the double-stranded helix to bind to the target fragment, single-stranded DNA binding protein wraps single-stranded DNA with high affinity to protect against degradation and secondary structure formation, and the polymerase of *Bacillus subtilis* recognizes the 3' end of the primer for rapid amplification (Supplementary Fig. S1A and B), which can produce a large number of amplification products in about 20 min at 30–45 °C [17]. The amplification products of RPA can be detected by gel electrophoresis, a fluorescence detector, or with the use of a lateral flow strip (LFS). However, gel electrophoresis and fluorescence detection is limited to laboratory settings. In contrast, LFS is suitable for in situ detection in field conditions and the results can be directly observed without a dedicated display device or the need for sophisticated thermal cycling equipment and highly trained technicians. Visualization of a LFS is dependent on labeling of the 5' and 3' ends of the probe with fluorescein isothiocyanate (FITC) and C3 blocking sites, respectively, while the reverse primer is labeled with biotin. Binding of the probe to the amplification strand is performed by cleavage of the middle tetrahydrofuran (THF) site of the probe by endonuclease IV (nfo gene of *Escherichia coli* K-12) to release the 3' end of the probe for extension and the 5' and 3' ends of the final amplification product are labeled with FITC and biotin, respectively. Gold nanoparticle (AuNP)-labeled anti-FITC antibody binds to the amplification product and is subsequently captured and aggregated by streptavidin on the detection line to produce a red positive signal, while the secondary antibody on the quality control line captures the AuNP-labeled anti-FITC antibody without binding the amplification product and produces a red signal regardless of the presence or absence of the amplification product (Supplementary Fig. S1C) [18, 19]. The RPA-LFS assay can achieve fast response times and good accuracy for the diagnosis of various infectious diseases.

In our study, RPA and qPCR primers were designed for the diagnosis of *C. parapsilosis* by *FKS2* sequence. To test the effectiveness of the assay system, primer and probe specificity screening, sensitivity testing, specificity analysis, and testing of clinical samples was performed to ensure that our established method could be used for the diagnosis of *Candida* infections caused by *C. parapsilosis*.

## Methods

### Strain acquisition

*Candida parapsilosis* (*C. parapsilosis* means *C. parapsilosis* sensu stricto unless otherwise specified) ATCC 22019/90018/200954/7330 was purchased from Shanghai Covey Chemical Technology Co., Ltd. (Shanghai, China) and 15 strains of *C. parapsilosis* were isolated from clinical samples collected from 2020 to 2021, and the authenticity of the strain was verified by qPCR. The specificity of the RPA-LFS assay was verified based on the *FKS2* gene (GenBank: EU221326.1) of 35 common pathogens stored in our laboratory, which included *Candida tropicalis* ATCC 20962, *C. albicans* ATCC 10231, *Candida auris*, *Candida dubliniensis*, *Candida krusei*, *Candida glabrata*, *Aspergillus fumigatus*, *Cryptococcus neoformans* ATCC 14116, *Enterococcus faecium*, *E. coli* O157, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus capitis*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus saprophyticus*, *Staphylococcus warneri*, *Stenotrophomonas maltophilia*, *Streptococcus pneumoniae*, *Viridans streptococci*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* ATCC 1960, *Candida metapsilosis*, *Candida orthopsilosis*, *Cryptococcus gattii*, *Acinetobacter calcoaceticus*, *Acinetobacter lwoffii*, *Acinetobacter haemolyticus*, *Acinetobacter junii*, *Acinetobacter johnsonii*, *Enterobacter cloacae*, *Mycobacterium tuberculosis* H37Ra, *Listeria monocytogenes*, *Neisseria meningitidis*. In addition to isolating 15 strains of *C. parapsilosis* and 35 common pathogens stored in our laboratory, an additional 281 strains in total (Blood specimen 124, sputum specimen 107, pus specimen 28, dermal specimen 22) were collected from patients with suspected *Candida* infection.

### Genomic DNA extraction

All bacterial strains were boiled at 100 °C for 10 min to release DNA for use as templates and the concentration was checked by nucleic acid quantification (UV260/280 ratio). If not otherwise specified, 1 µl of 10<sup>7</sup> copies/µl of heat-treated culture was used as a template. For *C. parapsilosis* and other fungi, genomic DNA was extracted and purified from cultures or clinical samples using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) in accordance with the manufacturer's instructions and quantified using a Invitrogen™ Qubit™ 4 Fluorometer (Thermo Fisher Scientific).

### Object and Probe Design and Screening

Two primer pairs based on the *FKS2* gene were designed for RPA using Primer Premier 5 software (Premier Biosoft,

Palo Alto, CA, USA) with the following parameters: product size, 80–150 bp; primer size, 30–35 bp; complementary pairing,  $\leq 3$  consecutive bases at the 3' end; maximum hairpin fraction, 5; maximum primer-dimer fraction, 5; and maximum poly-X, 5. The primers were designed based on sequences retrieved from the National Center for Biotechnology Information (NCBI) database (<https://www.ncbi.nlm.nih.gov/>). The species specificity of the primers and probes were confirmed using the NCBI primer designing tool (<https://www.ncbi.nlm.nih.gov/tools/primer-blast>). The performance of the forward primer, which was extended backward by 15–23 bp, was evaluated to theoretically avoid the formation of dimers and hairpin structures using Primer Premier 5 software. The size of the probe was 46–53 bp with a GC content of 30–80% and melting temperature of 57–80 °C. The 5' end of the probe was labeled with FITC, the 3' end was closed with a C3 spacer, the bases in the middle of the probe (at least 30 bases before the THF site and at least 15 afterward) were replaced with THF, and the 5' end of the reverse primer was labeled with biotin.

### RPA Reaction

RPA reactions were performed using the TwistAmp® Liquid DNA Amplification Kit (TwistDx Inc., Maidenhead, UK) in accordance with the manufacturer's instructions. Each 50- $\mu$ l reaction system contained 25  $\mu$ l of 2 $\times$  reaction buffer, 5  $\mu$ l of 10 $\times$ E-mix, 2.5  $\mu$ l of 20/ core mix, 2.4  $\mu$ l of 10  $\mu$ M forward primer, 2.4  $\mu$ l of 10  $\mu$ M reverse primer, and 9.2  $\mu$ l of distilled water, in addition to 2.5  $\mu$ l of 280 mM magnesium acetate and 1  $\mu$ l of template to the top of the reaction tube. After a short centrifugation step, the reaction mixture was incubated at 37 °C for 30 min. The RPA amplification products were purified using the PCR Cleaning Kit (Shanghai MEIJI Biotechnology Co. Ltd.) and then separated by electrophoresis on a 2% agarose gel.

### RPA-LFS Assay

RPA reactions were performed using the Twist Amp® DNA amplification nfo kit (TwistDx Ltd.) in accordance with the manufacturer's instructions. Each 50- $\mu$ L reaction mixture contained 2.1  $\mu$ l of each primer (10  $\mu$ M), 0.6  $\mu$ l of the probe (10  $\mu$ M), 2.0  $\mu$ l of the template, and other standard reaction components. Primers and probes were synthesized by Anhui General Biotechnology Co., Ltd (Chuzhou, China). Magnesium acetate (280 mM, 2.5  $\mu$ l) was added to initiate the reaction prior to incubation of the reaction mixture at 37 °C for 20 min. Then, 5  $\mu$ l of the amplification product were diluted 20-fold and spotted on the LFS (USTAR Biotechnology Co., Ltd., Hangzhou, China). The LFS consisted of a sample pad, a gold-labeled antibody pad (soaked with mouse-derived AuNP-labeled anti-FITC antibody), a test

line (coated with streptavidin), a control line (coated with anti-mouse antibody), and an absorption pad, arranged by the solvent migration pathway. The RPA amplification product was added to the sample pad of the LFS and the LFS was submerged into 100  $\mu$ l of solvent for approximately 2 min until the test and control lines became visible.

### Sensitivity and Specificity of the RPA-LFS Assay

A tenfold gradient dilution was tested from  $5.0 \times 10^2$  to  $5.0 \times 10^7$  copies/ $\mu$ l (reaction volume of 50  $\mu$ l containing 1  $\mu$ l of *C. parapsilosis* inactivation solution) and  $10^7$  copies/ $\mu$ l of an inactivating solution of another common pathogen (*C. albicans*) were prepared for the RPA-LFS reaction. The lower limit of detection (LOD) of the method was determined with a probit regression analysis of 10 independent experiments. To verify the specificity of the assay system, 35 common clinical fungi and bacterial were selected for RPA-LFS testing.

### Quantitative PCR (qPCR) analysis

The primers and probe for qPCR analysis are listed in Table 1. Specific primers and probe were targeted to *FKS2* of *C. parapsilosis* for qPCR detection. Each qPCR reaction mixture consisted of 12.5  $\mu$ l of MonAmp™ Taqman qPCR mixture (Tiangen Biotechnology Co., Ltd., Beijing, China), 0.5  $\mu$ M forward and reverse primers, 0.2  $\mu$ M probe, 1  $\mu$ l of genomic DNA, and distilled water to a final volume of 25  $\mu$ l. The cycling program consisted of an initial denaturation step at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 55 °C for 60 s. The qPCR reactions were conducted using a LightCycler® 480 System (Roche Diagnostics GmbH, Mannheim, Germany).

### Conventional Culture

According to Author Wang et al. [20], the 281 clinical samples were incubated on Stachybotrys medium for 24–48 h and then further inoculated on Candida chromogenic medium. All clinical samples (original specimens) were tested simultaneously using three methods: RPA-LFS, qPCR, and conventional culture.

## Results

### Primer Screening and Validation

*FKS2* was selected from the *C. parapsilosis* genome as a target gene for RPA-LFS detection. NCBI Primer-BLAST yielded two potential primer pairs for amplification of *FKS2* (Supplementary Table 1). These primers were initially

**Table 1** Bacterial strains used in this study

Species	Source	Strain designation	qPCR	RPA-LFS	UV260/280 ratio (ng/μl)
<i>C. parapsilosis</i>	Reference strain	22,019	Positive	Positive	N/A
<i>C. parapsilosis</i>	Reference strain	90,018	Positive	Positive	N/A
<i>C. parapsilosis</i>	Reference strain	200,954	Positive	Positive	N/A
<i>C. parapsilosis</i>	Reference strain	7330	Positive	Positive	N/A
<i>C. parapsilosis</i>	Sputum isolated strain	#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #14 #15 #16 #17	Positive	Positive	N/A
<i>C. tropicalis</i>	Reference strain	ATCC 20,962	Negative	Negative	N/A
<i>C. albicans</i>	Reference strain	ATCC 10,231	Negative	Negative	N/A
<i>C. auris</i>	Sputum isolated strain	N/A	Negative	Negative	N/A
<i>C. dubliniensis</i>	Sputum isolated strain	N/A	Negative	Negative	N/A
<i>C. krusei</i>	Sputum isolated strain	N/A	Negative	Negative	N/A
<i>C. glabrata</i>	Sputum isolated strain	N/A	Negative	Negative	N/A
<i>A. fumigatus</i>	Sputum isolated strain	N/A	Negative	Negative	N/A
<i>C. neoformans</i>	Reference strain	ATCC 14,116	Negative	Negative	N/A
<i>E. faecium</i>	Sputum isolated strain	N/A	Negative	Negative	27.3
<i>E. coli</i> O157	Sputum isolated strain	N/A	Negative	Negative	±68.4
<i>P. aeruginosa</i>	Sputum isolated strain	N/A	Negative	Negative	±73.9
<i>S. aureus</i>	Sputum isolated strain	N/A	Negative	Negative	±26.3
<i>S. capitis</i>	Sputum isolated strain	N/A	Negative	Negative	±27.1
<i>S. epidermidis</i>	Sputum isolated strain	N/A	Negative	Negative	±25.7
<i>S. haemolyticus</i>	Sputum isolated strain	N/A	Negative	Negative	±31.3
<i>S. hominis</i>	Sputum isolated strain	N/A	Negative	Negative	±28.3
<i>S. saprophyticus</i>	Sputum isolated strain	N/A	Negative	Negative	±25..2
<i>S. warneri</i>	Sputum isolated strain	N/A	Negative	Negative	±25.8
<i>S. maltophilia</i>	Sputum isolated strain	N/A	Negative	Negative	±78.4
<i>S. pneumonia</i>	Sputum isolated strain	N/A	Negative	Negative	±32.7
<i>V. streptococci</i>	Sputum isolated strain	N/A	Negative	Negative	±28.3
<i>K. pneumoniae</i>	Sputum isolated strain	N/A	Negative	Negative	±68.4
<i>A. baumannii</i>	Reference strain	ATCC 19,606	Negative	Negative	±64.9
<i>C. metapsilosis</i>	Sputum isolated strain	N/A	Negative	Negative	N/A
<i>C. orthopsilosis</i>	Sputum isolated strain	N/A	Negative	Negative	N/A
<i>C. gattii</i>	Sputum isolated strain	N/A	Negative	Negative	N/A
<i>A.A.A.A.A.calcoaceticus</i>	Sputum isolated strain	N/A	Negative	Negative	±68.4
<i>A. lwoffii</i>	Sputum isolated strain	N/A	Negative	Negative	±71.5
<i>A.A.A.A.A.haemolyticus</i>	Sputum isolated strain	N/A	Negative	Negative	±75.3
<i>A. junii</i>	Sputum isolated strain	N/A	Negative	Negative	±69.7
<i>A.A.A.A.A.johnsonii</i>	Sputum isolated strain	N/A	Negative	Negative	±65.2
<i>E. cloacae</i>	Sputum isolated strain	N/A	Negative	Negative	±75.6
<i>M.M.M.M.M.tuberculosis</i> H37Ra	Sputum isolated strain	N/A	Negative	Negative	±54.7
<i>L. monocytogenes</i>	Sputum isolated strain	N/A	Negative	Negative	±32.8
<i>N. meningitidis</i>	Sputum isolated strain	N/A	Negative	Negative	±67.2

ATCC, American Type Culture Collection (Manassas, VA, USA)

screened by amplification of target gene fragments and a no template control (NTC). The amplification products were separated by electrophoresis on an agarose gel to compare the amplification performance of the target gene with the formation of primer-dimers by the NTC. The best amplification

performance was obtained with the primer pair F2/R2 and no primer dimer was formed (Fig. 1a). The candidate probe was obtained by extending the 3' end of the forward primer F2 by 16 bp. All possible cross-dimers generated by this probe and the reverse primer were predicted for subsequent

modification of the base sequence (Supplementary Table 1) until no dimer could be formed (Fig. 1b). As shown in the Fig. 1b, there are four potential risks of dimer formation in theory before base replacement. Finally, the five forward primers were screened and tested upstream of the probe. The LFS results showed that the primer/probe sets F3/P/R2B, F4/P/R2B, F5/P/R2B, and F6/P/R2B effectively amplified the target gene fragment, while the negative controls F3/P/R2B, F4/P/R2B, and F5/P/R2B (NTC) produced false-positive signals. Hence, only F6/P/R2B met the requirements of the assay and had effectively amplified the *FKS2* fragment without amplification as a NTC (Fig. 1c). Therefore, the primer/probe set F6/P/R2B was used in the subsequent experiments.

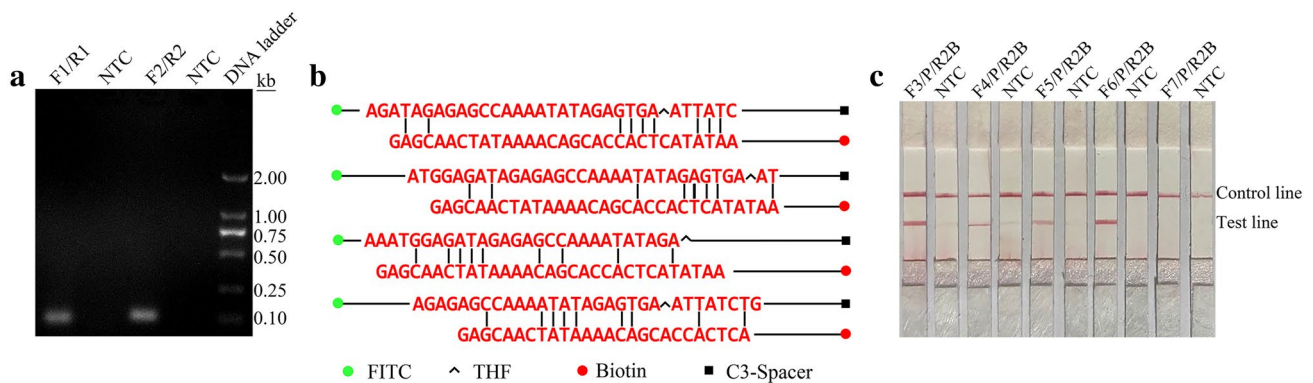
### RPA Reaction Temperature and LFS Chromogenic Time

The temperature parameters were set to 35–45 °C for 30 min. As shown in Fig. 2a, the signal on the test line appears on

each temperature gradient; the line is more obvious at 37 °C. Time parameters were set from 5 to 30 min. As shown in Fig. 2b, the pink test line appears at 10 min, and the line becomes clearer from 15 to 30 min. There is no significant difference between the 20 and 30 min lines. Therefore, a response time of 20 min was chosen for the RPA-LFS test of *C. parapsilosis*. Optimal conditions for *C. parapsilosis* RPA-LFS were determined to be incubation at 37 °C for 20 min.

### Sensitivity of the RPA-LFS Assay

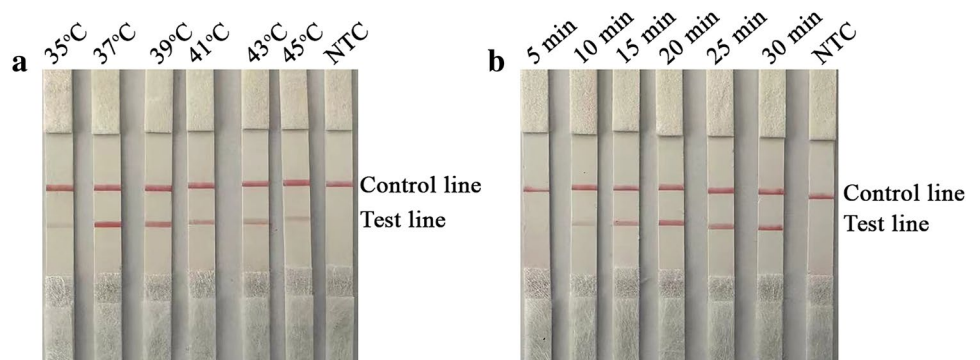
To determine the LOD of *C. parapsilosis*, a tenfold gradient dilution was tested from  $5.0 \times 10^2$  to  $5.0 \times 10^7$  copies/ $\mu$ l. A red band appeared on the test line at  $5.0 \times 10^2$  copies/ $\mu$ l. Moreover, the red band became darker with an increasing concentration of the *C. parapsilosis* solution (Fig. 3a). To determine whether the system is resistant to interference from other fungal DNA,  $5.0 \times 10^7$  copies/ $\mu$ l of *C. albicans* were added to the RPA reaction. The results showed that the *C. albicans* inactivation solution did not interfere with the



**Fig. 1** Screening of the primer–probe sets. **a** RPA results for two different primer sets targeting *FKS2*. The primer set is specified at the top of the corresponding lane. A NTC was used in all reactions, which were performed at 37 °C for 20 min. The results of three independent experiments are shown. **b** Pairwise analysis and sequence modification of the primer–probe sets for detection of *FKS2* using Primer Premier 5 software. The associated DNA base substitutions of

the probe and primers are shown. The DNA strands are shown as horizontal lines and the matching bases are indicated by vertical lines. Molecular markers are listed in (b). **c** Validity of the primer–probe sets for the RPA-LFS assay. The primer set is specified at the top of the corresponding lane. The positions of the test and control lines are shown on the right. All reactions were performed at 37 °C for 20 min. The results of three independent experiments are shown

**Fig. 2** RPA reaction temperature and LFS chromogenic time. **a** Performance of *C. parapsilosis* RPA-LFS at temperatures ranging from 35 to 45 °C. **b** Performance of *C. parapsilosis* RPA-LFS at times ranging from 5 to 30 min. NTC lane is a no template control



detection of *C. parapsilosis* (Fig. 3b). Hence, the LOD of the RPA-LFS system was  $5.0 \times 10^2$  copies/50  $\mu$ l per reaction and the presence of other fungi did not interfere with the detection sensitivity. However, not all assays yielded positive results when using  $5.0 \times 10^2$  (12.5%, 1/8 samples) and  $5.0 \times 10^3$  (75% positive results, 6/8 samples) copies, each sample had been finished ten independent experiments and the results of them were consistent. The results had been listed in Supplementary Table 2. To determine the exact LOD of the RPA-LFS assay, probit regression analysis of data from eight independent assays was performed using SPSS software (SPSS, Inc., Chicago, IL, USA). At 95% probability, the lowest LOD was  $5.85 \times 10^3$  copies per reaction (Fig. 3c).

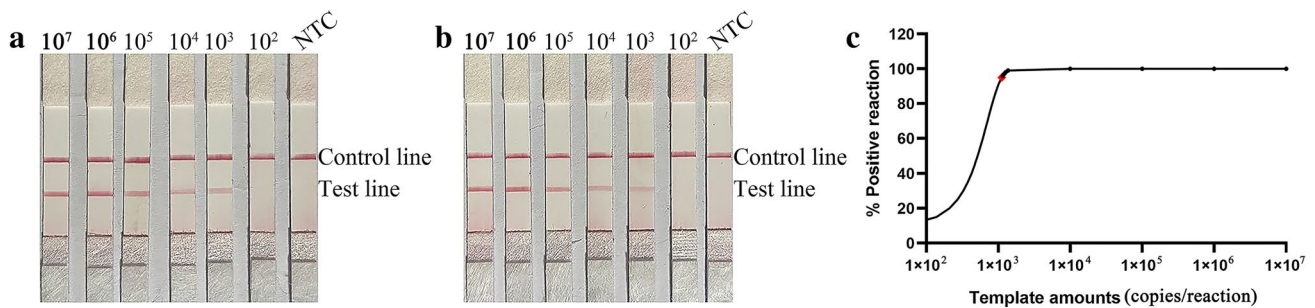
### Interspecies Specificity of the RPA-LFS Assay

To confirm the inclusivity and specificity of the primer–probe set F6/P/R2B, four reference strains, 17 clinical isolates, and other pathogenic bacteria were amplified (Table 1). We tested the nucleic acid concentration of

genomic DNA extracted by boiling method and carried out three technical and three biological repeats respectively. The results are shown in Supplementary Table 1. The four reference strains and 17 clinical isolates produced positive results (Fig. 4), while the results for all other pathogenic cultures were negative (Fig. 5a and b), indicating that the primer–probe set was specific for *C. parapsilosis* without cross-reactivity with other pathogens.

### Analysis of Clinical Samples

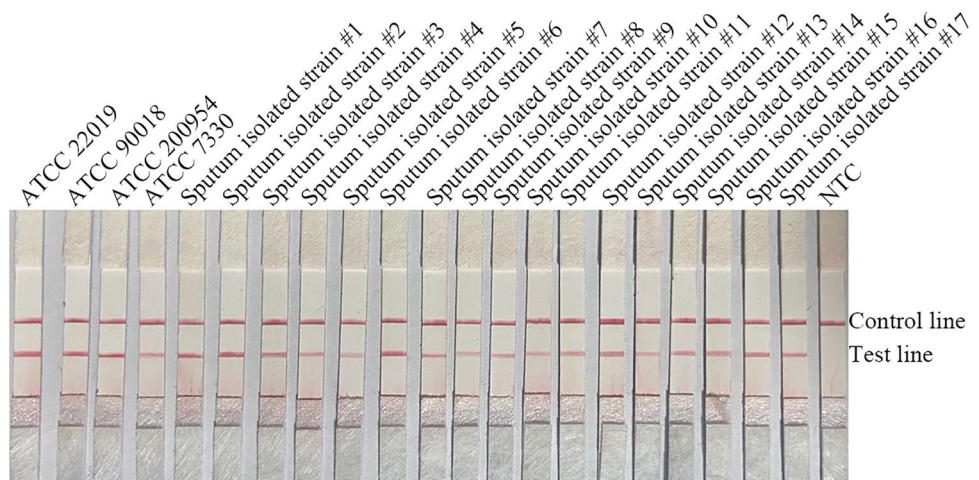
For verification, 281 clinical samples were collected for the RPA-LFS, qPCR, and traditional culture assays, respectively. The detection rate of 89 *C. parapsilosis* isolates of all the samples 281 clinical was 31.7%, consistent with the detection results of qPCR and traditional culture methods. The experimental results showed that the accuracy of the RPA-LFS assay was the same of that of qPCR and consistent with traditional culture methods.

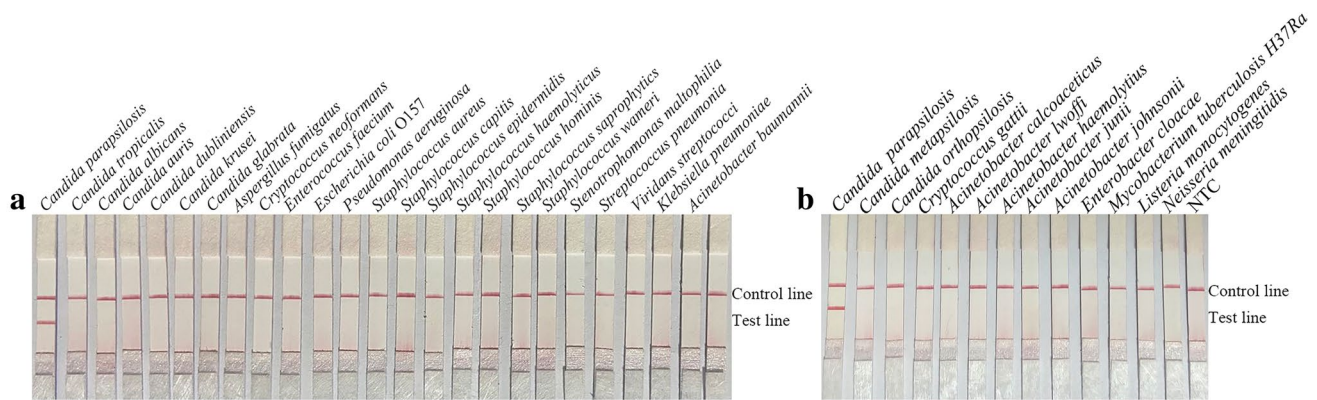


**Fig. 3** Determination of the LOD of the RPA-LFS assay for detection of *C. parapsilosis*. **a** The LOD of the *C. parapsilosis* RPA-LFS assay with the primer–probe set F6/P/R2B was determined from eight independent assays using an inactivating solution of *C. parapsilosis* with serial dilutions from  $5.0 \times 10^2$  to  $5.0 \times 10^7$  copies. The results of the

RPA-LFS assay are shown. The number of templates is indicated at the top of the bar graph. **b** The results of the RPA-LFS assay using primer–probe set F6/P/R2B and  $5.0 \times 10^7$  copies of *C. albicans* as interference. **c** Probit regression analysis of data collected from eight replicates was performed using SPSS software

**Fig. 4** Validation of the specificity of the primer–probe set F6/P/R2B for detection of *C. parapsilosis*. #1–#17 refers to 17 *C. parapsilosis* isolates from clinical samples. The positions of the test and control lines are marked on the right side of the bar graph. Reactions were performed at 37 °C for 20 min. The results of three independent experiments are shown. NTC, no template control





**Fig. 5** Specificity of the primer–probe set F6/P/R2B. **a, b** *C. parapsilosis* ATCC 22,019 was used as a positive control against other pathogenic bacteria. Species are identified at the top of each strip. The positions of the test and control lines are marked on the right side of

the bars. Reactions were performed at 37 °C for 20 min. The results of three independent experiments are shown. NTC, no template control

## Discussion

The incidence of hospital-acquired infections caused by *Candida* spp. continues to increase, most dramatically with *C. parapsilosis*, which is responsible for 10–25% of *Candida* infections of neonates and intensive care patients. *C. parapsilosis* infections are associated with the use of medical devices, especially central venous catheters, parenteral nutrition administration, and exposure to healthcare workers [21, 22].

In addition to traditional fungal culture methods to identify *C. parapsilosis*, molecular diagnosis based on nucleic acid detection is gaining importance, in particular, isothermal amplification technology and real-time qPCR technology. Isothermal amplification techniques can be divided into two categories based on the purpose of amplification: specific amplification and non-specific amplification. Besides RPA, LAMP, NASBA, SPIA, SDA, HDA, and RCA are also included. Among these amplification methods, only RPA is the only one that can automate isothermal amplification, while the others require DNA annealing and other necessary initial operations to achieve isothermal amplification, so RPA has a significant advantage in practical applications [14]. Real-time qPCR is specific and sensitive for large-scale detection of nucleic acids, especially for detection of novel coronaviruses, which are currently prevalent worldwide [23, 24]. RPA is tolerant of complex templates. Genomic DNA released by boiling method can be effectively detected. Release of DNA is more efficient with gram negative bacteria vs gram positive bacteria. Because the LOD of the RPA-LFS system was  $5.85 \times 10^3$  copies/50  $\mu$ l per reaction, the release of DNA of gram positive bacteria could not potentially impact the result of the assay. Due to the large-scale screening of nucleic acids conducted in various locations, the demand for laboratory testing by trained professionals

continues to increase. In addition, most screening methods require expensive instruments for nucleic acid extraction and amplification. Moreover, aerosol contamination is a potential problem associated with testing of nucleic acids in a laboratory setting.

RPA is an effective complement to qPCR for testing of nucleic acids and can be used for home testing of patients or on-site testing in harsh conditions. Notably, this method allows for exponential amplification of nucleic acids at 37 °C without the need for expensive thermal sequencing instruments and professional laboratory personnel. Enzyme-linked immunosorbent assays are suitable for in vitro quantitative detection of proteins as markers of pathogens in cell cultures, serum, plasma, and other biological fluids. The double antibody sandwich method to detect antigenic proteins employs a microtiter plate with wells coated with a high-affinity biotinylated antibody for detection of the sample or standard. The presence of the target protein in the sample is detected by a colorimetric reaction with a substrate solution that is terminated by the addition of a termination solution [25]. However, this method does not allow effective detection at the early stage of disease, thus delaying prognosis and threatening the health of the patient. To compensate for this shortcoming, RPA is a highly sensitive, rapid, specific, and portable method for detection of *C. parapsilosis*.

Molecular detection techniques require the selection of diagnostic amplification targets to effectively detect specific species. Many studies have evaluated various methods for detection of *C. parapsilosis*. In the present study, a primer–probe set was designed to target *FKS2* of *C. parapsilosis*. The results showed that there was no significant effect on the LOD for base mismatching and that the RPA-LFS assay accurately detected *C. parapsilosis*. The LOD of the RPA-LFS assay was  $5.85 \times 10^3$  copies, which was more sensitive than qPCR at  $10^2$ – $10^3$  copies per reaction. In addition,

the RPA-LFS assay for detection of *C. parapsilosis* is simple and rapid, as the assay can be performed in 30 min or less. In addition, this method requires an isothermal temperature of only 37 °C, whereas PCR, qPCR, and loop-mediated isothermal amplification require temperature control equipment and relatively long reaction times. Evaluation of clinical samples showed that the RPA-LFS assay was specific. Testing of samples from different patients showed that the results of the RPA-LFS and qPCR assays were comparable, demonstrating that the RPA-LFS assay is a rapid and useful alternative. The detection system we have established for *C. parapsilosis* is consistent with many literature reports, featuring fast, portable, and instrument-independent characteristics. However, there is also a risk of aerosol contamination in the laboratory, which can be largely avoided by home testing.

## Conclusions

The RPA-LFS assay developed in this study is a rapid highly specific and sensitive molecular technique for diagnosis of *C. parapsilosis* infection. Importantly, the test results are available within 40 min without the use of expensive instruments or trained laboratory personnel. Since testing can be conducted on-site, the proposed assay is useful for rapid detection of *Candida* spp. The established RPA-LFS assay is simple, rapid, and accurate, does not require laboratory facilities, and can be combined with a minimal and rapid DNA extraction method for home detection of *C. parapsilosis* infections for timely diagnosis to facilitate early treatment.

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**Author Contributions** LZ, KW, and CC designed the experiments and wrote the manuscript. BZ and KW collected the clinical samples. BZ, LW, and YYL performed the main experiments. BZ analyzed the data. All authors reviewed and approved the final version of the manuscript.

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**Data Availability** The data presented in this study are included in the article. Further inquiries should be directed to the corresponding authors.

## Declarations

**Competing interest** The authors declare that they have no competing interests.

**Ethics Approval** The study protocol was approved by the Medical Ethics Committee of the Second People's Hospital of Lianyungang City

(Lianyungang, Jiangsu, China) (permit number 2020013) and informed consent was obtained from patients prior to collection of clinical samples. All methods were carried out in accordance with relevant guidelines and regulations.

**Consent for Publication** Not applicable.

## References

1. Yacoub A, Moreland S, Jani D, Nanjappa S, Quilitz R, Carraway S, Sandin R, Greene J (2016) Candidemia in cancer patients: a retrospective analysis at a cancer center from 2001 to 2014. *Infect Dis Clin Pract* 24(5):273–277
2. Toth R, Toth A, Vagvolgyi C, Gacser A (2017) *Candida parapsilosis* secreted lipase as an important virulence factor. *Curr Protein Pept Sci* 18(10):1043–1049. <https://doi.org/10.2174/1389203717666160813163054>
3. Tóth R, Nosek J, Mora-Montes HM, Gabaldon T, Bliss JM, Nosanchuk JD, Turner SA, Butler G, Vágvolgyi C, Gácsér A (2019) *Candida parapsilosis*: from genes to the bedside. *Clin Microbiol Rev*. <https://doi.org/10.1128/CMR.00111-18>
4. Mesini A, Mikulska M, Giacobbe DR, Del Puente F, Gandolfo N, Codda G, Orsi A, Tassinari F, Beltramini S, Marchese A, Icardi G, Del Bono V, Viscoli C (2020) Changing epidemiology of candidaemia: increase in fluconazole-resistant *Candida parapsilosis*. *Mycoses* 63(4):361–368. <https://doi.org/10.1111/myc.13050>. (Epub 2020 Feb 20)
5. Guinea J, Mezquita S, Gómez A, Padilla B, Zamora E, Sánchez-Luna M, Sánchez-Carrillo C, Muñoz P, Escribano P (2021) Whole genome sequencing confirms *Candida albicans* and *Candida parapsilosis* microsatellite sporadic and persistent clones causing outbreaks of candidemia in neonates. *Med Mycol*. <https://doi.org/10.1093/mmy/myab068>
6. Neji S, Trabelsi H, Hadrich I, Cheikhrouhou F, Sellami H, Makni F, Ayadi A (2017) Molecular study of the *Candida parapsilosis* complex in Sfax, Tunisia *Med Mycol* 55(2):137–144. <https://doi.org/10.1093/mmy/myw063>
7. Al-Tekreeti A, Al-Halbosiy M, Dheeb B, Hashim A, Al-Zuhairi A, Mohammad F (2018) Molecular identification of clinical *Candida* isolates by simple and randomly amplified polymorphic DNA-PCR. *Arab J Sci Eng* 43(1):163–170. <https://doi.org/10.1007/s13369-017-2762-1>
8. Souza A, Ferreira R, Gonçalves S, Quindós G, Eraso E, Bizerra F, Briones M, Colombo A (2012) Accurate identification of *Candida parapsilosis* (sensu lato) by use of mitochondrial DNA and real-time PCR. *J Clin Microbiol* 50(7):2310–2314. <https://doi.org/10.1128/JCM.00303-12>
9. Feng X, Wu Z, Ling B, Pan S, Liao W, Pan W, Yao Z (2014) Identification and differentiation of *Candida parapsilosis* complex species by use of exon-primed intron-crossing PCR. *J Clin Microbiol* 52(5):1758–1761. <https://doi.org/10.1128/JCM.00105-14>
10. Ferreira L, Sánchez-Juanes F, Porrás-Guerra I, García-García M, García-Sánchez J, González-Buitrago J, Muñoz-Bellido J (2011) Microorganisms direct identification from blood culture by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Clin Microbiol Infect* 17(4):546–551. <https://doi.org/10.1111/j.1469-0691.2010.03257.x>
11. Fallahi S, Babaei M, Rostami A, Mirahmadi H, Arab-Mazar Z, Sepahvand A (2020) Diagnosis of *Candida albicans*: conventional diagnostic methods compared to the loop-mediated isothermal amplification (LAMP) assay. *Arch Microbiol* 202(2):275–282. <https://doi.org/10.1007/s00203-019-01736-7>



12. Huang C, Huang P, Yao J, Li Z, Weng L, Guo X (2019) Pooled analysis of nuclear acid sequence-based amplification for rapid diagnosis of *Mycoplasma pneumoniae* infection. *J Clin Lab Anal* 33(5):e22879. <https://doi.org/10.1002/jcla.22879>
13. Carinelli S, Kühnemund M, Nilsson M, Pividori M (2017) Yoctomole electrochemical genosensing of Ebola virus cDNA by rolling circle and circle to circle amplification. *Biosens Bioelectron* 93:65–71. <https://doi.org/10.1016/j.bios.2016.09.099>
14. Yang H, Wang Y, Yang Q, Fan H, Wang L, Zhang T, Li Z, Liu G, Zhao P, Wu H (2021) A rapid and sensitive detection method for *Pseudomonas aeruginosa* using visualized recombinase polymerase amplification and lateral flow strip technology. *Front Cell Infect Microbiol* 11:698929. <https://doi.org/10.3389/fcimb.2021.698929>
15. Jeong Y, Park K, Kim D (2009) Isothermal DNA amplification in vitro: the helicase-dependent amplification system. *Cellular Mol Life Sci: CMLS* 66(20):3325–3336. <https://doi.org/10.1007/s00018-009-0094-3>
16. Zhang M, Li R, Ling L (2017) Homogenous assay for protein detection based on proximity DNA hybridization and isothermal circular strand displacement amplification reaction. *Anal Bioanal Chem* 409(16):4079–4085. <https://doi.org/10.1007/s00216-017-0356-0>
17. Piepenburg O, Williams C, Stemple D, Armes N (2006) DNA detection using recombination proteins. *PLoS Biol.* 4(7):e204. <https://doi.org/10.1371/journal.pbio.0040204>
18. Lee H, Yi S, Kwon J, Choi J, Lee D, Lee S, Shin Y (2021) Rapid and highly sensitive pathogen detection by real-time DNA monitoring using a nanogap impedimetric sensor with recombinase polymerase amplification. *Biosens Bioelectron* 179:113042. <https://doi.org/10.1016/j.bios.2021.113042>
19. Yang X, Xie J, Hu S, Zhan W, Duan L, Chen K, Zhang C, Yin A, Luo M (2020) Rapid and visual detection of *enterovirus* using recombinase polymerase amplification combined with lateral flow strips. *Sens Actuators B: Chem.* 311:127903. <https://doi.org/10.1016/j.snb.2020.127903>
20. Wang K, Huo L, Li Y, Zhu L, Wang Y, Wang L (2022) Establishment of a rapid diagnosis method for *Candida glabrata* based on the ITS2 gene using recombinase polymerase amplification combined with lateral flow strips. *Front Cell Infect Microbiol.* 12:953302. <https://doi.org/10.3389/fcimb.2022.953302>
21. Arıkan K, Arıkan-Akdaglı S, Kara A (2019) 183. Candidaemia in children and importance of central venous catheter removal. *Open Forum Infect Dis* 6(Supplement 2):S112–S113. <https://doi.org/10.1093/ofid/ofz360.258>
22. Goemaere B, Becker P, Van Wijngaerden E, Maertens J, Spriet I, Hendrickx M, Lagrou K (2018) Increasing candidaemia incidence from 2004 to 2015 with a shift in epidemiology in patients pre-exposed to antifungals. *Mycoses* 61(2):127–133. <https://doi.org/10.1111/myc.12714>
23. Kim H, Schuck A, Lee S, Lee Y, Kang M, Kim Y (2021) Sensitive electrochemical biosensor combined with isothermal amplification for point-of-care COVID-19 tests. *Biosens Bioelectron* 182:113168. <https://doi.org/10.1016/j.bios.2021.113168>
24. Shelite T, Uscanga-Palomeque AC, Castellanos-Gonzalez A, Melby PC, Travi BL (2021) Isothermal recombinase polymerase amplification-lateral flow detection of SARS-CoV-2, the etiological agent of COVID-19. *J Virological Methods.* 296:114227. <https://doi.org/10.1016/j.jviromet.2021.114227>
25. Tsurusawa N, Chang J, Namba M, Makioka D, Yamura S, Iha K, Kyosei Y, Watabe S, Yoshimura T (2021) Modified ELISA for ultrasensitive diagnosis. *J Clin Med* 10(21):5197. <https://doi.org/10.3390/jcm10215197>

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