The challenges in implementing a new fungal diagnostic test in the routine lab – A real world experience

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Background

Treatment for candidaemia is time critical, with delayed treatment associated with higher hospital mortality. Culture-based tests lack sensitivity and prolong turnaround times, minimizing clinical utility. In this context, non-culture-based methods for the identification of *Candida*, such as PCR, represent a promising approach to allow rapid diagnosis and species identification, allowing the timely initiation of species-oriented therapy.

Our objective was to document the process of validating the OLM Diagnostics *Cand*ID commercial PCR test and its subsequent implementation into the busy routine testing laboratory. This included a retrospective evaluation of *Cand*ID for the diagnosis of invasive candidiasis (IC) prior to incorporation of this test into service, describing issues encountered in the transfer to routine diagnostic use, the steps taken to overcome these issues and the subsequent prospective evaluation and integration into a diagnostic pathway.

Materials/Methods

Routinely, patients were prospectively screened for IC by Bruker Fungiplex *Candida* PCR and Fungitell (1-3)-β-D-Glucan (BDG). A retrospective evaluation of the OLM *Cand*ID PCR assay was performed on 74 serum samples (28 from 22 IC cases, 46 from 45 controls) over a 4-month period, with samples either randomly selected or based on a previous positive Fungiplex result. Nucleic acid extraction of 0.5mL of serum was performed on the BioMerieux eMAG platform prior to *Cand*ID testing performed in duplicate on the Qiagen Rotorgene-Q , as per manufacturer's instructions. Performance parameters (Sensitivity, specificity Positive and negative likelihood and diagnostic odds ratios (LR+tive, LR-tive and DOR) of *Cand*ID were calculated, with IC defined as follows: Candidaemia (n=10) - *Candida* recovered by blood culture; Probable IC (n=12) - Clinical risk factor for invasive candidiasis (e.g. surgery, gut disruption, COVID-19), plus evidence of *Candida* colonization at ≥2 non-contiguous anatomical sites and a positive serum (1-3)-β-D-Glucan; or recovery of *Candida* from a central venous catheter or deep wound and a positive serum (1-3)-β-D-Glucan. *Candida* PCR testing played no role in defining IC.

On the basis of retrospective clinical performance (Table 1) it was decided to incorporate the *Cand*ID assay into routine service. However, after six weeks of testing the specificity was significantly reduced (68.8%). Environmental monitoring and investigation of extraction and amplification negative controls indicated the source of false positivity was associated with the nucleic acid extraction platform, where negative extraction controls and swabs from the eMAG platform were consistently positive for *C. parapsilosis,* which accounted for 73.7% of false positivity.

To maintain a service, a rapid technical evaluation of the Roche MagNA Pure 96 extractor was performed. After confirming satisfactory analytical performance it was incorporated into routine service for *Candida* PCR and a prospective consecutive evaluation of performance was conducted, involving 179 serum samples from 25 cases of IC and 78 control patients.

Population	Sensitivity (%)	Specificity (%)	LR+tive	LR-tive	DOR
Candidaemia (n=10) vs No IC (n=45)	**80 (49-94)**	**93 (82-98)**	**11.43**	**0.22**	**53.14**
Probable IC (n=12) vs No IC (n=45)	**92 (65-99)**	**93 (82-98)**	**13.14**	**0.09**	**152.79**
Combined IC (n=22) vs No IC (n=45)	**86 (67-95)**	**93 (82-98)**	**12.3**	**0.15**	**81.61**

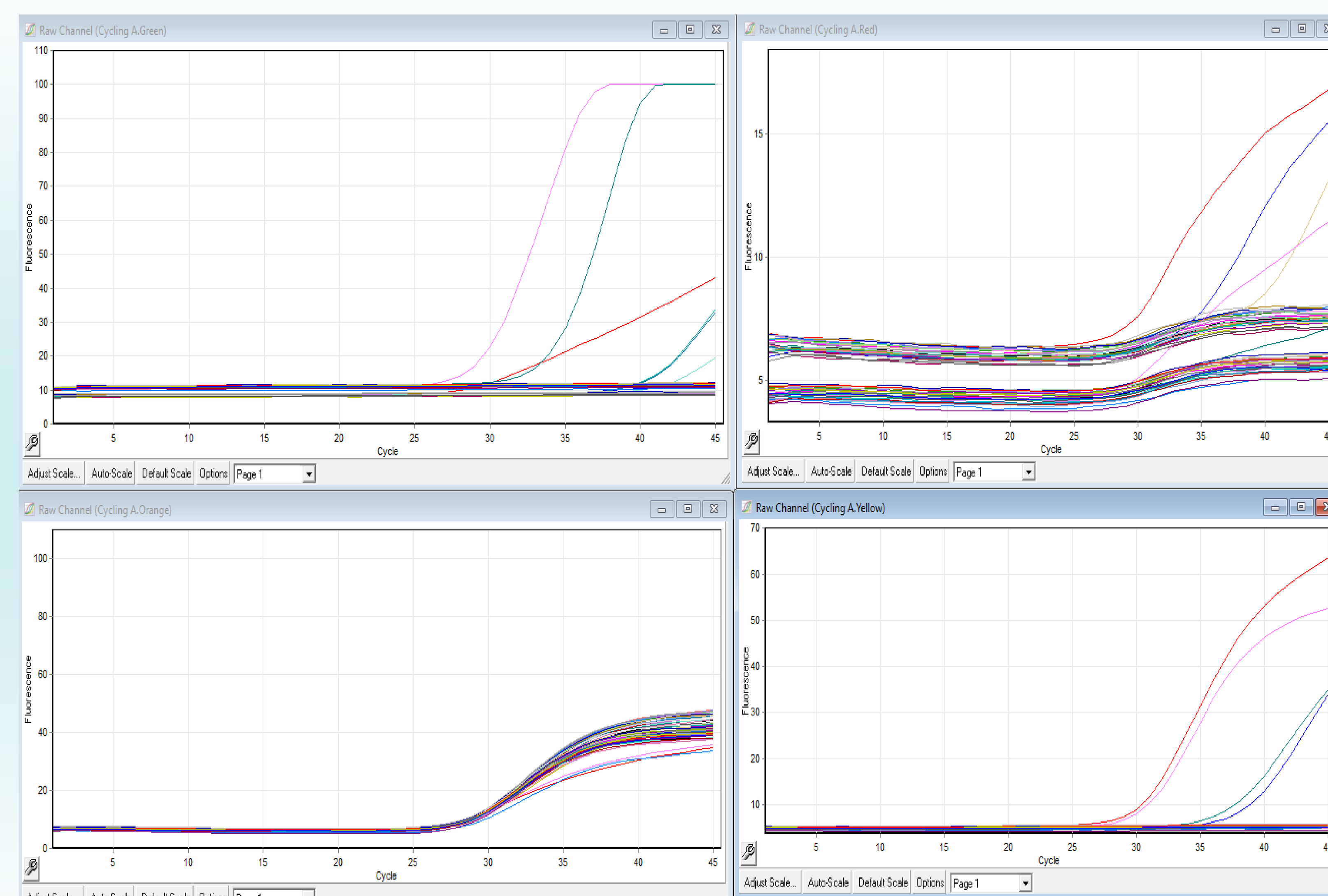
Table 1. Retrospective evaluation of *Cand*ID performance when testing serum samples

Results

Nucleic Acid extraction through PCR amplification and result interpretation was completed in less than 4 hours.

The retrospective positivity rate for samples from cases was 75% (21/28, 95% CI: 57-87), significantly greater than the false positivity rate (6.5%, 3/46, 95% CI: 2.2-17.5) for control samples. Clinical performance is shown in the table. Using a threshold of <37.5% cycles to define PCR positivity, specificity increased to 100%, with a slight reduction in sensitivity (77%). Species identification with *Cand*ID kits was 100% concordant with that obtained through culture identification (*C. albicans* (n=13), *C. glabrata* (n=4), *C. tropicalis* (n=1), *C.krusei* (n=1)). Two candidaemia cases falsely negative by PCR were also negative by BDG and routine PCR testing. One patient only had *Candida* recovered in 1/3 blood culture bottles, the other had received prior antifungal therapy.

Prospective testing generated a sensitivity and specificity of 84% (65-94) and 82% (72-89), respectively (LR +tive: 4.67; LR -tive: 0.19; DOR: 24.6). *C. parapsilosis* accounted for 50% of false positivity. Only 4/14 false positive results were positive in both replicates (Sp: 95%, LR +tive: 10.4) and no patients had multiple positive results (Sp: 100%, LR +tive >360), whereas 15/21 IC cases that were PCR positive met at least one of these criteria. Molecular ID confirmed the blood culture result in 3/4 candidaemia cases.



Conclusions

*Cand*ID provides excellent performance and a rapid time-to-result with accurate species identification that may prove beneficial in cases of suspected IC in the absence of *Candida* culture positivity, by supporting clinical decision-making and avoiding treatment delay or inappropriate therapy choices for IC caused by non-*albicans* species. The use of prior antifungal therapy compromises *Cand*ID sensitivity. However, a combined PCR/BDG and blood culture testing strategy avoids missing cases. Specificity can be improved by requiring multiple positive samples, or both PCR replicates to be positive, or by combining with BDG positivity.

In patients at high risk of IC, combining BDG testing with *Cand*ID PCR provides a strategy to both exclude and more so confirm IC, in the absence of culture.