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An in-house assay is superior to Sepsityper® for the direct MALDI-TOF identification of yeast species in blood culture

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Running Head: Direct identification of yeast in blood cultures

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Abstract – max 50 words

We developed an in-house assay for the direct identification, by MALDI-TOF, of yeasts in blood culture. Sixty-one representative strains from 12 species were analyzed in artificial blood cultures. Our assay accurately identified 95 of 107 (88.8%) positive blood cultures and outperformed the commercial Sepsityper® kit.

Prompt, appropriate, first-line antifungal treatment has been shown to improve outcome in patients with fungemia, but identification is currently delayed by the need for subculture from positive blood cultures (1, 2). Mass spectrometry techniques based on MALDI-TOF technology have made it possible to identify species directly from positive blood cultures (3). The sample contains human cells, and identification protocols must lyse these cells efficiently (without disrupting the microorganism) and eliminate the unwanted human proteins. In-house protocols based on the lytic agent saponin have been described for bacterial identification (4, 5), but only few assays have been developed for yeast-positive blood cultures (6–9). Bruker produces an easy-to-use kit, Sepsityper®, which has yielded good results for the characterization of both bacteria and yeasts (10, 11). However, this commercial test has never been compared, for yeast identification, with any in-house protocol. We developed an in-house protocol for the direct identification, by MALDI-TOF, of the yeast species commonly isolated from blood cultures. We compared the results obtained with our assay to those obtained with Sepsityper®.

We used an artificial blood culture protocol (blood from healthy volunteers), to facilitate the testing of large numbers of strains and species (12). We used 61 strains previously identified by MALDI-TOF and representative of 12 species commonly implicated in fungemia: *Candida albicans* (15), *C. glabrata* (9), *C. parapsilosis* (7), *C. kefyr* (6), *C. krusei* (6), *C. tropicalis* (7), *Cryptococcus neoformans* (4), *C. dubliniensis* (3), *C. utilis* (1), *C. guillermondii* (1), *C. inconspicua* (1), *and Saccharomyces cerevisiae* (1). Six were isolated directly from blood cultures from patients. The strains were used to

inoculate (10 yeast cells/flask) Mycosis IC/F and BACTEC Plus Aerobic/F or BACTEC plus Anaerobic/ F (*C. glabrata* only) flasks, and were incubated in BACTEC FX (Becton Dickinson) until the flask tested positive for their presence. Each positive flask was subjected to MALDI-TOF MS identification with a Microflex TM MALDI-TOF mass spectrometer (Bruker Daltonics, Germany). We first compared the following agents: 0.8% saponin (as described in ref (4)), 1% Triton, 1.8% SDS (two other commonly used lytic agents) and the Sepsityper® kit, on 17 strains. Two washing steps were included after lysis, because the inclusion of an additional washing step had been shown to improve identification scores ((11) and data not shown). Briefly, the lytic agent was added to 1 ml of medium from each positive flask, which was then vortexed and pelleted (16,000 RCF, two minutes). The supernatant was discarded and the pellet was washed twice in sterile distilled water (saponin, Triton and SDS1.8 protocol) or Sepsityper® washing buffer. It was then homogenized with an appropriate quantity of formic acid (from 2 µl to 15 µl, depending on pellet volume; mean of 5 µl) and an equal volume of acetonitrile was added. Following a final centrifugation (16,000 RCF, 2 min) 1 µl of supernatant was dispensed, in duplicate, on a polished steel target and covered with 1 µl α-cyano-4-hydroxycinnamic acid (HCCA) for MALDI-TOF analysis. The spectra obtained were analyzed with the BioTyper 2.0 database (Bruker Daltonics). The thresholds for interpretation were adapted as previously suggested (8, 13, 14). Identification scores were classified into four categories: A [>2]; B [1.7-2]; C [1.4-1.7], with the expected species proposed first and four times in a row; D [< 1.4] or not identified. The first three categories yielded acceptable results for identification to species level. In total, 305 tests were carried out (Table 1). According to our adapted scores,

95/107 (88.8%), 94/115 (81.7%), 16/42 (38%), 12/41 (29.2%) tests led to correct identification for the SDS1.8, Sepsityper®, saponin and Triton protocols, respectively. Protocols based on saponin and Triton were the least efficient for yeast identification in blood cultures, possibly because they lysed human cells less efficiently, leading to a mixed (yeast and human) and, thus, less specific spectrum profile. The spectrum obtained with the SDS1.8 protocol was very similar to that obtained with the Sepsityper® kit (Figure 1). Based on these results, we limited subsequent comparisons to the Sepsityper® and SDS1.8 protocols.

We analyzed 44 strains grown in both Mycosis IC/F and BACTEC Plus/F flasks with the SDS1.8 and Sepsityper® protocols (176 tests). Scores are reported as median values (interquartile range). Score was transformed into a qualitative value, with four classes corresponding to the identification categories (see above). The Wilcoxon signed rank sum test and Spearman's rank correlation analysis were used for quantitative scores and Fisher's exact test was used for qualitative scores. The results obtained with the two protocols were well correlated (Spearman's correlation coefficient r=0.70 p<0.001). Median score was significantly higher with the SDS protocol (1.9 [1.7; 2.1]) than with Sepsityper® (1.8 [1.55; 2.0]) (Wilcoxon test p=0.003). This difference remained significant if separate analyses were carried out by vial type (Mycosis IC/F p<0.001 and BACTEC Plus p=0.04) (figure 2) and for the non-*Candida albicans* yeasts (*C. albicans*: p=0.16; non-*C. albicans* yeasts: p<0.001) (data not shown). A subgroup analysis by vial type showed that score class results were also better for the SDS assay than for the Sepsityper® assay for Mycosis IC/F (p=0.04) (Figure 3).

This is the first comparison of an in-house protocol with the Sepsityper® kit for the MALDI-TOF identification of yeasts in positive blood cultures. We found that lysis with 1.8% SDS was globally superior to the Sepsityper® kit, particularly for yeasts isolated from Mycosis IC/F medium and for non-*albicans* species of *Candida*. The two protocols had similar durations (30 minutes), but the SDS1.8 protocol was cheaper than the Sepsityper® protocol (by a factor of 220: €0.025 vs. €5.5 euros per test). The SDS protocol yielded a correct identification rate of 100% (mean score 2 ± 0.19) in clinical practice, in tests on nine flasks from six patients, but a prospective study is required to confirm this performance in other vials containing patient's blood. As the 0.8% saponin procedure gave poorer results than previously reported for bacteria, rapid identification procedures for positive blood cultures will probably need to be adapted according to the results of direct examination. The SDS1.8 protocol appears to be an effective, cheap alternative for yeast identification in positive blood cultures.

Acknowledgments

Authors have no conflict of interest to declare

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Figure legends

Figure 1 Spectral profile obtained for *Candida krusei* isolated from Mycosis® blood culture and subjected to various lysis protocols. SDS1.8 = 1.8% SDS; NC= negative control (Mycosis® blood culture negative on day 6). Arrows indicate the three principal nonspecific spectral signals present in negative controls but also identified in the saponin and Triton protocols.

Figure 2: A. Boxplots of scores by protocol: **median score is significantly higher with the SDS protocol than with Sepsityper® (p = 0.003). **B.** Boxplots of scores by protocols and type of blood culture flasks: * significant difference between the two protocols for BACTEC Plus (p=0.04) and **significant difference between the two protocols for Mycosis IC/F (p<0.001).

Figure 3: Diagram showing the distribution of identification score classes, by protocol and by type of culture vial. The classes correspond to the following scores: A: > 2; B: 1.7-2; C >1.4; D<1.4. * Subgroup analysis: class to class comparison was significantly different between SDS1.8 and sepsityper® in the mycosis media (p = 0.04)

Table 1: **Detailed results for each species and each protocol. SEP: Sepsityper®, SDS: 1.8% SDS, SAP: 0.8% saponin, TRI: 1% Triton. M= Mycosis® media, B: bacterial media, NI= not identified.**

SPECIES		***C. albicans***								***C. glabrata***								***C. parapsilosis***								***C. kefyr***								***C. krusei***			
PROTOCOL		SEP		SDS		SAP		TRI		SEP		SDS		SAP		TRI		SEP		SDS		SAP		TRI		SEP		SDS		SAP		TRI		SEP		SDS	
FLASKS		M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B
CLASS	NI	1	2	1	2	1	1	1	2	1	2	1	1	1	1	2	1					1		2			4	1	1	3	1	3	1				
	<1.4		1		1		2	1	1	1								1				1	1		1		1		1	2	1	2	1				
	1.4-1.7	2	2		2	2	1	1	1	4				2		1				1	1			1				1			1		2				
	1.7-2	6	4	4	3	2	1	1		1	3	5	4		1		1	4	2	2		1	1		1	6	1	1	1		1			4	2	2	2
	>2	4	4	6	3					2	4	3	4		1		1	2	3	4	4							2	2				1	2	4	3	3
n	total =305	13	13	11	11	5	5	4	4	9	9	9	9	3	3	3	3	7	5	7	5	3	2	3	2	6	6	5	5	5	4	5	5	6	6	5	5

SPECIES		***C. tropicalis***								***Cryptococcus neoformans***								***C. dubliniensis***								***C. utilis***				***C. inconspicua***				***C. guillermondii***			
PROTOCOL		SEP		SDS		SAP		TRI		SEP		SDS		SAP		TRI		SEP		SDS		SAP		TRI		SEP		SDS		SEP		SDS		SEP		SDS	
FLASKS		M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B
CLASS	NI	1	1	1		1		1			1		2	2	2	2	2					1	1	1													
	<1.4		1								2								1						1												
	1.4-1.7	1		1	2					2		2	1					2	1		1									1							
	1.7-2	5	5	4	5		1			2	1	2	1					1		3	1							1			1	1	1	1	1	1	1
	>2			1					1																	1	1		1								
n	total =305	7	7	7	7	1	1	1	1	4	4	4	4	2	2	2	2	3	2	3	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

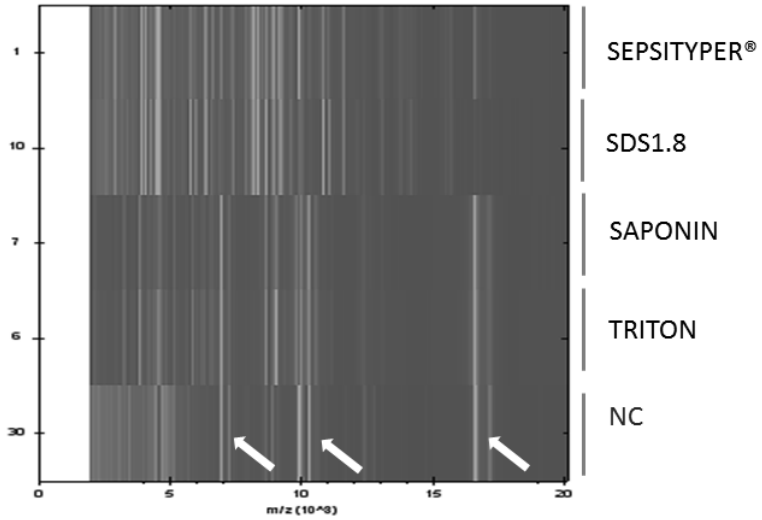
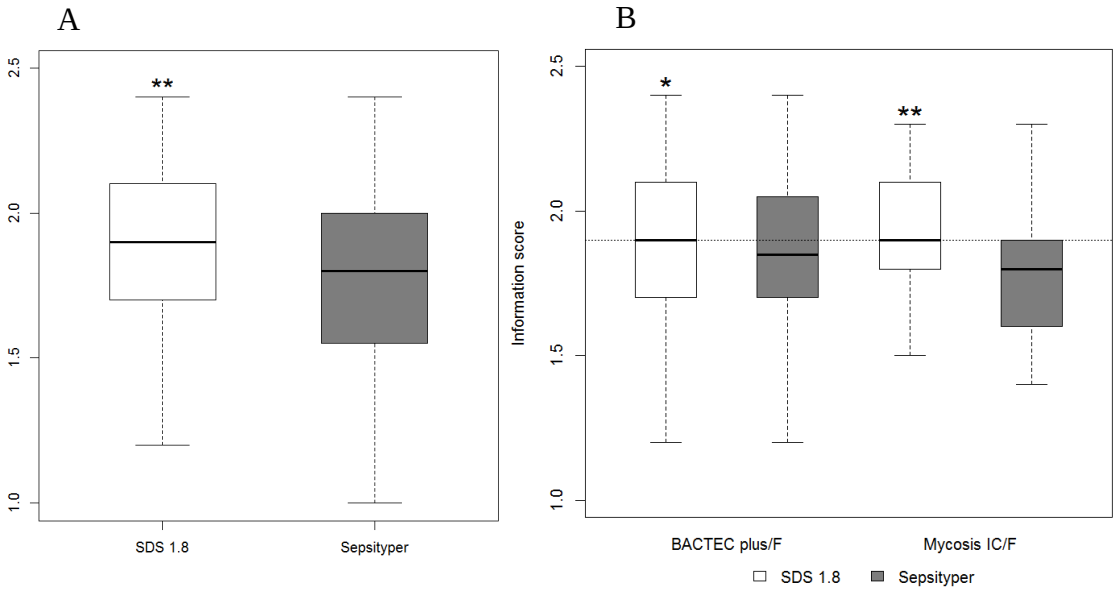


Figure 4 Spectral profile obtained for *Candida krusei* isolated from Mycosis® blood culture and subjected to various lysis protocols. SDS1.8 = 1.8% SDS; NC= negative control (Mycosis® blood culture negative on day 6). Arrows indicate the three principal nonspecific spectral signals present in negative controls but also identified in the saponin and Triton protocols.



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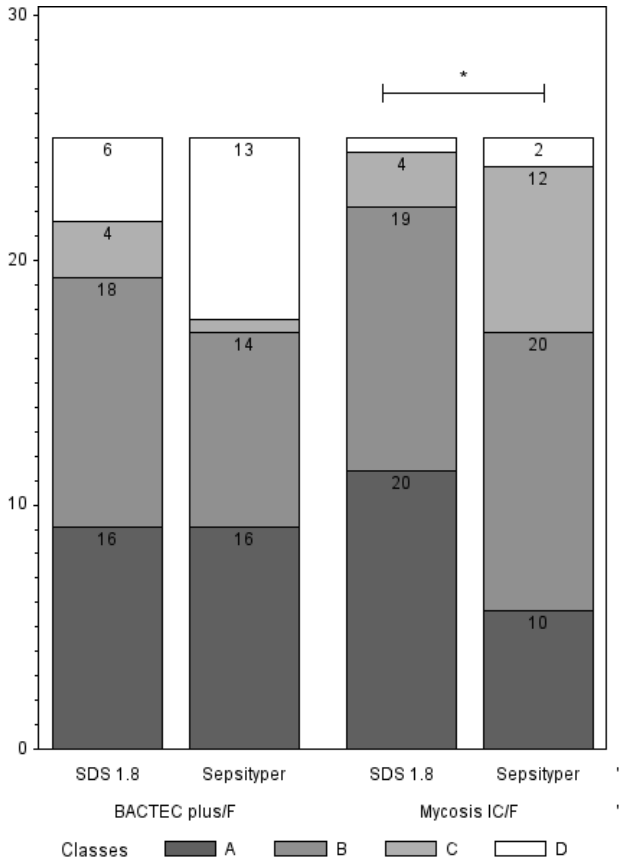

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