A novel processing technology for simple and rapid detection of Candida albicans and fungal pathogens in blood by qPCR, opening the field for new molecular diagnosis of candidiasis.

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INTRODUCTION

Candida species are common human commensals that can cause disseminated infection. In fact, *Candida albicans* (the major cause of invasive candidiasis), has become one of the pathogens most frequently isolated from the blood of postoperative and immunocompromised patients during the last decade (1, 2). However, diagnosis remains difficult and tests have been developed to detect circulating Candida antigens for rapid diagnosis of disseminated candidiasis, but no PCR-based test has set the standard for molecular detection yet (3). In this work, a rapid system for blood processing is described that allows extraction and stabilisation of all fungal genomic targets. This novel technology has produced a proof of concept by teaming up with a new specific and sensitive qPCR test for the timely and accurate diagnosis Candida and concomitant fungal infections (see Table 1). A finer analysis within a small cohort of clinical specimens has allowed to set a basic LOD on par with blood culture, requiring a total time of 3 hours to complete the entire process (extraction and detection).

RESULTS

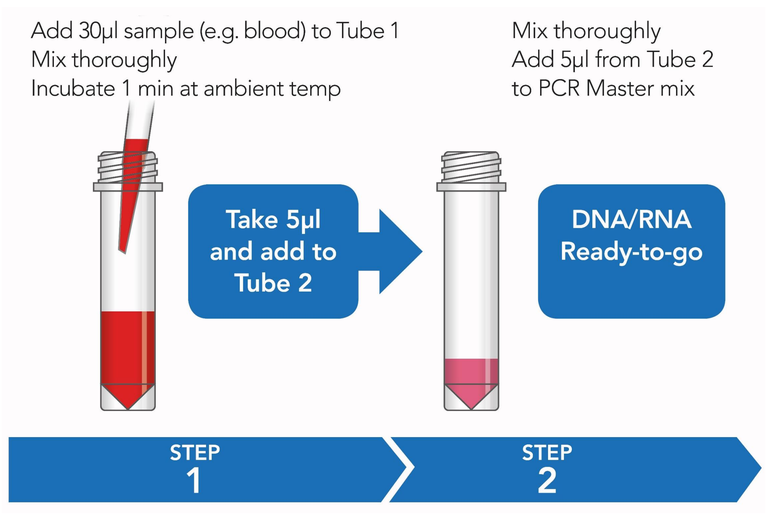

Figure 1: Rapid sample processing protocol. See 'Methods' for further detail

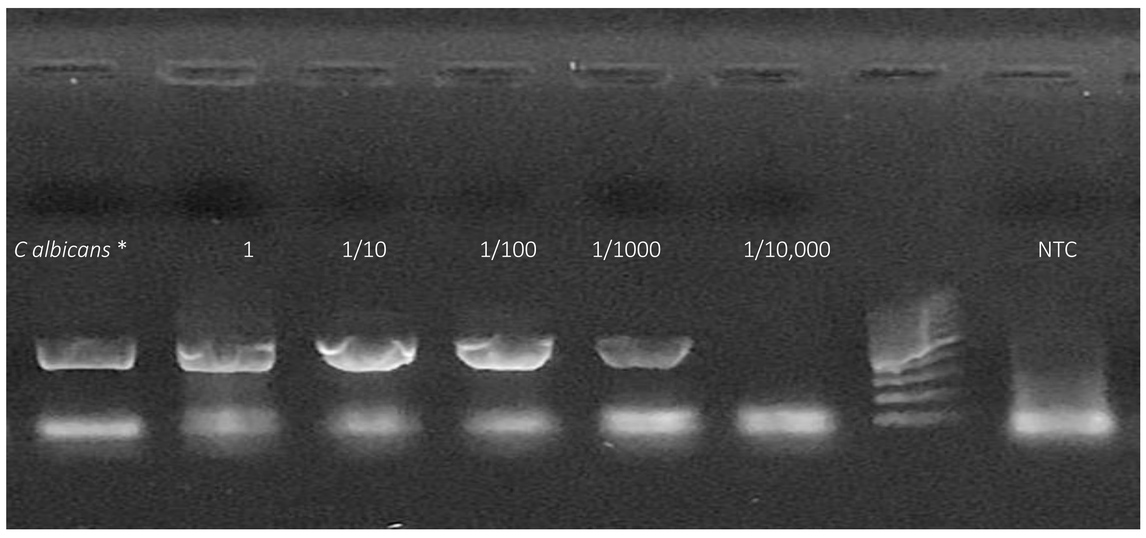

Figure 2:End point PCR results. A dilution series of C. albicans spiked into whole blood

Micro and macro identification (Microbiology)	PCR	Sequencing results from ARCIS extraction
C. albicans (958)	+	*C. albicans, C. dublinensis, C. parapsilosis*
A. fumigatus(959)	+	*A. sojae, A tamarii, A.flavus, A.fumigatus*
Candida spp (7)	+	*C. albicans, C. dublinensis*
A. flavus (8)	+	*A. sojae, A tamarii, A.flavus, A.fumigatus,*
A. niger (10)	+	*A. niger, A terreus, A thuringiensis*
Penicillium sp. (11)	+	*A. sydowii, A. versicolor, Ascomycota, A. terreus*
Alternaria (12)	+	*Alternaria alternata*
Trichophyton rubrum (13)	+	*T. rubrum, T.soudanense, T. mentagrophytes*
Trichophyton rubrum (14)	+	*T. rubrum, T. schoenleinii T.soudanense*

Table 1: Sequencing analysis. All samples were processed rapidly and subjected to PCR and Pyrosequencing. All samples were PCR positive and correctly identified by sequencing analysis.

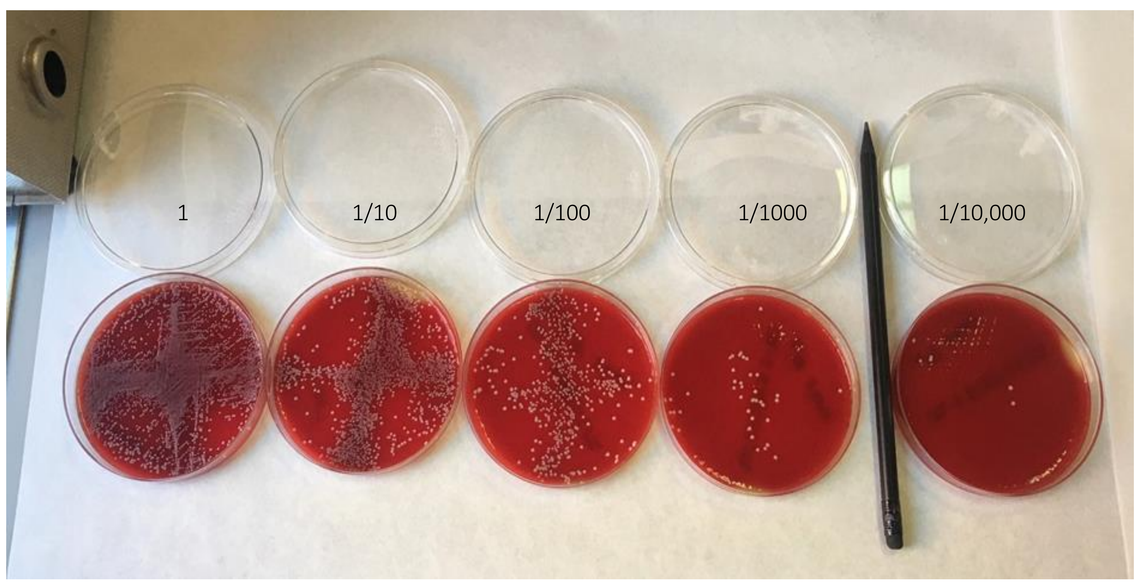

Figure 3: Colony counting on agar plates. A dilution series of C. albicans spiked into whole blood, plated and counted by eye.

LOD calculation: 43 colonies counted at 1/1000 dilution from plating 5µl of inoculated blood, hence the dilution contained 43/5= approx./ 8 CFUs/ µl

MINIMUM CONCENTRATION OF CANDIDA IN BLOOD DETECTABLE BY THE PROCEDURE (1/1000 diluted stock) = 8 CFUs/ µl

30µl of that spiked dilution of blood with 8 CFUs/ µl were added to ARCIS extraction mix (Vf 200µl) accounting for (30µl x8 CFUs/ µl) / 200 µl = 1.2 CFUs/ µl in the final extract. 5µl of ARCIS extract were added to the qPCR, 1.2 CFUs/ µl x 5µl = 6 CFUs

MINIMUM AMOUNT OF TARGET NEEDED FOR THE PCR TO PRODUCE A POSITIVE RESULT (analytical limit of detection for the PCR reaction) = 6CFUs

METHODS

1. A 2-McFarland dilution of C. albicans in water was produced as starting point.
2. 150µl of this titrated dilution was added to 150µl of fresh peripheral blood to generate the Dilution 1.
3. A serial dilution in fresh peripheral blood was produced to reach log1 to log4 (ie. 1/10,000) to generate Dilution 2, 3 and 4.
4. Each dilution was plated for counting adding 5µl of each dilution to blood agar plates and incubated for 48h at 37 deg. C
5. Each dilution was extracted adding 30µl of spiked blood + 170µl of ARCIS Reagent 1. Incubating 10' at room temperature with vortex mixing at the start, middle and end of the incubation
6. Extracts were washed with ARCIS washing solution (Reagent 2)
7. 5µl of washed extracts were added to ARCIS fungal detection kit*.

*Detailed description in ***ADVANCED ALTERNATIVES****; New technology for easy extraction of sequence-able DNA from medically relevant fungi and induction of competence in top-fermenting strains*. Innovations in Pharmaceutical Technology, number 62.

CONCLUSIONS

Results shown above were obtained using clinical fungi specimens, supporting two main conclusions:

ARCIS extraction via a modification of the ultra-fast sample prep process provides fungal genetic material that is fully compatible with end-point PCR,and qPCR showing an analytical LOD in blood of 6 CFUs.

All fungal amplification products obtained are also fully compatible with sequencing and pyrosequencing, allowing a double-validation within this study.

REFERENCES

1. Burgener-Kairuz P, Zuber J P, Jaunin P, Buchman T G, Bille J, Rossier M. Rapid detection and identification of Candida albicans and Torulopsis (Candida) glabrata in clinical specimens by species-specific nested PCR amplification of a cytochrome P-450 lanosterol-α-demethylase (L1A1) gene fragment. J Clin Microbiol. 1994;32:1902–1907. 2. Burnie J P, Golbang N, Mathews R C. Semiquantitative polymerase chain reaction enzyme immunoassay for diagnosis of disseminated candidiasis. Eur J Clin Microbiol Infect Dis. 1997;16:346–350 3. Chryssanthou E, Anderson B, Petrint B, Lofdahl S, Tollemar J. Detection of Candida albicans DNA in serum by polymerase chain reaction. Scand J Infect Dis. 1994;26:479–485.