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An In-House Assay Is Superior to Sepsityper for Direct Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) Mass Spectrometry Identification of Yeast Species in Blood Cultures

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

3

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14

15 Running Head: Direct identification of yeast in blood cultures

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20

21 **Abstract – max 50 words**

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

26

27

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

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

50 inoculate (10 yeast cells/flask) Mycosis IC/F and BACTEC Plus Aerobic/F or BACTEC
51 plus Anaerobic/ F (*C. glabrata* only) flasks, and were incubated in BACTEC FX (Becton
52 Dickinson) until the flask tested positive for their presence. Each positive flask was
53 subjected to MALDI-TOF MS identification with a Microflex™ MALDI-TOF mass
54 spectrometer (Bruker Daltonics, Germany). We first compared the following agents:
55 0.8% saponin (as described in ref (4)), 1% Triton, 1.8% SDS (two other commonly used
56 lytic agents) and the Sepsityper® kit, on 17 strains. Two washing steps were included
57 after lysis, because the inclusion of an additional washing step had been shown to
58 improve identification scores ((11) and data not shown). Briefly, the lytic agent was
59 added to 1 ml of medium from each positive flask, which was then vortexed and pelleted
60 (16,000 RCF, two minutes). The supernatant was discarded and the pellet was washed
61 twice in sterile distilled water (saponin, Triton and SDS1.8 protocol) or Sepsityper®
62 washing buffer. It was then homogenized with an appropriate quantity of formic acid
63 (from 2 µl to 15 µl, depending on pellet volume; mean of 5 µl) and an equal volume of
64 acetonitrile was added. Following a final centrifugation (16,000 RCF, 2 min) 1 µl of
65 supernatant was dispensed, in duplicate, on a polished steel target and covered with 1 µl
66 α-cyano-4-hydroxycinnamic acid (HCCA) for MALDI-TOF analysis. The spectra
67 obtained were analyzed with the BioTyper 2.0 database (Bruker Daltonics). The
68 thresholds for interpretation were adapted as previously suggested (8, 13, 14).
69 Identification scores were classified into four categories: A [>2]; B [1.7-2]; C [1.4-1.7],
70 with the expected species proposed first and four times in a row; D [< 1.4] or not
71 identified. The first three categories yielded acceptable results for identification to species
72 level. In total, 305 tests were carried out (Table 1). According to our adapted scores,

73 95/107 (88.8%), 94/115 (81.7%), 16/42 (38%), 12/41 (29.2%) tests led to correct
74 identification for the SDS1.8, Sepsityper®, saponin and Triton protocols, respectively.
75 Protocols based on saponin and Triton were the least efficient for yeast identification in
76 blood cultures, possibly because they lysed human cells less efficiently, leading to a
77 mixed (yeast and human) and, thus, less specific spectrum profile. The spectrum obtained
78 with the SDS1.8 protocol was very similar to that obtained with the Sepsityper® kit
79 (Figure 1). Based on these results, we limited subsequent comparisons to the Sepsityper®
80 and SDS1.8 protocols.

81 We analyzed 44 strains grown in both Mycosis IC/F and BACTEC Plus/F flasks
82 with the SDS1.8 and Sepsityper® protocols (176 tests). Scores are reported as median
83 values (interquartile range). Score was transformed into a qualitative value, with four
84 classes corresponding to the identification categories (see above). The Wilcoxon signed
85 rank sum test and Spearman's rank correlation analysis were used for quantitative scores
86 and Fisher's exact test was used for qualitative scores. The results obtained with the two
87 protocols were well correlated (Spearman's correlation coefficient $r=0.70$ $p<0.001$).
88 Median score was significantly higher with the SDS protocol (1.9 [1.7; 2.1]) than with
89 Sepsityper® (1.8 [1.55; 2.0]) (Wilcoxon test $p=0.003$). This difference remained
90 significant if separate analyses were carried out by vial type (Mycosis IC/F $p<0.001$ and
91 BACTEC Plus $p=0.04$) (figure 2) and for the non-*Candida albicans* yeasts (*C. albicans*:
92 $p=0.16$; non-*C. albicans* yeasts: $p<0.001$) (data not shown). A subgroup analysis by vial
93 type showed that score class results were also better for the SDS assay than for the
94 Sepsityper® assay for Mycosis IC/F ($p=0.04$) (Figure 3).

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

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109 **Acknowledgments**

110 Authors have no conflict of interest to declare

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175 **Figure legends**

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

181

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

187

188 **Figure 3:** Diagram showing the distribution of identification score classes, by protocol
189 and by type of culture vial. The classes correspond to the following scores: A: > 2; B:
190 1.7-2; C >1.4; D<1.4. * Subgroup analysis: class to class comparison was significantly
191 different between SDS1.8 and sepsityper® in the mycosis media (p = 0.04)

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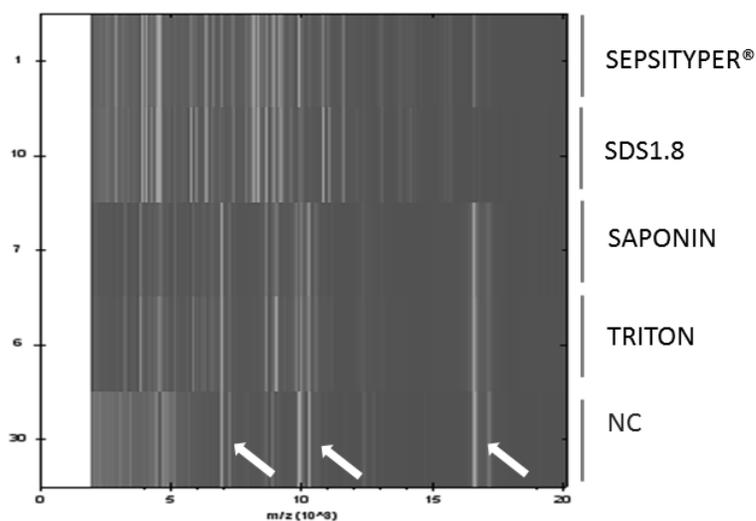
Table 1: Detailed results for each species and each protocol. SEP: Sepsityper®, SDS: 1.8% SDS, SAP: 0.8% saponin, TRI: 1% Triton. M= Mycosis® media, B: bacterial media, NI= not identified.

SPECIES		<i>C. albicans</i>								<i>C. glabrata</i>								<i>C. parapsilosis</i>								<i>C. kefyr</i>								<i>C. krusei</i>							
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				
NI		1	2	1	2	1	1	1	2	1	2	1	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		1		2		1		1									
CLASS		1.4-1.7		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		4	4	6	3					2	4	3	4		1		1	2	3	4	4				2	2					1	2	4	3							
n		total =305		13	13	11	11	5	5	4	4	9	9	9	9	3	3	3	3	3	3	7	5	7	5	3	2	3	2	6	6	5	5	5	4	5	5	6	6	5	

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SPECIES		<i>C. tropicalis</i>								<i>Cryptococcus neoformans</i>								<i>C. dubliniensis</i>								<i>C. utilis</i>								<i>C. inconspicua</i>								<i>C. guillermoidii</i>							
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												
NI		1	1	1		1		1		1		2	2	2	2	2				1	1	1																											
CLASS		<1.4		1						2								1				1						1																					
1.4-1.7		1		1	2					2		2	1					2	1		1									1		1	1	1	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												
>2		1				1												1		1		1																											
n		total =305		7	7	7	7	1	1	1	1	1	1	4	4	4	4	2	2	2	2	2	2	2	2	3	2	3	2	1	1	1	1	1	1	1	1												

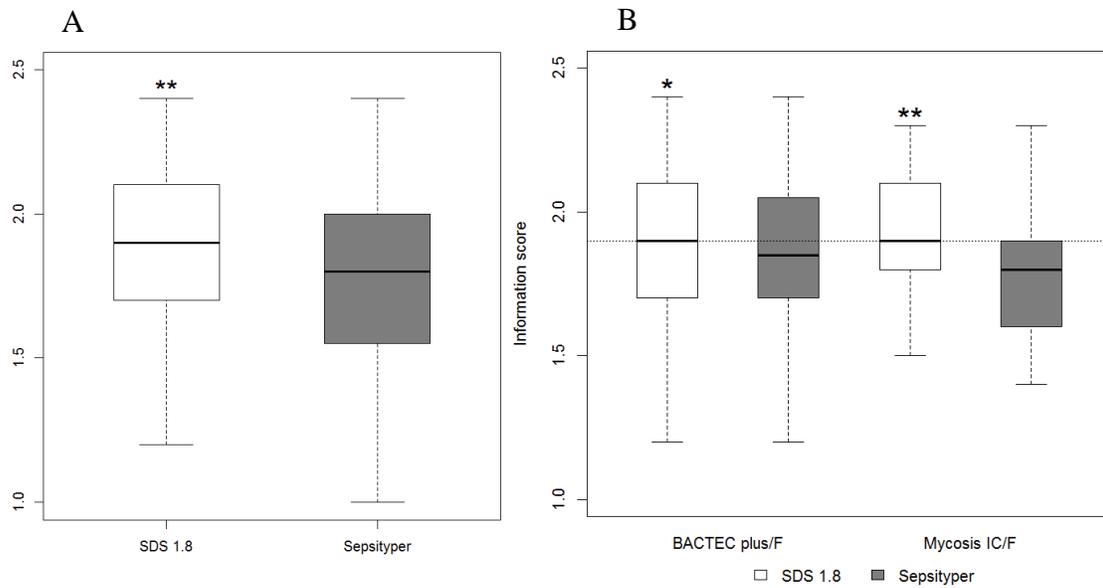
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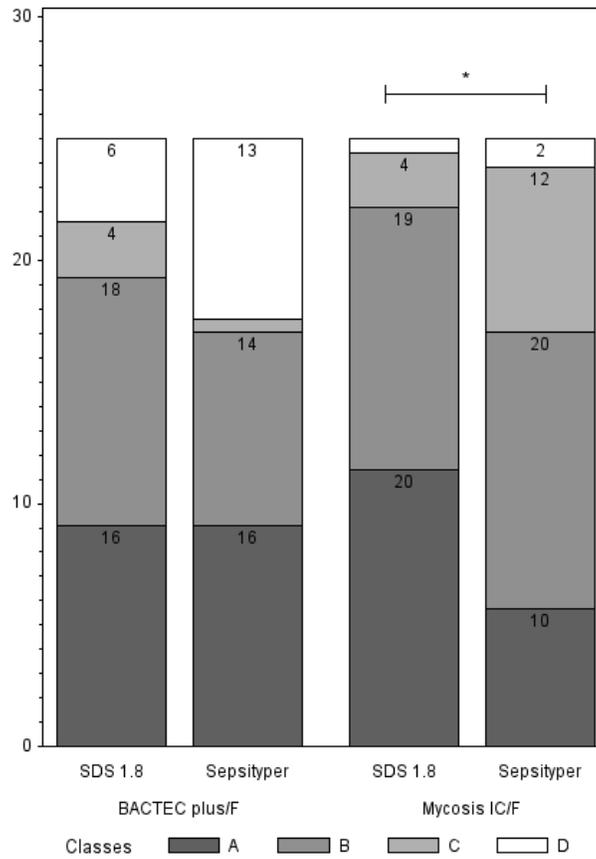
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