

Hayette¹ M.P., Wéry¹ M., Boreux¹ R., Peeters¹ S., Stirling³ E., Stevenson³ R., Layios² N.

¹Medical Microbiology, ²Intensive Care Unit, University Hospital of Liège, Belgium, ³Renishaw Diagnostics Limited, Glasgow, UK

Objectives

Microbiological diagnosis of invasive candidiasis is still dependent on culture-based methods. The use of beta-D-glucan antigen detection is included in the EORTC microbiological diagnostic criteria but is rarely available in the clinical labs. On the other hand, PCR-based methods lack standardization. The *RenDx Fungiplex*[®] is a new commercially available semi-automated PCR SERS assay designed for the detection of *Aspergillus sp.* and *Candida sp.* including the differentiation of resistant strains as *C. glabrata*, *C. krusei* and *A. terreus*. This study was performed for sensitivity and reproducibility testing of the method on 8 different *Candida* species.

Methods

The study was conducted on EDTA-blood collected from a healthy donor. Blood samples were spiked with 10 *Candida* reference strains. Spiked samples were diluted at final concentrations ranging from 1 CFU/mL to 1000 CFU/mL. Cultures on Sabouraud dextrose agar were performed in parallel to control yeasts dilutions. DNA extraction was performed by using proteinase K-based method followed by purification on QIAcube automate. The *RenDx*[®] Fungiplex kit (Renishaw Diagnostics) was used for the amplification process and final detection on spiked blood. Reproducibility testing was performed on the three *C. albicans* reference strains by repeating each test 5 times.

Results

<i>Candida strains</i>	1000 CFU/mL	100 CFU/mL	10 CFU/mL	1 CFU/mL	<i>C. albicans ref. strains</i>	Extraction procedures performed on:				
						ATCC 10231	Day 1	Day2	Day 3	Day 4
<i>C. glabrata</i> ATCC 90030	Detected	Detected	Detected	ND	1000 CFU	Detected	Detected	Detected	Detected	Detected
<i>C. krusei</i> ATCC 6258	Detected	Detected	Detected	ND		Detected	Detected	Detected	Detected	Detected
<i>C. tropicalis</i> NEQAS 1036	Detected	Detected	Detected	Detected		Detected	Detected	Detected	Detected	Detected
<i>C. guilliermondii</i> NEQAS 1035	Detected	Detected	ND*	ND		ND	Detected	ND	ND	ND
<i>C. parapsilosis</i> ATCC 22019	Detected	Detected	Detected	ND		ND	ND	ND	ND	ND
<i>C. lusitaniae</i> NEQAS 1511	Detected	Detected	Detected	Detected	NEQAS 1206	Day 1	Day2	Day 3	Day 4	Day 5
<i>C. dubliniensis</i> IHEM 14280	Detected	Detected	Detected	ND		Detected	Detected	Detected	Detected	Detected
<i>C. albicans</i> ATCC 10231	Detected	Detected	ND	ND		Detected	Detected	Detected	Detected	Detected
<i>C. albicans</i> NEQAS 1206	Detected	Detected	ND	ND		ND	ND	ND	ND	Detected
<i>C. albicans</i> NEQAS 2359	Detected	Detected	ND	ND		ND	ND	ND	ND	ND
					1 CFU	Day 1	Day2	Day 3	Day 4	Day 5
						Detected	Detected	Detected	Detected	Detected
						Detected	Detected	Detected	Detected	Detected
						ND	ND	Detected	ND	ND
						ND	ND	ND	ND	ND
					NEQAS 2359	Day 1	Day2	Day 3	Day 4	Day 5
						Detected	Detected	Detected	Detected	Detected
						Detected	Detected	Detected	Detected	Detected
						ND	ND	Detected	ND	ND
						ND	ND	ND	ND	ND

*ND: not detected

Fig 2 : Results of *RenDx Fungiplex* assay performed on blood spiked with 10 *Candida sp.* reference strains

A total of 142 samples were tested in the study including positive and negative controls (figures 2,3) . A sensitivity of 10 CFU/mL was reached for *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. dubliniensis* spiked blood samples while *C. lusitaniae* and *C. tropicalis* performed better at 1 CFU/mL. The three *C. albicans* reference strains and *C. guilliermondii* gave the lowest sensitivity (100 CFU/mL). The reproducibility of the assay was 96% (figure 3).

*ND: not detected

Fig 3 : Reproducibility testing performed on blood spiked with three *C. albicans* reference strains.

Conclusions

RenDx Fungiplex[®] kit allows the detection of the most frequent *Candida* species responsible for invasive candidiasis in spiked blood samples. The **sensitivity of the test is comprised between 10 and 100 CFU/mL for most *Candida sp.*** and **reproducibility is very high**. Because of the high sensitivity of the test, these results allow us to consider this commercial kit for inclusion in a clinical study on invasive candidiasis in comparison with non-molecular diagnostic assays including beta-D-glucan detection test. Meanwhile, further assays including a greater number of *C. albicans* routine strains are being tested.

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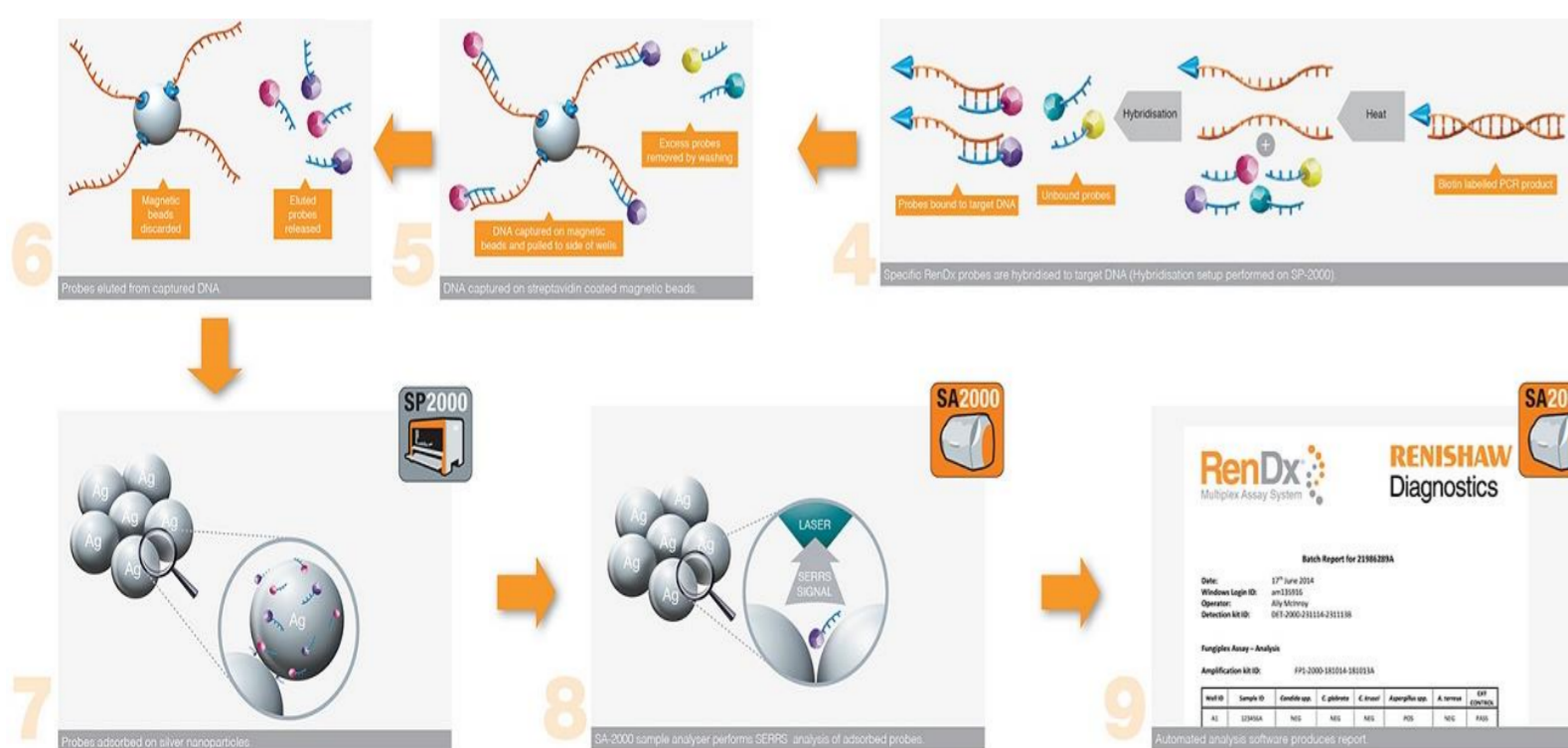
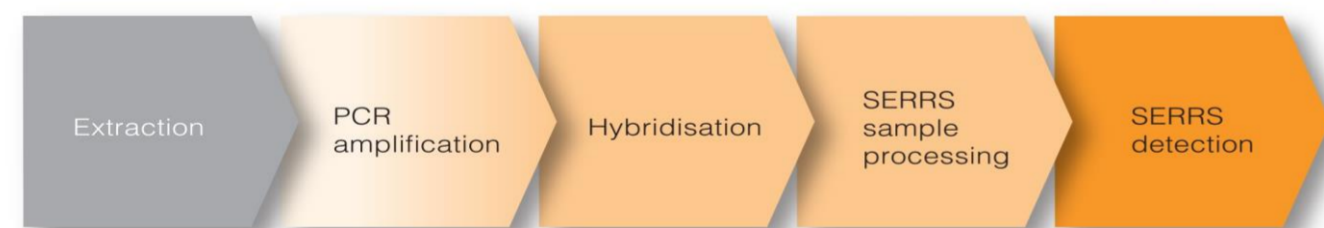


Figure 1 : Summary of the work flow.