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Chemical extraction *versus* direct smear for MALDI-TOF mass spectrometry identification of anaerobic bacteria

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ABSTRACT

In the present study, two pre-analytic processes for mass spectrometric bacterial identification were compared: the time-consuming reference method, chemical extraction, and the direct smear technique directly using cultured colonies without any further preparation. These pre-analytic processes were compared in the identification of a total of 238 strains of anaerobic bacteria representing 34 species. The results showed that 218/238 strains were identified following chemical extraction, 185 identifications (77.7%) were secured to both genus and species [log(score) > 2.0] whereas 33 identifications (14%) were secured to genus only [log(score) between 1.7 and 2.0]. Following direct smear, 207/238 anaerobic bacteria were identified, 158 identifications (66.4%) were secured to both genus and species [log(score) > 2.0] whereas 49 identifications were secured to genus only [log(score) between 1.7 and 2.0]. Twenty strains were not identified [log(score) < 1.7] by MALDI-TOF MS following chemical extraction whereas 31 strains were not identified with the direct smear technique. Although direct smear led to a significant decrease of the log(score) values for the *Clostridium* genus and the Gram positive anaerobic bacteria (GPAC) group ($p < 0.0001$, Wilcoxon test), identification to both species and genus were not changed. However these differences were not statistically significant ($p = 0.1$, Chi square). Therefore, MALDI-TOF MS identification following the direct smear technique appears to both non-inferior to the reference method and relevant for anaerobic bacteria identification.

Matrix-assisted laser desorption and ionisation time-of-flight mass spectrometry (MALDI-TOF MS) is an emerging method for routine bacterial identification. Briefly, a single colony or a centrifuged aliquot of liquid culture can be either directly or following a chemical extraction step applied on a MALDI target plate matrix in a thin film. Laser dependant ionization of bacterial peptides and proteins generates species-specific profiles of spectra allowing bacterial identification by comparison with profiles from a database of reference strains. Thus, MALDI-TOF MS has been proven reliable for accurate and rapid identification of various microorganisms, such as Gram-positive bacteria [1–4], *Enterobacteriaceae* [5], non-fermenting bacteria [6–8], and mycobacteria [9–11]. Identification performed by MALDI-TOF MS was also demonstrated to outperform conventional phenotypical methods and may perform as well as bacterial gene amplification, which remains the gold-standard for bacteria identification [12]. Additionally, in the case of fastidious bacteria, MALDI-TOF MS has been shown to decrease the need for more expensive and time-consuming tools [12,13]. Recently, MALDI-TOF MS has been found to identify anaerobic bacteria as accurately as 16S rDNA [2,14,15] but more rapidly in hours *versus* days. However, a majority of studies used chemical extraction for bacterial preparation before MALDI-TOF MS identification which requires additional hands-on time reducing the time saved by MALDI-TOF MS identification by several hours [16]. The timesaving alternative is to directly smear the cultured colony onto the MALDI matrix without any additional preparation with extraction [17]. Therefore, we compared the identification of clinically relevant anaerobic bacteria by MALDI-TOF MS (Bruker Daltonik, Wissembourg, France) of a colony directly smeared onto the matrix (direct smear) or a culture aliquot following bacterial preparation by chemical extraction (chemical extraction).

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Table 1
Effect of sample preparation on identification of anaerobic bacteria by MALDI-TOF MS.

MALDI-TOF analysis[a]						
Biotyper 3.0 interpretation[b]	Log(score) after chemical extraction			Log(score) on direct smear		
	Score >2	1.7> Score <2	Score <1.7	Score >2	1.7> Score <2	Score <1.7
Bacteroides fragilis	45	0		43	1	
Bacteroides ovatus	8	0		7	0	
Bacteroides thetaiotamicron	8	3		10	1	
Bacteroides vulgatus	7	0		6	1	
Bacteroides distasonis	5	0		5	0	
Bacteroides nordii	1	1		1	1	
Bacteroides uniformis	2	0		2	0	
Bacteroides intestinalis	2	0		0	1	
Clostridium difficile	17	0		11	6	
Clostridium perfringens	20	0		19	0	
Clostridium ramosum	2	0		2	1	
Clostridium paraputrificum	2	0		2	0	
Clostridium innocuum	0	4		0	4	
Tissierella praeacuta	2	0		2	0	
Clostridium clostridioforme	0	2		3	0	
Clostridium hathewayi	2	0		3	0	
Clostridium butyricum	2	0		2	0	
Clostridium subterminale	2	0		0	2	
Clostridium tertium	2	0		2	0	
Clostridium novyi	2	0		0	1	
Clostridium sporogenes	2	0		0	2	
Finegoldia magna	17	3		9	7	
Peptoniphilus harei	9	1		9	1	
Parvimonas micra	4	1		2	3	
Anaerococcus hydrogenalis	0	2		0	1	
Anaerococcus octavius	2	0		0	1	
Anaerococcus murdochii	2	0		0	1	
Peptostreptococcus anaerobius	2	0		2	0	
Eggerthella lenta	2	0		2	0	
Propionibacterium acnes	2	12		4	10	
Fusobacterium naviforme	2	1		1	2	
Fusobacterium necrophorum	0	2		2	0	
Veillonella atypica	7	0		4	1	
Veillonella parvula	3	1		3	1	
No identification	–	–	20	–	–	31

[a] Isolates were tested in duplicate for MALDI-TOF MS identification. No uniform results between duplicate results were scores.
[b] No difference in the genus and species proposed by Biotyper 3.0 was observed between strain identified with a log(score) in the range of 1.7–2.0 and strains identified with a log(score) up to 2.0.

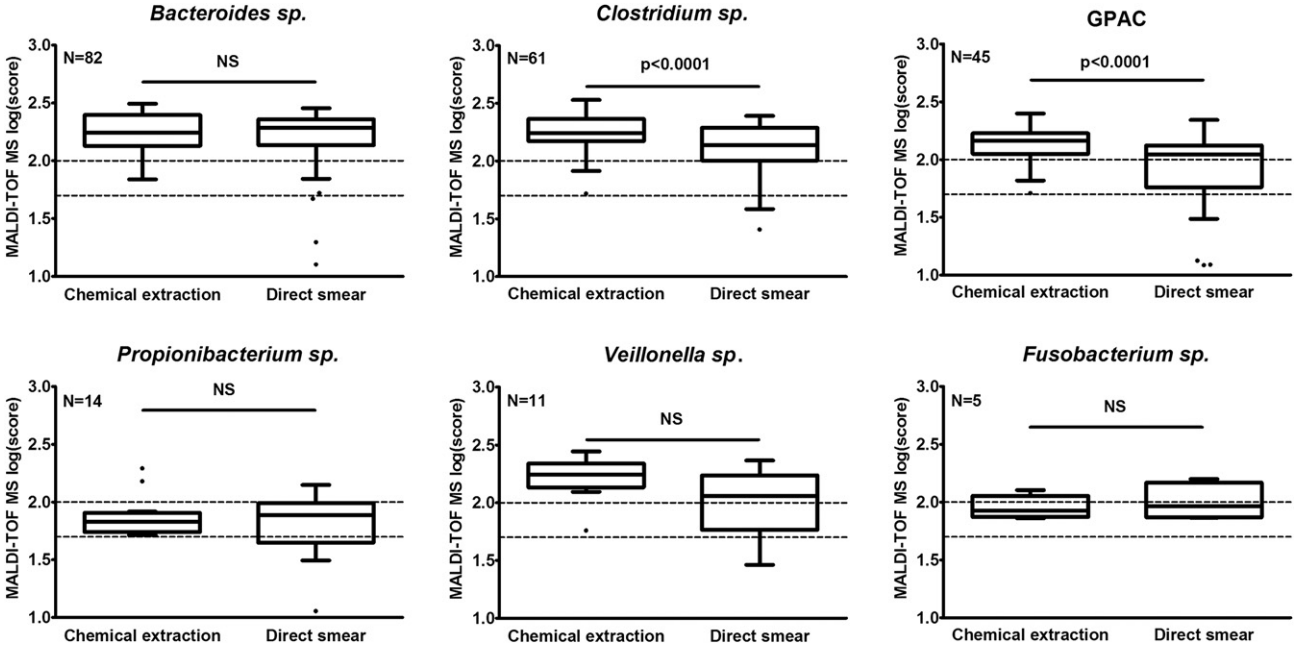

Fig. 1. Identifications by direct smear and chemical extraction. Log(score) assessed by Biotyper software following either direct smear or chemical extraction represented in boxplots. Bottom and top of boxes are lower 25th quartile (Q1), and the upper 75th quartile (Q3). Horizontal lines within the box are the median (Q2). Minimum is represented by the lowest datum still within 1.5 times the interquartile range of the lower quartile, and maximum represents the highest datum still within 1.5 times the interquartile range of the upper quartile. Isolated dots situated upper and down the maximum or minimum values represents every point more than 3/2 times the interquartile range (outliers). Median values were compared with a Wilcoxon statistical test.