Evaluation of new multiplex PCR-based blood assays for detection of candidemia

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Background - Aim

Blood cultures have been considered as diagnostic gold standard for candidemia. Compared to cultures, assays based on polymerase chain reaction (PCR) may be useful to shorten the time to diagnosis of invasive candidiasis and to initiation of antifungal therapy. Recently, the new *Cand*ID® and *Cand*ID PLUS® kits (OLM Diagnostics, Newcastle Upon Tyne, England) for molecular detection of different *Candida spp*. have been developed. In this study, the analytical and clinical performance of the new kits was investigated. Reference material and clinical specimens were used.

Methods

Nucleic acid extraction was performed on the EMAG® platform (bioMérieux, Marcy-l´Etoile, France; Figure 1a) using the specific B protocol. Real-time PCR (qPCR) and detection with the *Cand*ID® and *Cand*ID PLUS® kits were performed on the Light Cycler® 480 II CE/IVD instrument (Roche Diagnostics, Penzberg, Germany; Figure 1b). Both of the kits are based on multiplex qPCR providing detection of three different *Candida spp*. each and an internal extraction control: *Candida albicans*, *Candida glabrata*, *Candida parapsilosis* with the *Cand*ID® and *Candida tropicalis*, *Candida krusei*, *Candida dubliniensis* with the *Cand*ID PLUS®.

The accuracy of the new kits was determined utilizing the Quality Control for Molecular Diagnostics (QCMD) 2018 *Candida spp*. EQA Programme. The panel consisted of 10 members including *Candida albicans*, *Candida auris*, *Candida glabrata*, *Candida krusei*, and vials without *Candida spp*. The clinical performance of the new kits was determined with specimens derived from the NOBIS and NOBICS studies [1, 2]. Specimens had been obtained from patients with culture-proven candidemia (*n*=23; EDTA whole blood specimens) and patients without candidemia but bacteremia (*n*=31; serum samples). All blood samples were collected at the same day as the corresponding blood cultures.

Results

With the quality control program, all panel members were correctly identified by the new PCR assays (Table 1). With the clinical study, 2 EDTA whole blood samples obtained from patients with candidemia were found to be inhibited and thus excluded from further analysis. In patients with candidemia (mean age, 67 years, range 20 to 89 years), 14 of 21 samples (67%) gave a positive result when employing the new PCR assays (Figure 2, Table 2). All *Candida spp*. from candidemic patients were correctly identified with the PCR assays. In patients with bacteremia (mean age, 62 years, range 23 to 91 years), 1 of 31 samples gave a false-positive result with the *Cand*ID® assay. The average turn-around time with *Cand*ID® and *Cand*ID PLUS® kits was 2.5 h.

Figure 1: The EMAG® platform (**a**) and the Lightcycler 480 II instrument (**b**).

(**a**)

(**b**)

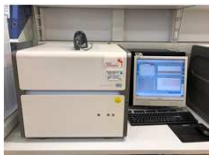


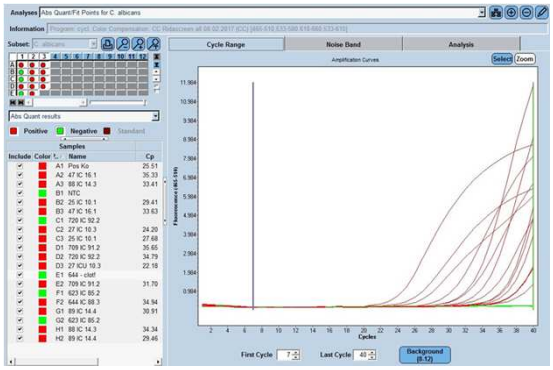
Table 1: Results of accuracy testing utilizing the Quality Control for Molecular Diagnostics (QCMD) 2018 *Candida spp*. EQA Programme.

Vial no.	Sample content	Matrix	Result obtained[1]
1	*Candida albicans*	Plasma	*Candida albicans*
2	*Candida albicans*, *Candida glabrata*	Plasma	*Candida albicans*, *Candida glabrata*
3	*Candida glabrata*	Plasma	*Candida glabrata*
4	*Candida albicans*	Plasma	*Candida albicans*
5	Negative	Plasma	Negative
6	*Candida albicans*	Synthetic BAL	*Candida albicans*
7	*Candida auris*	Plasma	Negative[2]
8	*Candida albicans*	Plasma	*Candida albicans*
9	*Candida krusei*	Plasma	*Candida krusei*
10	*Saccharomyces cervisiae*	Plasma	Negative

[1]Combined result obtained by the *Cand*ID® and *Cand*ID PLUS® kits.
[2]Correct result as both of the kits do not provide detection of *Candida auris*.

Figure 2: Lightcycler qPCR results for *C. albicans* (**a**) and for the internal extraction control (**b**) with the *Cand*ID® kit.

(**a**)



(**b**)

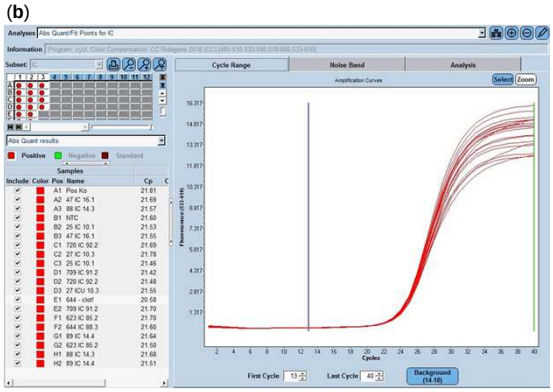


Table 2: Results of samples from patients with culture-proven candidemia obtained by the new *Cand*ID® and *Cand*ID PLUS® kits.

Vial no.	Pathogens in blood culture	Result obtained with molecular assays*
1	*Candida albicans*	Negative
2	*Candida parapsilosis*	*Candida parapsilosis*
3	*Candida glabrata*	*Candida glabrata*
4	*Candida albicans*	*Candida albicans*
5	*Candida albicans*	*Candida albicans*
6	*Candida glabrata*	*Candida glabrata*
7	*Candida albicans*	*Candida albicans*
8	*Candida albicans*	*Candida albicans*
9	*Candida albicans*	Negative
10	*Candida glabrata*	Negative
11	*Candida albicans*	Negative
12	*Candida glabrata*	*Candida glabrata*
13	*Candida albicans*	*Candida albicans*
14	*Candida albicans*	Negative
15	*Candida albicans*	*Candida albicans*
16	*Candida albicans*	*Candida albicans*
17	*Candida albicans*	Negative
18	*Candida albicans*	*Candida albicans*
19	*Candida albicans*	*Candida albicans*
20	*Candida glabrata*	Negative
21	*Candida albicans*, *Candida krusei*	*Candida albicans*, *Candida krusei*

*Combined result obtained by the *Cand*ID® and *Cand*ID PLUS® kits.

Conclusions

When compared to blood culture, two thirds of samples obtained from patients with candidemia were found positive with the new *Cand*ID® or *Cand*ID PLUS® kits. In samples obtained from patients with bacteremia, one false-positive result was observed with the new PCR assays. With introduction of the new assays, the time to results could be reduced to 2.5 hours.

[1] Hoenigl M et al. Procalcitonin fails to predict bacteremia in SIRS patients: a cohort study. Int J Clin Pract 2014;68:1278-81
[2] Zurl C et al. T2 Candida Magnetic Resonance in Patients with Invasive Candidiasis: Strengths and Limitations. Medical Mycology (submitted)

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