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Original article

Performance evaluation of the Becton Dickinson Kiestra™ IdentifA/SusceptA

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ABSTRACT

Objectives: New automated modules are required to provide fully automated solutions in diagnostic microbiology laboratories. We evaluated the performance of a Becton Dickinson Kiestra™ IdentifA/SusceptA prototype for MALDI-TOF identification (ID) and Phoenix™ antibiotic susceptibility testing (AST).

Methods: The performance of the IdentifA/SusceptA coupled prototype was compared with manual processing for MALDI-TOF ID on 1302 clinical microbial isolates or ATCC strains and for Phoenix™ M50 AST on 484 strains, representing 61 species.

Results: Overall, the IdentifA exhibited similar ID performances than manual spotting. Higher performances were observed for Gram-negative bacteria with an ID at the species level (score >2) of 96.5% (369/382) and 86.9% (334/384), respectively. A significantly better performance was observed with the IdentifA (95.2%, 81/85) compared with manual spotting (75.2%, 64/85) from colonies on MacConkey agar. Contrariwise, the IdentifA exhibited lower ID performances at the species level than manual processing for streptococci (76.1%, 96/126 compared with 92%, 115/125), coagulase-negative staphylococci (73.3%, 44/60 compared with 90%, 54/60) and yeasts (41.3%, 19/46 compared with 78.2%, 36/46). *Staphylococcus aureus* and enterococci were similarly identified by the two approaches, with ID rates of 92% (65/70) for the IdentifA and 92.7%, (64/69) for manual processing and 94.8%, (55/58) for the IdentifA and 98.2%, (57/58) for manual processing, respectively. The SusceptA exhibited an AST overall essential agreement of 98.82% (6863/6945), a category agreement of 98.86% (6866/6945), 1.05% (6/570) very major errors, 0.16% (10/6290) major errors, and 0.91% (63/6945) minor errors compared to the reference AST.

Conclusions: Overall, the automated IdentifA/SusceptA exhibited high ID and AST performances. **Damien Jacot, Clin Microbiol Infect 2021;27:1167.e9–1167.e17**

Introduction

Automation in clinical bacteriology is currently revolutionizing laboratory operations by allowing standardization, traceability, reduced human errors and possibly decreased turnaround time (TAT) [1–7]. Even though these systems allow automation of some laboratory processes, most of them remain manual for processes such as microbial identification (ID) by MALDI-TOF and antibiotic susceptibility testing (AST). These manual processes are labour intensive and are prone to multiple possible errors because multiple individual manual steps are required. The development of additional automated solutions is therefore required to provide fully automated solutions with limited manual human intervention offering complete standardization and traceability from sample inoculation to final ID and AST results.

In this study, we evaluated the performance of a Becton Dickinson Kiestra™ IdentifA/SusceptA prototype for automatic colony picking, bacterial suspension preparation, MALDI-TOF target plate spotting and Phoenix™ M50 AST panel preparation. These two modules can be coupled to a BD Kiestra™ TLA system for a complete automation process from specimen processing to susceptibility testing.

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Methods

Inoculation and incubation of clinical specimen and microbial isolates

A total of 169 de-identified clinical specimens, 199 clinical isolates and 17 ATCC microbial strains representing 18 different specimen types (Supplementary Table S1) have been inoculated with the BD Kiestra InoqulA (BD Kiestra, Drachten, Netherlands) in duplicate on BD™ Columbia Agar with 5% Sheep Blood (COL, Cat. No. 254071), BD™ Chocolate agar with IsoVitaleX (CHOC, Cat. No. 254060), BD™ MacConkey II Agar (MAC, Cat. No. 254025) and BD™ CHROMagar™ Orientation Medium (CHROM, Cat. No. 257481). The agar media were incubated at 37°C for 18 h in BD Kiestra ReadA Compact incubators in normal (MAC and CHROM) or in 5% CO_2 atmosphere (COL and CHOC). One plate of each medium was used for automated IdentifA/SusceptA processes and one plate was used for manual processes. A total of 1302 microbial isolates or ATCC strains were recovered from the four different inoculated media representing 61 species (Supplementary Tables S2 and S3); 1302 were analysed for Bruker MALDI-TOF identification (Supplementary Table S2) and 484 for Phoenix M50 AST with the SusceptA and manually (Supplementary Table S3). Different numbers of isolated colonies for each organism have been recovered from the different agar media due to (a) differences in microbial growth on the different media types, (b) number of recovered discrete colonies on each duplicated plate, (c) the specimen types and, (d) the use of selective media.

Automated processing with the IdentifA and SusceptA

Digital image acquisition of incubated plates were taken with the ImagA BT using the OPTIS™ software (BD Kiestra, Drachten, Netherlands) from the BD Kiestra system used for routine diagnostic in our laboratory. A software package, consisting of the SHQI viewer, the RUO import/export tool and the BD Epicenter, was created to enable all necessary steps from colony picking to ID and AST data collection. The SHQI viewer was used to display the images taken by the ReadA Compact and mark the locations for colony picking. The RUO import/export tool was used to set up the samples, fill in the required test per sample and export this data to the IdentifA/SusceptA and to Epicenter. The import functionality was used to pull the data from the IdentifA/SusceptA and Epicenter and make it available for investigation. Epicenter was used to interface with the Bruker MALDI-TOF and BD Phoenix M50. A total of nine isolated microbial colonies or spotting regions were selected with the SHQI viewer. Selected colonies were automatically picked by the IdentifA to prepare a microbial suspension in 300 µL of deionized water in eight-well cuvettes. If a bacterial suspension of 1.6 McFarland or greater was achieved after picking three colonies, the system was directly initiating the MALDI target plate spotting and/or AST Phoenix M50 panel preparation. If the 1.6 McFarland target was not achieved, a second round of six colony picking was initiated. Depending on the microbial suspension concentration, 1–3 µl were sequentially spotted (layering) on a 96-well MALDI-TOF target plate (MBT Biotarget 96, Cat No. 1839298, Bruker Daltonik, Bremen, Germany) followed by 1 µl of formic acid and 1 µl of HCCA (α-cyano-4-hydroxycinnamic acid) matrix (Bruker, catalog No. 8255344). A bacterial suspension of 0.25 McFarland in BD Phoenix Broth was prepared in the SusceptA from the 300-µL microbial suspension in deionized water and loaded into the Phoenix panels for Gram-negative (NMIC-408), Gram-positive (PMIC-88) and streptococci (SMIC-101).

Manual processing

A direct spotting of picked selected colonies was performed on the Bruker MALDI-TOF target plate before sequential application of 1 µL of formic acid and 1 µL of HCCA matrix. A bacterial suspension of 0.25 McFarland in BD Phoenix Broth was manually prepared from picked colonies as described by the manufacturer and manually loaded in the Phoenix panels for Gram-negative (NMIC-408), Gram-positive (PMIC-88) and streptococci (SMIC-101).

MALDI-TOF identification and Phoenix M50 AST

Bruker MALDI target plates prepared with the IdentifA or manually were loaded on the same Bruker MALDI-TOF Biotyper 2.3 and identification was carried out using the MBT Compass Library, Revision E, MBT7854 MSP Library BTyp2.0 Sec. Library 1.4 V8.0.0.0_7311-7854. Similarly, AST panels prepared by the SusceptA or manually were loaded on the same BD Phoenix™ M50 system. EUCAST 2019 criteria were used to define the interpretative categories S (Susceptible, standard dosing regimen), I (Susceptible, increased exposure) and R (Resistant). Essential agreement (EA), category agreement (CA), minor errors (mE), major errors (ME) and very major errors (VME) were used according to the definitions given by the US Food and Drug Administration [8]. VME and ME discrepant results were further investigated by Etest testing (bioMérieux, Marcy-l'Etoile, France), considered here as the reference result.

Statistical analyses

All data were processed using GraphPad Prism 8.3.0. Significance was assessed between the two methods using a Kruskal–Wallis test and the p-values interpretation were written on the graph.

Ethical statement

This study was evaluated by our Ethics Committee (CER-VD) and did not deserve a specific approval being only a quality assessment of diagnostic tests.

Results

MALDI-TOF identification

The performance of the IdentifA prototype was compared with conventional manual processing for MALDI-TOF identification. Overall, the IdentifA exhibited comparable performances to manual processing with the identification of bacteria at the species level (score >2) of, respectively, 87.1% (749/653) and 89.0% (667/749) (Table 1, Fig. 1). Better performances were observed for the IdentifA compared with manual processing with Gram-negative bacteria, with an identification at the species level (score >2) of 96.5% (382/369) and 86.9% (334/384) ($p \leq 0.0001$), respectively (Table 1, Fig. 1). Specifically, an improved performance was observed for bacteria isolated from McConkey agar (score >2 of, respectively, 95.2% (81/85) and 75.2% (64/85), $p \leq 0.0001$). In contrast, for Gram-positive bacteria, the IdentifA exhibited an overall lower identification performance compared with manual processing with, respectively, 82.5% (265/321) and 93.1% (297/319) ($p \leq 0.0001$) identifications of specimens with a score >2 (Table 1, Fig. 1). For yeasts identification, manual processing of samples performed better than the automated IdentifA with 78.2% (36/46) and 41.3% (19/46) of correct

Table 1
Performance of the IdentifA and manual identification based on MALDI-TOF Bruker identification cut-offs

Families	Methods	CHOC % (*n*)				COL % (*n*)				MAC % (*n*)				CHROM % (*n*)				Total all media % (*n*)			
		n	<1.7	1.7–2	>2	*n*	<1.7	1.7–2	>2	*n*	<1.7	1.7–2	>2	*n*	<1.7	1.7–2	>2	*n*	<1.7	1.7–2	>2
Overall	IdentifA	181	8.83 (16)	3.31 (6)	87.8 (159)	288	8.68 (25)	3.47 (10)	87.8 (253)	85	4.70 (4)	0 (0)	95.2 (81)	195	10.2 (20)	7.69 (15)	82.0 (160)	749	8.67 (65)	4.13 (31)	87.1 (653)
	Manual	181	5.52 (10)	4.41 (8)	90.0 (163)	289	8.99 (26)	1.03 (3)	89.9 (260)	85	18.8 (16)	5.88 (5)	75.2 (64)	194	2.06 (4)	5.15 (10)	92.7 (180)	749	7.47 (56)	3.47 (26)	89.0 (667)
GN Total	IdentifA	91	3.29 (3)	0 (0)	96.7 (88)	106	1.88 (2)	0 (0)	98.1 (104)	85	4.70 (4)	0 (0)	95.2 (81)	100	3 (3)	1 (1)	96 (96)	382	3.14 (12)	0.26 (1)	96.5 (369)
	Manual	91	7.69 (7)	2.19 (2)	90.1 (82)	108	15.7 (17)	0.92 (1)	83.3 (90)	85	18.8 (16)	5.88 (5)	75.2 (64)	100	1 (1)	1 (1)	98 (98)	384	10.6 (41)	2.34 (9)	86.9 (334)
Enterobacterales	IdentifA	65	3.07 (2)	0 (0)	96.9 (63)	79	1.26 (1)	0 (0)	98.7 (78)	67	5.97 (4)	0 (0)	94.0 (63)	79	3.79 (3)	0 (0)	96.2 (76)	290	3.44 (10)	0 (0)	96.5 (280)
	Manual	65	7.69 (5)	0 (0)	92.3 (60)	81	18.5 (15)	1.23 (1)	80.2 (65)	67	23.8 (16)	7.46 (5)	68.6 (46)	80	1.25 (1)	0 (0)	98.7 (79)	293	12.6 (37)	2.04 (6)	85.3 (250)
nf GN	IdentifA	20	5 (1)	0 (0)	95 (19)	24	4.16 (1)	0 (0)	95.8 (23)	18	0 (0)	0 (0)	100 (18)	21	0 (0)	4.76 (1)	95.2 (20)	83	2.40 (2)	1.20 (1)	96.3 (80)
	Manual	20	10 (2)	5 (1)	85 (17)	24	8.33 (2)	0 (0)	91.6 (22)	18	0 (0)	0 (0)	100 (18)	20	0 (0)	5 (1)	95 (19)	82	4.87 (4)	2.43 (2)	92.6 (76)
Other GN	IdentifA	6	0 (0)	0 (0)	100 (6)	3	0 (0)	0 (0)	100 (3)	0	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	9	0 (0)	0 (0)	100 (9)
	Manual	6	0 (0)	16.6 (1)	83.3 (5)	3	0 (0)	0 (0)	100 (3)	0	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	9	0 (0)	11.1 (1)	88.8 (8)
GP total	IdentifA	77	12.9 (10)	3.89 (3)	83.1 (64)	166	12.0 (20)	3.01 (5)	84.9 (141)					78	17.9 (14)	5.12 (4)	76.9 (60)	321	13.7 (44)	3.73 (12)	82.5 (265)
	Manual	77	3.89 (3)	5.19 (4)	90.9 (70)	165	4.24 (7)	1.21 (2)	94.5 (156)					77	3.89 (3)	3.89 (3)	92.2 (71)	319	4.07 (13)	2.82 (9)	93.1 (297)
S. aureus	IdentifA	20	0 (0)	0 (0)	100 (20)	30	6.66 (2)	3.33 (1)	90 (27)					20	5 (1)	5 (1)	90 (18)	70	4.28 (3)	2.85 (2)	92.8 (65)
	Manual	20	0 (0)	0 (0)	100 (20)	30	10 (3)	0 (0)	90 (27)					19	5.26 (1)	5.26 (1)	89.4 (17)	69	5.79 (4)	1.44 (1)	92.7 (64)
ConS	IdentifA	17	23.5 (4)	17.6 (3)	58.8 (10)	24	4.16 (1)	12.5 (3)	83.3 (20)					19	10.5 (2)	15.7 (3)	73.6 (14)	60	11.6 (7)	15 (9)	73.3 (44)
	Manual	17	5.88 (1)	17.6 (3)	76.4 (13)	24	0 (0)	4.16 (1)	95.8 (23)					19	0 (0)	5.26 (1)	94.7 (18)	60	1.66 (1)	8.33 (5)	90 (54)
Streptococci	IdentifA	22	22.7 (5)	0 (0)	77.2 (17)	90	17.7 (16)	1.11 (1)	81.1 (73)					14	57.1 (8)	0 (0)	42.8 (6)	126	23.0 (29)	0.79 (1)	76.1 (96)
	Manual	22	9.09 (2)	4.54 (1)	86.3 (19)	89	3.37 (3)	1.12 (1)	95.5 (85)					14	14.2 (2)	7.14 (1)	78.5 (11)	125	5.6 (7)	2.4 (3)	92 (115)
Enterococci	IdentifA	15	0 (0)	0 (0)	100 (15)	19	5.26 (1)	0 (0)	94.7 (18)					24	8.33 (2)	0 (0)	91.6 (22)	58	5.17 (3)	0 (0)	94.8 (55)
	Manual	15	0 (0)	0 (0)	100 (15)	19	5.26 (1)	0 (0)	94.7 (18)					24	0 (0)	0 (0)	100 (24)	58	1.72 (1)	0 (0)	98.2 (57)
GPB	IdentifA	1	100 (1)	0 (0)	0 (0)	3	0 (0)	0 (0)	100 (3)					1	100 (1)	0 (0)	0 (0)	5	40 (2)	0 (0)	60 (3)
	Manual	1	0 (0)	0 (0)	100 (1)	3	0 (0)	0 (0)	100 (3)					1	0 (0)	0 (0)	100 (1)	5	0 (0)	0 (0)	100 (5)
GPC	IdentifA	2	0 (0)	0 (0)	100 (2)	0	0 (0)	0 (0)	0 (0)					0	0 (0)	0 (0)	0 (0)	2	0 (0)	0 (0)	100 (2)
	Manual	2	0 (0)	0 (0)	100 (2)	0	0 (0)	0 (0)	0 (0)					0	0 (0)	0 (0)	0 (0)	2	0 (0)	0 (0)	100 (2)
Yeast	IdentifA	13	23.0 (3)	23.0 (3)	53.8 (7)	16	18.7 (3)	31.2 (5)	50 (8)					17	17.6 (3)	58.8 (10)	23.5 (4)	46	19.5 (9)	39.1 (18)	41.3 (19)
	Manual	13	0 (0)	15.3 (2)	84.6 (11)	16	12.5 (2)	0 (0)	87.5 (14)					17	0 (0)	35.2 (6)	64.7 (11)	46	4.34 (2)	17.3 (8)	78.2 (36)

CHOC, BD Chocolate agar; CHROM, BD CHROMagar Orientation Medium; COL, BD Blood agar; ConS, coagulase-negative Staphylococci; GN, Gram-negative bacteria; GP, Gram-positive bacteria; GPB, Gram-positive bacilli (*Bacillus* spp., *Corynebacterium* spp.); GPC, Gram-positive Cocci (*Rothia* spp.); MAC, BD MacConkey agar; nf GN, non-fermentative Gram-negative bacteria; Other GN, other Gram-negative bacteria (*Haemophilus* spp., *Moraxella* spp., *Neisseria* spp.).

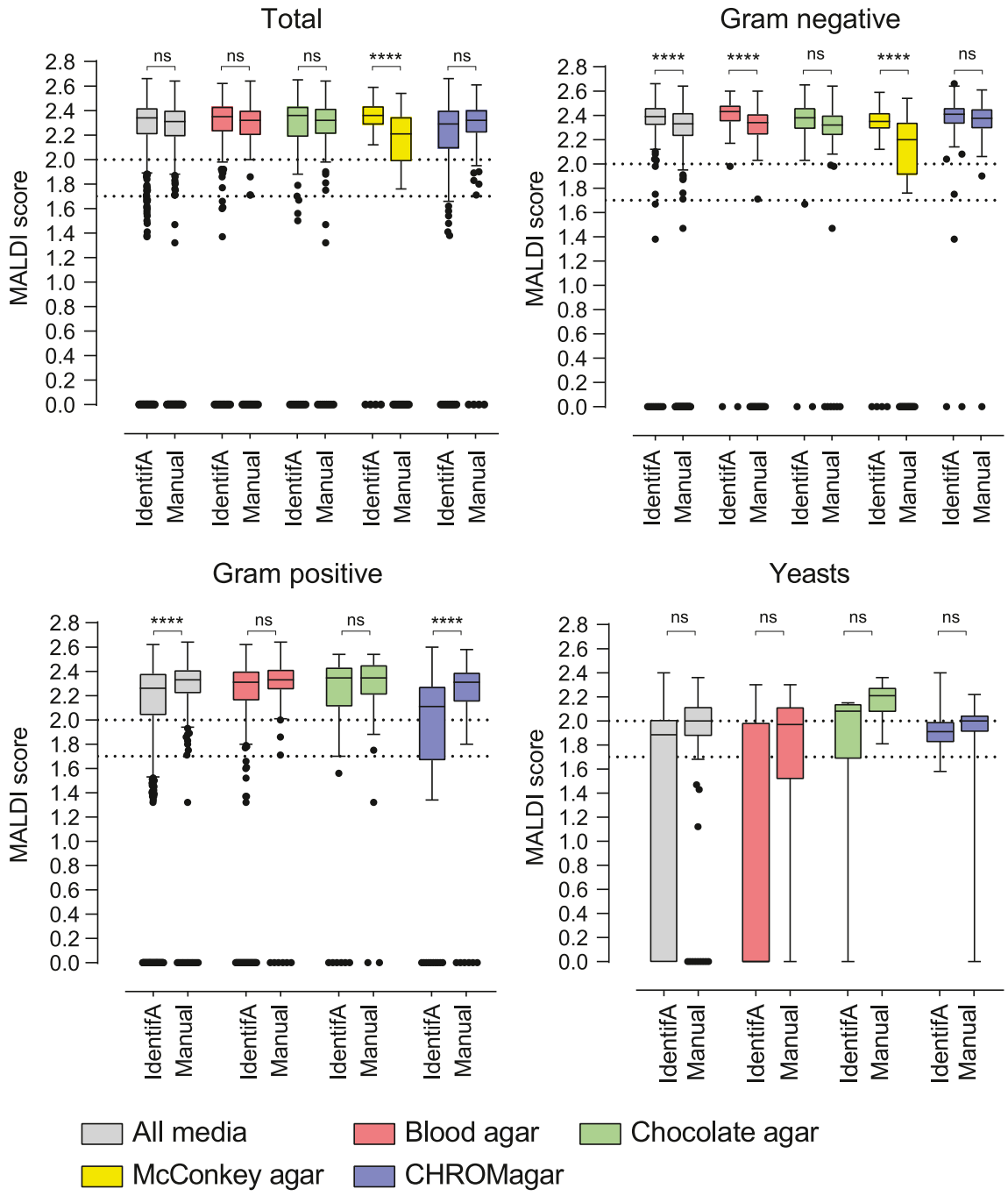

Fig. 1. Tukey box plot analyses of the MALDI-TOF scores for the identification of Gram-negative bacteria and Gram-positive bacteria and yeasts using either the manual method or the automated IdentifA process. Upper and lower horizontal dashed lines correspond to thresholds of 2 and 1.7, respectively. The sum of all the selective media are represented by 'all media'. Non-significant (ns): $p > 0.05$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$. ConS, coagulase-negative staphylococci. The middle of the box corresponds to the median. The box extends from the 25th to 75th percentiles. The upper whisker corresponds to the 75th percentile plus 1.5 of the inter-quartile distance (IQR). Any values greater are plotted as individual points. The lower whisker corresponds to the 25th percentile minus 1.5 IQR. Any values lower are plotted as individual points.

species identification with a score above 2. However, this difference was not statistically significant (Table 1, Fig. 1).

Detailed analyses showed enhanced species identification with the IdentifA with *Enterobacterales* colonies picked on MacConkey agar (IdentifA 94% (63/67) vs manual 68.6% (46/67), $p \leq 0.0001$) and blood agar (IdentifA 98.7% (78/79) vs manual 80.2% (65/81), $p \leq 0.01$) (Table 1, Fig. 2). *Staphylococcus aureus* and enterococci were similarly identified by the two approaches, with ID rates of 92% (65/70) for the IdentifA and 92.7% (64/69) for manual processing, and 94.8% (55/58) for the IdentifA and 98.2% (57/58) for manual processing, respectively. Conversely, automated processing of streptococci and coagulase-negative staphylococci (ConS) showed lower performance with the IdentifA compared with the manual method with 76.1% (96/126) vs 92% (115/125) ($p \leq 0.0001$) and 73.3% (44/60) vs 90% (54/60) ($p \leq 0.0001$), respectively. However, this inferiority was mostly observed on CHROMagar plates (73.6% (14/19) vs 94.7% (18/19), $p \leq 0.0001$ for ConS and 42.8% (6/14) vs 78.5% (11/14) $p \leq 0.0001$ for streptococci).

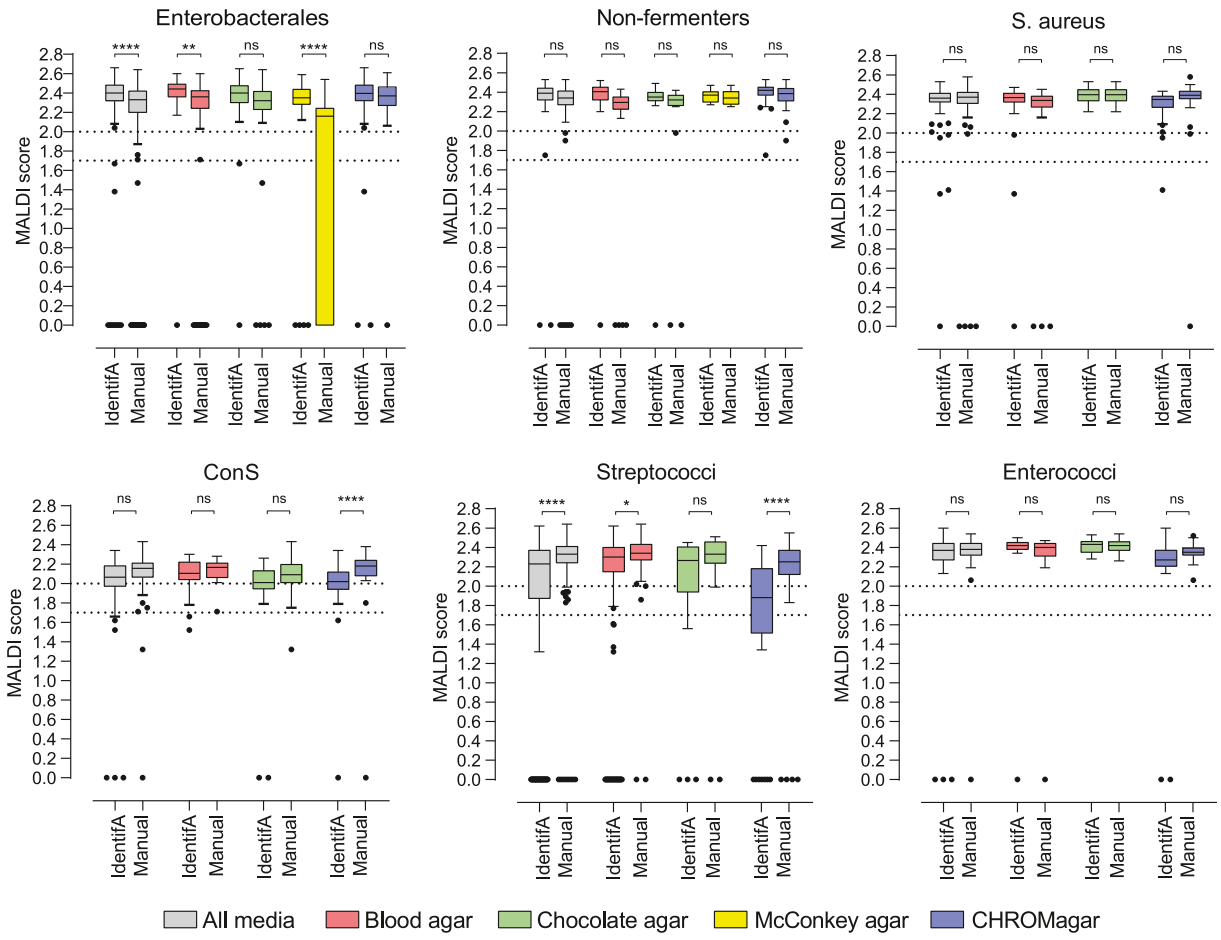

Fig. 2. Detailed Tukey box plot analyses of the MALDI-TOF scores for the identification of Enterobacterales, non-fermenters, *S. aureus*, ConS, Streptococci, Enterococci and yeasts using either the manual method or the automated IdentifA process. Upper and lower horizontal dashed lines correspond to thresholds of 2 and 1.7, respectively. The sum of all the selective media are represented by 'all media'. Non-significant (ns): $p > 0.05$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$. ConS, coagulase-negative staphylococci. The line in the middle of the box is plotted at the median. The box extends from the 25th to 75th percentiles. The upper whisker corresponds to the 75th percentile plus 1.5 of the inter-quartile distance (IQR). Any values greater are plotted as individual points. The lower whisker corresponds to the 25th percentile minus 1.5 IQR. Any values lower are plotted as individual points.

Table 2
Gram-negative bacteria antibiotic susceptibility testing (AST) results (*Enterobacterales* and non-fermentative bacteria)

Drug	Total tested	# EA	% EA	# CA	% CA	Total R	Total S	# VME	% VME	# ME	% ME	# mE	% mE
Ceftazidime	248	246	99.19%	248	100.00%	0	248	0	0.00%	0	0.00%	0	0.00%
Ceftriaxone	231	227	98.27%	222	96.10%	39	189	0	0.00%	3	1.59%	6	2.60%
Ertapenem	230	230	100.00%	227	98.70%	17	205	0	0.00%	0	0.00%	3	1.30%
Amoxicillin–clavulanate	233	227	97.42%	221	94.85%	129	94	2	1.55%	2	2.13%	8	3.43%
Amikacin	278	277	99.64%	278	100.00%	0	278	0	0.00%	0	0.00%	0	0.00%
Ciprofloxacin	275	275	100.00%	275	100.00%	0	275	0	0.00%	0	0.00%	0	0.00%
Cefoxitin	233	230	98.71%	223	95.71%	78	141	0	0.00%	2	1.42%	8	3.43%
Gentamicin	257	257	100.00%	257	100.00%	0	257	0	0.00%	0	0.00%	0	0.00%
Levofloxacin	274	274	100.00%	274	100.00%	0	274	0	0.00%	0	0.00%	0	0.00%
Tobramycin	265	263	99.25%	265	100.00%	0	265	0	0.00%	0	0.00%	0	0.00%
Trimethoprim–sulphamethoxazole	242	242	100.00%	242	100.00%	35	207	0	0.00%	0	0.00%	0	0.00%
Tigecycline	187	187	100.00%	187	100.00%	0	187	0	0.00%	0	0.00%	0	0.00%
Cefuroxime	129	128	99.22%	129	100.00%	0	129	0	0.00%	0	0.00%	0	0.00%
Cefepime	289	285	98.62%	287	99.31%	16	269	0	0.00%	1	0.37%	1	0.35%
Imipenem	277	268	96.75%	260	93.86%	50	206	0	0.00%	0	0.00%	17	6.14%
Piperacillin–tazobactam	244	243	99.59%	244	100.00%	0	244	0	0.00%	0	0.00%	0	0.00%
Meropenem	292	289	98.97%	290	99.32%	25	262	0	0.00%	0	0.00%	2	0.68%
Ampicillin	53	53	100.00%	53	100.00%	0	53	0	0.00%	0	0.00%	0	0.00%
Piperacillin	191	187	97.91%	191	100.00%	0	191	0	0.00%	0	0.00%	0	0.00%
Total	4428	4388	99.10%	4373	98.76%	389	3974	2	0.51%	8	0.20%	45	1.02%

CA, category agreement; EA, essential agreement; mE, minor errors; ME, major errors; S, susceptible, standard dosing regimen; R, resistant; VME, very major errors. Errors are defined by the US Food and Drug Administration.

Identification of other Gram-negative and Gram-positive rods and cocci showed, on average, comparable results although the limited number of samples did not allow assessing their statistical significance (Table 1).

One misidentification at the genus level (*Mycoplasma* spp. with a score of 1.75 instead of *Candida krusei*) was observed with the IdentifA. For Bruker scores above 2, two identifications resulted in differences at the species level (*Bacillus cereus* vs *Bacillus thuringiensis*) following automated or manual preparation whereas 14 isolates were identified at the species level by one of the two methods and reported as undefined species (spp) by the other method (Supplementary Table S4).

Antibiotic susceptibility testing (AST)

For AST, 484 isolates including 297 Gram-negative and 187 Gram-positive bacteria representing 46 bacterial species and 6945 antibiotics tests were investigated (Supplementary Table S5). Overall, an EA of 98.82% (6863/6945) and a CA of 98.86% (6866/6945) were observed with 1.05% (6/570) very major errors (VMEs), 0.16% (10/6290) major errors (MEs) and 0.91% (63/6945) minor errors (mEs) (Supplementary Tables S5 and S6). For Gram-negative bacteria, the EA and CA were 99.1% (4388/4428) and 98.74% (4373/4428), respectively, with VMEs, MEs and mEs of 0.51% (2/389), 0.20% (8/3974) and 1.02% (45/4428), respectively (Table 2). For AST of Gram-positive bacteria, the EA and CA were 98.3% (2475/2517) and 99.05% (2493/2517), respectively, with VMEs, MEs and mEs of 2.21% (4/181), 0.09% (2/2316) and 0.72% (18/2517), respectively (Table 3). All VMEs and MEs are described in Table 4.

Discussion

Main findings

Compared with manual processing, the IdentifA prototype exhibited excellent performances for the identification of Gram-negative bacteria and outperformed manual processing especially for *Enterobacterales* identification from colonies grown onto MacConkey agar. Indeed, during manual MALDI-TOF plate spotting, agar media contaminants are often inadvertently picked with microbial material and can interfere with MALDI-TOF ID [9]. The IdentifA system is designed to only pick microbial colonies avoiding any contact with the culture media thanks to conductance sensing tips. For Gram-positive bacteria, the IdentifA showed lower identification rates but mostly limited to the identification of streptococci and ConS and particularly from bacterial colonies growing on CHROMagar. This lower performance was mainly observed with tiny colonies obtained after 18 h of incubation (Supplementary Fig. S1). Small colonies or insufficient colony selection result in harvesting of insufficient biomass needed to prepare a highly concentrated microbial suspension required for MALDI-TOF identification as indicated by the IdentifA densimeter (Supplementary Fig. S2). Usually, around 10^7–10^8 microbial cells are required to obtain accurate identification with score over 2.0 with the Bruker MALDI-TOF biotyper [9]. For ConS the average score for MALDI-TOF identification is usually lower than for other bacterial genus for both manual and automated processes resulting with a more stringent cut-off for score over 2 (Fig. 2) [10]. Although not investigated here, a longer incubation period for streptococci and ConS or the identification from selected larger colonies growing on rich

Table 3
Gram-positive bacteria antibiotic susceptibility testing (AST) results

Drug	Total tested	# EA	% EA	# CA	% CA	Total R	Total S	# VME	% VME	# ME	% ME	# mE	% mE
Ceftriaxone	58	58	100.00%	58	100.00%	0	58	0	0.00%	0	0.00%	0	0.00%
Ciprofloxacin	126	124	98.41%	125	99.21%	26	99	0	0.00%	0	0.00%	1	0.79%
Gentamicin	82	81	98.78%	82	100.00%	0	82	0	0.00%	0	0.00%	0	0.00%
Levofloxacin	58	56	96.55%	58	100.00%	2	56	0	0.00%	0	0.00%	0	0.00%
Trimethoprim–sulphamethoxazole	95	93	97.89%	95	100.00%	7	88	0	0.00%	0	0.00%	0	0.00%
Tigecycline	78	78	100.00%	78	100.00%	0	78	0	0.00%	0	0.00%	0	0.00%
Cefepime	58	58	100.00%	58	100.00%	0	58	0	0.00%	0	0.00%	0	0.00%
Meropenem	58	57	98.28%	58	100.00%	0	58	0	0.00%	0	0.00%	0	0.00%
Ampicillin	37	36	97.30%	36	97.30%	6	31	1	16.67%	0	0.00%	0	0.00%
Moxifloxacin	100	99	99.00%	100	100.00%	0	100	0	0.00%	0	0.00%	0	0.00%
Daptomycin	129	127	98.45%	129	100.00%	0	128	0	0.00%	0	0.00%	0	0.00%
Fosfomycin with G6P	23	23	100.00%	23	100.00%	0	23	0	0.00%	0	0.00%	0	0.00%
Gentamicin	37	37	100.00%	37	100.00%	14	23	0	0.00%	0	0.00%	0	0.00%
Linezolid	179	178	99.44%	179	100.00%	0	179	0	0.00%	0	0.00%	0	0.00%
Erythromycin	114	108	94.74%	106	92.98%	9	97	0	0.00%	0	0.00%	8	7.02%
Nitrofurantoin	129	129	100.00%	125	96.90%	5	120	0	0.00%	0	0.00%	4	3.10%
Teicoplanin	126	123	97.62%	126	100.00%	0	126	0	0.00%	0	0.00%	0	0.00%
Vancomycin	185	183	98.92%	185	100.00%	0	185	0	0.00%	0	0.00%	0	0.00%
Penicillin G	126	124	98.41%	122	96.83%	50	72	1	2.00%	2	2.78%	1	0.79%
Rifampicin	91	89	97.80%	91	100.00%	0	91	0	0.00%	0	0.00%	0	0.00%
Oxacillin	91	87	95.60%	89	97.80%	32	59	2	6.25%	0	0.00%	0	0.00%
Ceftaroline	51	51	100.00%	51	100.00%	0	51	0	0.00%	0	0.00%	0	0.00%
Tetracycline	146	143	97.95%	145	99.32%	19	126	0	0.00%	0	0.00%	1	0.68%
Mupirocin (high dose)	92	92	100.00%	92	100.00%	2	90	0	0.00%	0	0.00%	0	0.00%
Clindamycin	132	128	96.97%	130	98.48%	9	123	0	0.00%	0	0.00%	2	1.52%
Amoxicilline	58	57	98.28%	57	98.28%	0	57	0	0.00%	0	0.00%	1	1.72%
Cefotaxime	58	56	96.55%	58	100.00%	0	58	0	0.00%	0	0.00%	0	0.00%
Total	2517	2475	98.33%	2493	99.05%	181	2316	4	2.21%	2	0.09%	18	0.72%

CA, category agreement; EA, essential agreement; mE, minor errors; ME, major errors; S, susceptible, standard dosing regimen; R, resistant; VME, very major errors. Errors are defined by the US Food and Drug Administration.

Table 4
List of very major errors (VMEs) and major errors (ME)

Error	Organismal	Drug	MIC BD Kiestra SusceptA	MIC manual	MIC Etest	SIR MIC Etest	SIR BD Kiestra SusceptA	SIR manual + Etest	Comments	Error corrected before result transmission
VME	*Escherichia coli*	AMC	8/2	>32/2	12	R	S	R	Eucast MIC breakpoints for AMC: S ≤ 8, R > 8. MIC by Etest = 12 (Etest and SusceptA: less than 1 dilution difference, Etest and manual: more than 2 dilutions difference)	No
VME	*Citrobacter freundii*	AMC	8/2	32/2	16	R	S	R	Eucast MIC breakpoints for AMC: S ≤ 8, R > 8 + *C. freundii* → AMP/S corrected to Amp/R since natural resistance	Yes
VME	*Enterococcus faecium*	Ampicillin	≤2	>8	>256	R	S	R	*E. faecium* AMP/S → Automatically checked by Etest and corrected	Yes
VME	*Staphylococcus epidermidis*	Penicillin G	0.125	0.25	0.19	R	S	R	*S. epidermidis* isolate is sensitive to methicillin (Oxacillin/S). According to Eucast, no currently available methods can reliably detect penicillinase production in ConS. The MIC breakpoint of resistance >0.125 in Eucast is only valid for *S. aureus* and *S. lugdunensis.* In our laboratory, a Cefoxitin screen is performed and Penicillin is not reported.	Yes
VME	*Staphylococcus epidermidis*	Oxacillin	≤0.25	>2	2	R	S	R	*S. epidermidis*: Oxacillin MIC in Methicillin resistant strain is > 0.25 mg/L MRSE screen of the Phoenix panel prepared by the SusceptA was negative	No
VME	*Staphylococcus capitis*	Oxacillin	≤0.25	>2	8.7	R	S	R	*S. capitis*: Oxacillin MIC in Methicillin resistant strain is > 0.25 mg/L MRSE screen of the Phoenix panel prepared by the SusceptA was negative	No
ME	*Escherichia coli*	Ceftriaxone	>4	≤1	0.032	S	R	S	Multi-sensitive *E. coli* isolate with only CRO/R. CRO would have been checked by disk diffusion or Etest and corrected.	Yes
ME	*Klebsiella pneumoniae*	AMC	>32/2	≤2/2	2	S	R	S	Isolate AMC/R and	No
ME		Cefoxitin	>16	≤4	2	S	R	S	Cefoxitin/R but TZP/S, C2G/S, C3G/S and C4G/S. Resistance to AMC and Cefoxitin would have been reported without additional verification (high-level penicillinase).	No
ME	*Klebsiella pneumoniae*	AMC	>32/2	≤2/2	0.75	S	R	S	Resistance to AMC would have been reported without additional verification.	No
ME	*Citrobacter koseri*	Cefoxitin	>16	8	6.9	S	R	S	Multi-sensitive *C. koseri* isolate with only Cefoxitin/R. Cefoxitin would have been checked by disk diffusion or Etest and corrected.	Yes
ME	*Enterobacter cloacae*	Ceftriaxone	>4	≤1	0.38	S	R	S	Group 3 *Enterobacterales* with inducible or constitutive cephalosporinase (AmpC). The use of C3G is thus not recommended for Group 3 *Enterobacterales*. In our lab, CRO is not reported when S but reported when R.	Yes
ME	*Serratia marcescens*	Ceftriaxone	>4	≤1	2	S	R	S		Yes

(*continued on next page*)

Table 4 (*continued*)

Error	Organismal	Drug	MIC BD Kiestra SusceptA	MIC manual	MIC Etest	SIR MIC Etest	SIR BD Kiestra SusceptA	SIR manual + Etest	Comments	Error corrected before result transmission
ME	*Pseudomonas aeruginosa*	Cefepime	>8	8	8	S	R	S	ME reported without additional verification. Only one dilution difference between SusceptA MIC (16) and manual reference MIC (8) verified by Etest.	No
ME	*Staphylococcus epidermidis*	Penicillin G	0.25	≤0.0625	0.094	S	R	S	*S. epidermidis* isolates sensitive to methicillin (Oxacillin/S). According to Eucast, no currently available methods can reliably detect penicillinase production in ConS. The MIC breakpoint of resistance >0.125 in EUCAST is only valid for *S. aureus* and *S. lugdunensis.* In our laboratory, a Cefoxitin screen is performed and Penicillin is not reported.	Yes
ME	*Staphylococcus epidermidis*	Penicillin G	>0.25	≤0.0625	0.25	S	R	S		Yes

AMC, amoxicillin–clavulanate; CRO, ceftriaxone; MIC, minimum inhibitory concentration. SIR: interpretation S (susceptible, standard dosing regimen), I (susceptible, increased exposure) and R (resistant) according to EUCAST MIC breakpoints. Error corrected before result transmission: error corrected with additional routine AST verification tests (Disk diffusion or Etest) upon unusual AST profile.

agar media allowing optimal microbial growth such as COL or CHOC agar (Fig. 2) could be a simple alternative to increase the biomass and thus the performance of the IdentifA system.

Yeast automated identification gave results below manual processing. This lower performance is likely multifactorial and may include a reduced number of yeast cells in the biomass picked by the system compared with bacteria and rapid sedimentation in the microbial suspension. Our laboratory standard operating procedure (SOP) allows the ID of yeast cells at the species level with score >1.7 providing that a difference of at least 0.2 is observed with the second best identified species and that the morphology of the colony is compatible with the ID [11]. This approach would allow a yeast identification at the species level of 80.4% (37/46) with the IdentifA and of 95.5% (44/46) manually instead of 41.3% (19/46) and 78.2% (36/46), respectively.

The misidentification of a *Mycoplasma* spp. instead of *Candida krusei* is surprising because no *Mycolplasma* spp. isolates have been observed in this study or would have grown on the used media plates in less than 24 h incubation. Yeast extract is an important component of *Mycoplasma* spp. culture media and it cannot be excluded that the *Mycoplasma* spp. reference spectra in the Bruker database contains yeast proteins residues that could lead to misidentification [12]. Alternatively, Lagacé-Wiens et al. have shown that non-specific agar medium specific peaks can result in misidentification of *Mycoplasma* spp [13]. *Bacillus cereus* and *B. thuringensis* cannot be distinguished with a high confidence level with the Bruker MALDI-TOF Biotyper 2.3 and are usually reported as *Bacillus cereus* complex/group.

Evaluation of the SusceptA prototype for Phoenix™ M50 AST showed a very high correlation compared with manual AST panel preparation. After verification of discrepant results by Etest MICs, we observed only six VME (1.05%) and 10 ME (0.16%) out of 6945 antibiotics tested. In our laboratory, three VME (50%) and six ME (60%) would have been corrected due to unusual profiles, natural resistance or routine laboratory confirmatory procedures (Table 4). One VME, an *Escherichia coli* with an amoxicillin/clavulanate MIC of 8 mg/L being at the limit of the EUCAST MIC breakpoint ($S \leq 8, R > 8$), represents an expected analytical variation as the measured MICs are correct at best in a two dilution (plus and minus 1) window (eucast.org).

Limitations

This study was performed on a standalone IdentifA/SusceptA prototype controlled by dedicated research software. This complex network using multiple data import/export prevented the assessment of several critical laboratory parameters such as throughput for ID and AST, TAT and hands-on time as well as the integration of these modules into BD Kiestra TLA automation workflows.

Implications

The implementation of the IdentifA and SusceptA either as a standalone module or integrated in a complete BD Kiestra WCA or TLA automated systems should provide essential added values for diagnostic laboratories including high quality results for most micro-organisms as well as optimal standardization, reproducibility and traceability of the laboratory processes for both ID and AST [1,4,5,14]. The use of the same bacterial suspension for ID and AST eliminates the potential for selecting different organisms for each process. In addition, a significant reduction of both hands-on time and human-induced errors such as sample inversion, incorrect colony picking and/or inappropriate compliance of SOP is expected. In our laboratory, a complete manual process for MALDI-TOF ID from colony selection to final results includes seven to nine human interventions with potential risks of errors or mistakes. The implementation of the IdentifA should reduce the number of human interventions to only one process (transfer of the plate to the Bruker MALDI-TOF).

Noteworthy, the implementation of the IdentifA and SusceptA will also induce additional consumables cost compared to manual preparation.

Conclusions

Overall, this study demonstrate very good analytical performances of the IdentifA/SusceptA prototype compared to manual processing. The implementation of the IdentifA/SusceptA on a BD Kiestra TLA system together with the addition of digital imaging

monitored by expert image analysis applications [1,15–18] represent the required tools to finally achieve a real total laboratory automation.

Author contributions

D.J. and A.C. wrote the first draft. All authors critically reviewed the manuscript.

Transparency declaration

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2020.09.050.

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