Applied Microbiology and Biotechnology

Dual One-step Recombinase-aided PCR for Rapid Detection of *Candida* in Blood

Supplementary Material

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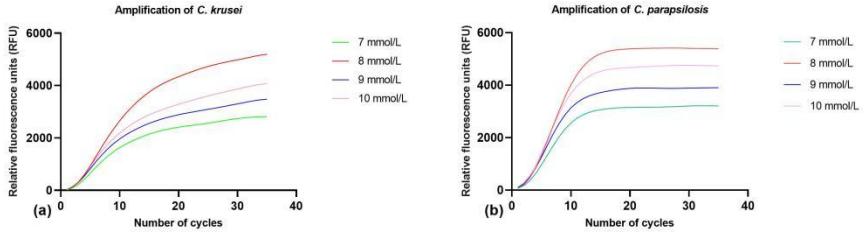
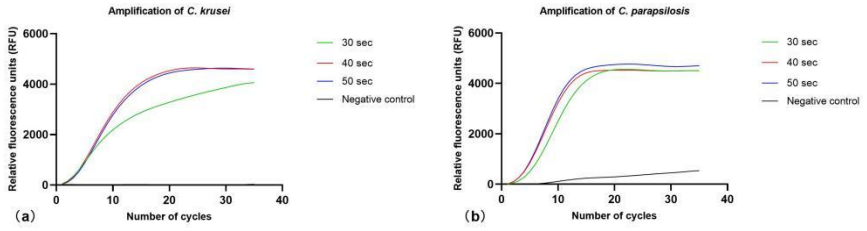
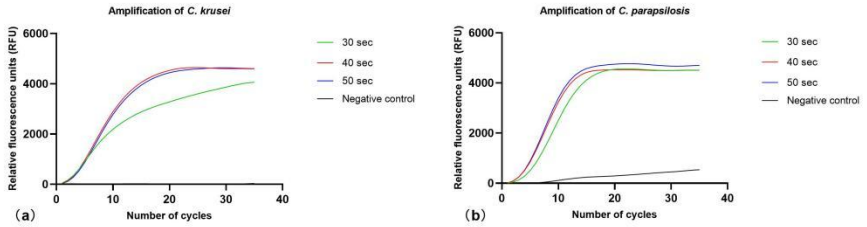


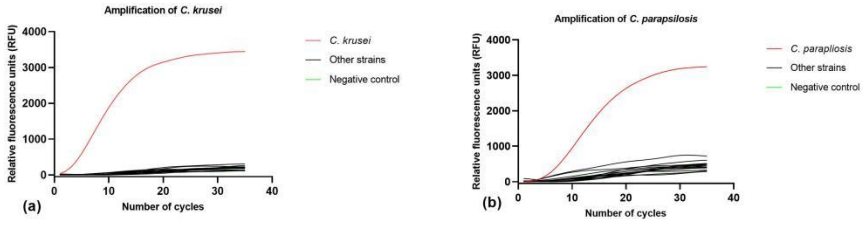
Supplementary Fig. S1 (a) Optimization of magnesium concentration for DO-RAP for *C. krusei* detection
(b) Optimization of magnesium concentration for DO-RAP for *C. parapsilosis* detection



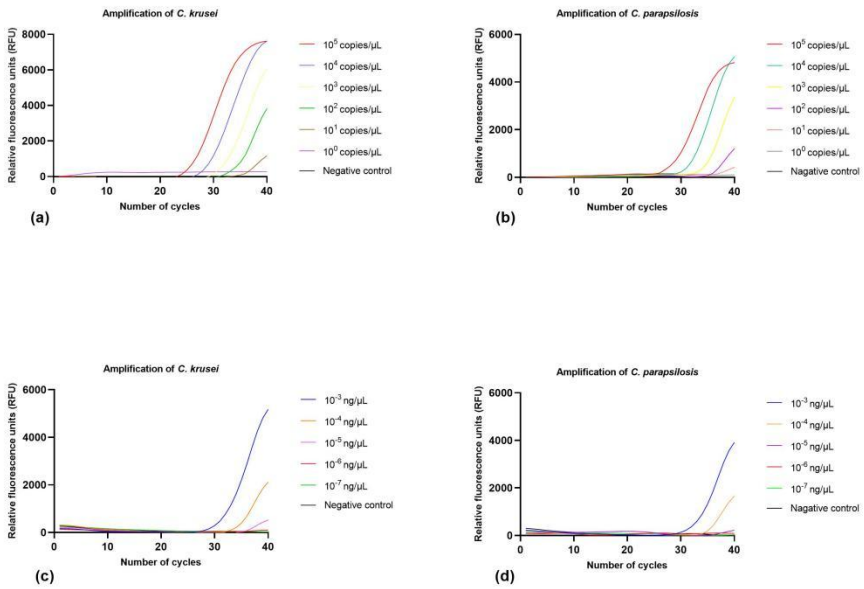
Supplementary Fig. S2 (a) Optimal PCR annealing time for DO-RAP for *C. krusei* detection
(b) Optimal PCR annealing time for DO-RAP for *C. parapsilosi*s detection



Supplementary Fig. S3 (a) Optimal RAA reaction time for DO-RAP for *C. krusei* detection
(b) Optimal RAA reaction time for DO-RAP for *C. parapsilosis* detection



Supplementary Fig. S4 Specificity evaluation
Evaluation of the specificity of DO-RAP for the detection of *C. krusei* (a) and *C. parapsilosis* (b).



Supplementary Fig. S5 Quantitative PCR sensitivity analysis

The sensitivity of detecting *C. krusei* and *C. parapsilosis* was evaluated using two different templates: plasmid (10^1 copies/μL for both *C. krusei* in (a) and *C. parapsilosis* in (b)) and genomic DNA (10^{-5} ng/μL for *C. krusei* in (c) and 10^{-4} ng/μL for *C. parapsilosis* in (d)).

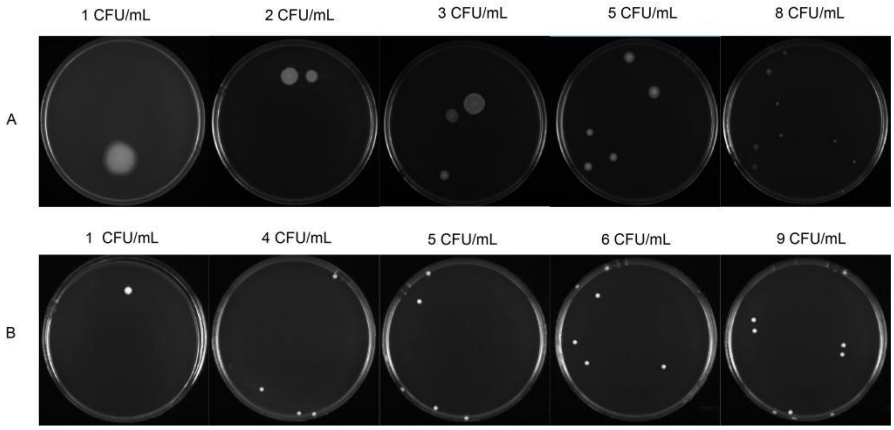
Supplementary Table S1 Bacterial and *Candida* strains used in the specificity test

Strains	Origin	DO-RAP (*C. krusei*)	DO-RAP (*C. parapsilosis*)
Candida krusei	ATCC 6258	Pos (8/8[a])	Neg (0/8)
Candida parapsilosis	ATCC 22019	Neg (0/8)	Pos (8/8)
Candida albicans	ATCC 753	Neg (0/8)	Neg (0/8)
Candida tropicalis	ATCC 750	Neg (0/8)	Neg (0/8)
Candida glabrata	ATCC 2001	Neg (0/8)	Neg (0/8)
Staphylococcus aureus	ATCC 29213	Neg (0/8)	Neg (0/8)
Pseudomonas aeruginosa	ATCC 27853	Neg (0/8)	Neg (0/8)
Klebsiella pneumoniae	ATCC 11296	Neg (0/8)	Neg (0/8)
Escherichia coli	ATCC 25922	Neg (0/8)	Neg (0/8)
Staphylococcus epidermidis	ATCC 35984	Neg (0/8)	Neg (0/8)
Enterococcus faecium	ATCC 19434	Neg (0/8)	Neg (0/8)
Enterococcus faecalis	ATCC 29212	Neg (0/8)	Neg (0/8)
Enterobacter cloacae	ATCC 13047	Neg (0/8)	Neg (0/8)
Streptococcus pneumoniae	ATCC 6303	Neg (0/8)	Neg (0/8)
Mycobacterium tuberculosis	ATCC 25177	Neg (0/8)	Neg (0/8)
Listeria monocytogenes	ATCC 19111	Neg (0/8)	Neg (0/8)

Note. [a]The first number indicates the number of times a positive result was obtained. The second number indicates the number of times a negative result was obtained. Neg, negative; Pos, positive; DO-RAP, dual one-step recombinase-aided PCR.

Supplementary Table S2 The reproducibility of DO-RAP

Standard DNA (copies/μL)	*C. krusei*	*C. parapsilosis*
10^5	8/8	8/8
10^4	8/8	8/8
10^3	8/8	8/8
10^2	8/8	8/8
10^1	8/8	8/8
10^0	7/8	6/8



Supplementary Fig. S6 Colony count after culturing <10 CFU/mL PBS simulated samples on Sabouraud agar of *C. krusei* (A) and *C. parapsilosis* (B)