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Factors affecting the quality and reproducibility of MALDI-TOF MS identification for human *Capnocytophaga* species

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ABSTRACT

Reproducibility and quality of MALDI-TOF MS spectra are critical in the identification process, however, information on the factors affecting the identification scores are scarce. Here, we studied the influence of various factors during the identification process of human oral *Capnocytophaga* species. The influence of two incubation times, plate-spotting reproducibility of two examiners, extraction technique, storage period of plates, and different laser repetition rates on the quality of MALDI-TOF MS identification of 34 human *Capnocytophaga* strains (including *C. gingivalis, C. granulosa, C. haemolytica, C. leadbetteri, C. ochracea, C. sputigena,* and *Capnocytophaga* genospecies AHN8471) was examined. The identification rate did not show a significant difference ($P = 0.05$) between the two incubation times, except that *C. haemolytica* needed a longer incubation time to be recognized at the genus level. The reproducibility of spotting between two examiners was ensured by following the manufacturer's instructions. At the species level, formic acid extraction improved the identification of species with limited representation in the database, such as *C. haemolytica* and *C. granulosa*. The storage of plates for one week decreased the identification scores. No significant difference ($P = 0.39$) was observed between the 60 Hz and 120 Hz laser repetition rates for identifying *Capnocytophaga* species to the genus or species level. In conclusion, the MALDI TOF MS offers a reliable *Capnocytophaga* identification after following the universal protocol, while the formic acid extraction is restricted to species with a limited number of strains in the database.

1. Introduction

Since the 90s, the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been predicted as a promising identification technique for many microorganisms at the species level (Vargha et al., 2006). Currently, MALDI-TOF MS is considered a rapid and sensitive commercial diagnostic method that carries many superiorities over conventional ones, such as colony patterns, staining techniques, and biochemical tests. Despite the remarkable ability of MALDI-TOF MS to identify numerous bacterial species, the quality and reproducibility of identification at the species level can be influenced by various factors. Fluctuations in experimental conditions can produce diverse spectrum qualities from a single bacterial cell, frequently leading to unreliable specimen identification in most cases (Sauer and Kliem, 2010). Factors affecting the reproducibility and quality of MALDI-TOF MS identification can be divided into three categories. The first one is related to the MALDI-TOF MS instrument: the type of laser beam used, the condition of the instrument, laser repetition rates, and detector voltage, and the number of selected bacteria in the database (Williams et al., 2003; Alatoom et al., 2011; Spraggins and Caprioli, 2011) as well as the enriched database with the appropriate number of specific bacterial species and their spectra profiles (Alatoom et al., 2011; Veloo et al., 2016). The second one is related to the sample preparation, including the reproducibility of smear application, the spotting technique, and the drying and storage time of MALDI-TOF MS plates (Jackson et al., 2005; Liu et al., 2007; Veloo et al., 2014). The last one is related to bacterial characteristics, such as their ribosomal structure and protein profile, which are affected by the type of culture media and incubation time (Goldstein et al., 2013). Since all these factors can affect the selection of the sample preparation method and the analysis technique, establishing one standardized MALDI-TOF MS identification protocol to cover different bacterial species is difficult to apply. Therefore, customized protocols have been employed to ensure a qualitative, quantitative, and reproducible identification of selected

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target microorganisms (Tudó et al., 2015).

Members of the genus *Capnocytophaga* are Gram-negative, facultatively anaerobic, capnophilic, thin fusiform bacterial rods. They belong to the resident oral microbiota in humans and animals but are also present as opportunistic pathogens in various infections, including abscesses, septicemia, and adverse pregnancy outcomes, especially in immunocompromised patients (Jolivet-Gougeon et al., 2007; Lopez et al., 2010; Alhifany et al., 2017; Lawal and Baer, 2021). *Capnocytophaga* species of human origin isolated from oral samples, typically from dental plaque, currently include *C. gingivalis*, *C. granulosa*, *C. haemolytica*, *C. leadbetteri*, *C. ochracea*, *C. sputigena* (Frandsen et al., 2008), and the recently described *C. periodontitidis* (Zhang et al., 2021). In addition, there is one taxon without species recognition, *Capnocytophaga* genospecies AHN8471 (Frandsen et al., 2008).

The identification of *Capnocytophaga* to the species level by using technically challenging and time-consuming biochemical characterization has been difficult (Jolivet-Gougeon et al., 2007; Frandsen et al., 2008). Nowadays, MALDI-TOF MS is in routine use in clinical microbiology laboratories, enabling rapid identification of isolates from clinical specimens, however, the reproducibility and quality of MALDI-TOF MS spectra are critical in this identification process. In addition to the limited information regarding the use of MALDI-TOF MS in identifying *Capnocytophaga* species, the factors affecting the reproducibility and quality of identification scores have not been in focus in this context. Based on this background, we aimed to determine the influence of the incubation time, spotting reproducibility, extraction technique, drying time, and the storage period of plates, as well as different laser repetition rates on the quality and reproducibility of MALDI-TOF MS identification of human oral *Capnocytophaga* species.

2. Materials and methods

2.1. Clinical and type strains

Twenty-seven clinical bacterial strains, originally isolated from dental plaque samples of healthy Caucasian women (Gürsoy et al., 2009) and identified as *Capnocytophaga* spp. based on their colony morphology and routine biochemical tests (Socransky et al., 1979), were chosen. Six human *Capnocytophaga* type strains, including *C. gingivalis* CCUG 9715, *C. granulosa* CCUG 32991, *C. haemolytica* CCUG 32990b, *C. leadbetteri* AHN 8855, *C. sputigena* CCUG 9714, and *Capnocytophaga* genospecies AHN8471, and *C. ochracea* AHN 37380, a clinical strain previously confirmed by 16S rRNA sequencing, were included as a reference.

For each test, all strains were subcultured on non-selective Brucella agar plates supplemented with hemin (5 μg/ml) and vitamin K1 (10 μg/ml) and incubated in an anaerobic chamber (Whitley A35 Anaerobic Workstation, Don Whitley Scientific Ltd., Shipley, West Yorkshire, UK) with an atmosphere of 10% H_2, 5% CO_2, and 85% N_2 at 37 °C.

2.2. MALDI-TOF MS analysis

For each experiment, direct spotting was performed by transferring bacterial colonies in triplicate onto the MALDI-TOF MS stainless steel plates and covered by 1 μl of matrix HCCA (α-cyano-4-hydroxycinnamic acid in 50% acetonitrile/2.5% trifluoroacetic acid). Measurements were performed using MALDI-TOF MS Biotyper® Microflex LT and MALDI Biotyper MBT Smart system and MALDI-TOF MS Biotyper software version 3.1 and MBT Compass Library (DB-6903 MSP, # 1829023). Based on the manufacturer's recommendation, the following identification score values were used: ≥2.00 for the species level, 1.70–1.99 for the genus level, and < 1.70 indicating no reliable identification.

In the MALDI-TOF MS Biotyper® software version 3.1 and MBT Compass Library (DB-6903 MSP, #1829023), the strains *C. leadbetteri* AHN8855 and *Capnocytophaga* genospecies AHN8471 are identified as *Capnocytophaga* sp. and *C. sputigena*, respectively.

2.3. Parameters tested for reproducibility and quality of MALDI-TOF MS identification

2.3.1. Incubation time

Since *Capnocytophaga* species are slow-growing microorganisms, all strains were incubated in duplicate sets at two different incubation times: 48 and 96 h. Each group was directly spotted in triplicate on MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) stainless steel plates according to the manufacturer's protocol.

2.3.2. Spotting reproducibility

After all strains were incubated for 48 h, two to three visible colonies were directly spotted in triplicate on the plate. The spotting was performed twice by a professional hospital microbiologist and a well-trained researcher, and the plates were analyzed immediately.

2.3.3. Extraction methods

After 48 h of incubation, colonies were spotted in triplicate on two MALDI-TOF MS plates. One plate was used for the direct extraction method protocol and the other for the standard formic acid extraction protocol, as described previously by Alatoom et al. (2011).

2.3.4. Laser repetition rate

After 48 h of incubation, colonies of each strain were spotted using the direct method and analyzed with two Bruker MALDI-TOF MS instruments; one with a 60 Hz laser repetition rate (Microflex® LT), and the other with a 120 Hz laser repetition rate (MALDI Biotyper MBT Smart).

2.3.5. Storage and drying time

After the laser repetition rate experiment, MALDI-TOF MS plates were stored in a dry place at room temperature for one week and then reanalyzed to evaluate the effect of storage time on MALDI-TOF MS identification scores.

2.4. Statistical analysis

Using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA), the Pearson's Chi-Square test was used for comparing the genus and species level identification between all groups. The results were considered statistically significant when the *P*-value was <0.05.

3. Results

The identification scores of 34 *Capnocytophaga* strains are summarized in Table 1. The comparison of two incubation times showed that the genus-level identification was slightly better after 48 h than after 96 h of incubation (96% vs. 92%, respectively, $P = 0.05$). At the species level, the identification rates were the same (71.5%) between the two incubation times. The type strain of *C. haemolytica* was not identified to the species level, and this microorganism needed a longer incubation time to be recognized at the genus level.

Testing of spotting reproducibility did not result in significantly different score values of species-level identification between the two examiners (Table 2).

Both direct spotting and formic extraction methods identified 97% of the strains to the genus level (Table 3). The formic acid extraction method improved the identification of *C. haemolytica* and *C. granulosa* at the species level.

No significant differences were obtained between the instruments, MALDI Biotyper MBT Smart LS LS and Microflex® LT, in their capabilities of genus- and species-level identifications (97% vs. 94% and 79% vs. 85%, respectively, $P = 0.39$). However, the quality of identification score of Microflex® LT seemed to be slightly higher (Fig. 1). After one week of storage, the identification scores for both instruments of the

Table 1
The identification scores of human oral *Capnocytophaga* stains (n = 34) after 48 h and 96 h incubation time. All tests were performed in triplicate (n = 102).

		Incubation time 48 h					Incubation time 96 h				
No. of strains	Organism	ID score Range	SD	No reliable ID (n)	Genus ID (n)	Species ID (n)	ID score Range	SD	No reliable ID (n)	Genus ID (n)	Species ID (n)
26	*C. ochracea*	1.71-2.41	0.495	0	78	63	1.37- 2.44	0.757	6	72	64
3	*C. sputigena*	1.79-2.37	0.410	0	9	3	1.83-2.38	0.389	0	9	3
1	*C.* genospecies 8471*	1.88-2.03	0.106	0	3	1	1.85- 1.88	0.021	0	3	0
1	*C. gingivalis*	2.06-2.20	0.099	0	3	3	1.87- 2.07	0.141	0	3	1
1	*C. granulosa*	1.58-1.78	0.141	1	2	0	1.99-2.16	0.120	0	3	2
1	*C. haemolytica*	1.30-1.56	0.184	3	0	0	1.43- 1.81	0.269	2	1	0
1	*C. leadbetteri***	2.07-2.10	0.021	0	3	3	2.02- 2.14	0.085	0	3	3
34				4 (4%)	98 (96%)	73 (71.5%)			8 (8%)	94 (92%)	73 (71.5%)

* Identified as *C. sputigena*
** Identified as *Capnocytophga* sp

Table 2
The range scores and identification results of spotting human oral *Capnocytophaga* strains (n = 34) by two examiners. All tests were performed in triplicate (n = 102).

		Examiner 1					Examiner 2				
No. of strains	Organism	ID score Range	SD	No reliable ID (n)	Genus ID (n)	Species ID (n)	ID score Range	SD	No reliable ID (n)	Genus ID (n)	Species ID (n)
26	*C. ochracea*	1.71–2.41	0.495	0	78	63	1.32–2.5	0.834	2	76	66
3	*C. sputigena*	1.79–2.37	0.410	0	9	3	1.77–2.45	0.481	0	9	5
1	*C.* genospecies 8471*	1.88–2.03	0.106	0	3	1	0–2.02	1.428	1	2	1
1	*C. gingivalis*	2.06–2.20	0.099	0	3	3	2.06–2.20	0.099	0	3	3
1	*C. granulosa*	1.58–1.78	0.141	1	2	0	0–2.15	1.520	1	2	1
1	*C. haemolytica*	1.30–1.56	0.183	3	0	0	1.23–1.83	0.424	1	3	0
1	*C. leadbetteri***	2.07–2.10	0.021	0	3	3	2.14–2.23	0.063	0	3	3
34				4 (4%)	98 (96%)	73 (71.5%)			5 (5%)	98 (96%)	79 (77%)

* Identified as *C. sputigena.*
** Identified as *Capnocytophaga* sp.

Table 3
MALDI-TOF MS identification scores of human oral *Capnocytophaga* stains (n = 34) by direct colony and formic acid extraction techniques.

No. of strains	Organism	Direct colony method			Formic acid extraction method			No. of strains in the database
		No reliable ID (%)	Genus ID (%)	Species ID (%)	No reliable ID (%)	Genus ID (%)	Species ID (%)	
26	*C. ochracea*	0 (0%)	26 (76%)	23 (67%)	1 (3%)	25 (73%)	21 (61%)	5
3	*C. sputigena*	0 (0%)	3 (9%)	1 (3%)	0 (0%)	3 (9%)	3 (9%)	10
1	*C.* genospecies 8471*	0 (0%)	1 (3%)	1 (3%)	0 (0%)	1 (3%)	0 (0%)	0[a]
1	*C. gingivalis*	0 (0%)	1 (3%)	1 (3%)	0 (0%)	1 (3%)	1 (3%)	6
1	*C. granulosa*	0 (0%)	1 (3%)	0 (0%)	0 (0%)	1 (3%)	1 (3%)	2
1	*C. haemolytica*	1 (3%)	0 (0%)	0 (0%)	0 (0%)	1 (3%)	1 (3%)	1
1	*C. leadbetteri***	0 (0%)	1 (3%)	1 (3%)	0 (0%)	1 (3%)	1 (3%)	2[b]
34		1 (3%)	33 (97%)	27 (79%)	1 (3%)	33 (97%)	28 (82%)	

* Identified as *C.sputigena*
** Identified as *Capnocytophga* sp
[a] Not included in the database.
[b] *Capnocytophaga* species

reanalyzed plates were decreased significantly ($P < 0.001$) (Table 4). Still, it remained evident that the Microflex LT instrument delivered a remarkable ability to identify isolates compared to the MALDI Biotyper MBT Smart instrument ($P < 0.001$).

4. Discussion

The reproducibility of human *Capnocytophaga* species identification is crucial in avoiding delays in clinical diagnosis and selecting suitable treatment regimens for *Capnocytophaga*-related infections. In the present study, after examining potential methodological factors affecting the performance of MALDI-TOF MS in identifying the human *Capnocytophaga* species, we found that the quality and reproducibility of MALDI-TOF MS and spectra of human *Capnocytophaga* species can be ensured after following the universal identification protocol for anaerobic bacteria. Formic acid extraction is only necessary for species with a limited number of strains in the database, such as *C. haemolytica* and *C. granulosa*.

Despite the ability of MALDI-TOF MS to identify an increasing variety of bacterial species quickly and correctly by using a universal protocol (Liu et al., 2007), some genera still need modifications in the universal protocol to ensure the maximum identification quality of relevant clinical species (Tudó et al., 2015). Modifications required to change protocols include an extended incubation time and protection from oxygen exposure of anaerobic species (Veloo et al., 2014). In addition, the inter- and intra-examiner reproducibility of the spot, type of MALDI-TOF MS instrument used, storage time of MALDI-TOF MS plate, and a limited number of targeted species and their spectra profiles in the database are considered essential factors affecting the quality of MALDI-TOF MS identification (Alatoom et al., 2011; Veloo et al., 2016).

The incubation time is a critical factor influencing the physical characteristics of bacteria, which can be reflected in the identification score value. For example, if subjected to different growth conditions, the same bacterial isolate can be identified with differing score values

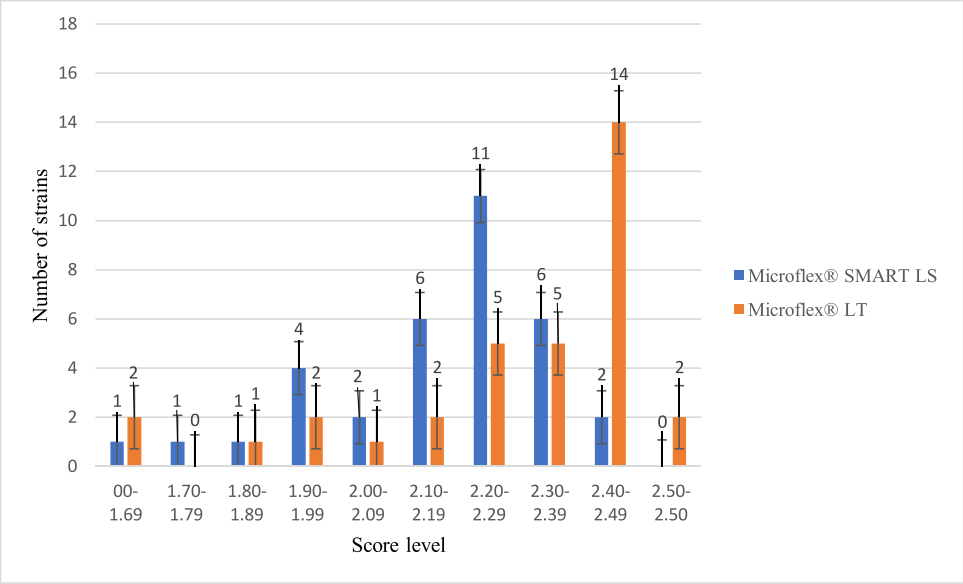

Fig. 1. Identification scores quality of human oral *Capnocytophaga* stains (n = 34) by using the Microflex® SMART LS (120 Hz) and Microflex® LT (60 Hz) instruments.

Table 4
The identification scores of human oral *Capnocytophaga* strains (n = 34) by the Microflex® SMART LS (120 Hz) and Microflex® LT (60 Hz) instruments after immediate run and one week storage time run.

No. of strains	Organism	Microflex® SMART LS					Microflex® LT					
		ID score Range	SD	No reliable ID (n)	Genus ID (n)	Species ID (n)	ID score Range	SD	No reliable ID (n)	Genus ID (n)	Species ID (n)	
26	*C. ochracea*	1.71-2.41	0.495	0	78	63	1.55- 2.53	0.650	1	77	71	
3	*C. sputigena*	1.79-2.37	0.410	0	9	3	1.85- 2.45	0.424	0	9	3	
1	*C.* genospecies 8471*	1.88-2.03	0.106	0	3	1	1.77- 2.02	0.177	0	3	2	
1	*C. gingivalis*	2.06-2.20	0.099	0	3	3	2.09- 2.22	0.092	0	3	3	Immediate run
1	*C. granulosa*	1.58-1.78	0.141	1	2	0	1.19-1.22	0.021	3	0	0	
1	*C. haemolytica*	1.30-1.56	0.184	3	0	0	1.27-1.35	0.057	3	0	0	
1	*C. leadbetteri***	2.07-2.10	0.021	0	3	3	2.10- 2.13	0.021	0	3	3	
34				4 (4%)	98 (96%)	73 (71.5%)			7 (7%)	95 (93%)	82 (80%)	
26	*C. ochracea*	0.0- 2.28	1.612	12	66	42	0.0- 2.48	1.754	2	76	66	
3	*C. sputigena*	0.0- 2.32	1.640	4	5	3	1.73- 2.57	0.594	0	9	3	
1	*C.* genospecies 8471*	0.0- 1.91	1.350	1	2	0	0.0- 0.0	0	3	0	0	
1	*C. gingivalis*	0.0- 2.13	1.506	1	2	1	0.0- 2.11	1.492	2	1	1	Run post 1 week storage
1	*C. granulosa*	0.0- 1.72	1.216	2	1	0	1.21-1.6	0.276	3	0	0	
1	*C. haemolytica*	0.0- 0.0	0	3	0	0	0.0- 1.4	0.989	3	0	0	
1	*C. leadbetteri***	1.88- 1.97	0.063	0	3	0	1.96-2.18	0.156	0	3	2	
34				23 (23%)	79 (77%)	46 (45%)			13 (13%)	89 (87%)	72 (70%)	

* Identified as *C.sputigena*
** Identified as *Capnocytophga* sp

(Sauer and Kliem, 2010). The growth rate of most anaerobic bacteria is slow and differs from one species to another. Some species are identified well after 24 h of incubation; others need up to 96 h to be identified (Veloo et al., 2014). Human *Capnocytophaga* species are slow-growing microorganisms; during the first 24 h of incubation, the plates have tiny pinpoint colonies. The colony size increases during the 48 h and reaches its maximum size after 96 h of incubation (Socransky et al., 1979). The present study found that although the shape and size of colonies were more defined and determined at 96 h than colonies incubated at 48 h, the morphological differences did not affect the identification rate (Table 1). Only the *C. haemolytica* was identified at the genus level after 96 h of incubation. However, the ability of MALDI-TOF MS to identify *C. haemolytica* at the genus level only after prolonged incubation time indicated that other factors are responsible for the low identification percentage. Veloo and coauthors studied the influence of incubation time on the MALDI-TOF MS identification quality of anaerobic bacteria; it was concluded that the suitable incubation time for most anaerobic bacteria is 48 h (Veloo et al., 2014). Furthermore, the spotting accuracy and the need for chemical extraction were considered more critical than an extended incubation time. Thus, we assumed that 48 h incubation time is enough for human oral *Capnocytophaga* species.

The smear quality referred to the amount of bacterial cells spotted on the MALDI-TOF MS plate. In the literature, the thickness of the smear has been highlighted as an important factor for the identification quality (Williams et al., 2003; Ford and Burnham, 2013). Once the direct spotting of the bacterial smear is entirely manual, it depends on the examiner's previous experience with the morphology and growth behavior of the target species on the agar plate (Veloo et al., 2014). Most human *Capnocytophaga* colonies have a defined convex shape; however, some *Capnocytophaga* species have colonies with flat, ill-defined boundaries, and colonies are subsurface in nature, affecting the spectra distortion possibilities, and therefore, special care is necessary

for picking up colonies without transferring agar media on the plate. We examined the inter- and intra-reproducibility of spotting between two examiners, a professional lab technician and a well-trained researcher. The lab technician was faster, while the latter followed the manufacturer's instructions for direct spotting slowly and carefully. As a result, the regular, well-defined colonies were identified without any statistical difference between the two examiners. On the other hand, the researcher was more familiar with the morphology and growth characteristics of human *Capnocytophaga*, thus leading to better scores with *C. haemolytica*, *C. gingivalis*, and *C. leadbetteri* isolates with irregular colonies and pitting on the agar surface (Table 2).

Although formic acid extraction results in more accurate and reliable identification scores than the direct method, its use is limited to the difficult-to-identify isolates because of the need for more preparation steps than the direct method (Bizzini et al., 2010; Fournier et al., 2012; Theel et al., 2012). Nevertheless, there is a general agreement on the advantage of using formic acid extraction for microbial species with a limited representation in the database (Bizzini et al., 2010; van Veen et al., 2010; Theel et al., 2012; Gürsoy et al., 2017). Regarding human *Capnocytophaga*, we did not find a significant advantage in using formic acid extraction compared to the direct method. Both methods resulted in similar identification rates at the genus level (97%) and species level (82% and 79%, respectively). According to our findings, formic acid extraction played a fundamental role in identifying *C. haemolytica* and *C. granulosa*, as these species are seldom found in the database and cannot be identified to the species level using the direct method (Table 3). By employing the formic acid extraction method, MALDI-TOF MS achieved a 100% identification score for both species, whether at the genus or species level. A study comparing these methods in identifying gram-positive cocci demonstrated the superiority of formic acid extraction over the direct method when the database contains $\leq$5 spectra (Alatoom et al., 2011).

Commercially, MALDI-TOF MS Microflex series BioTyper® (Bruker Daltonics, Bremen, Germany) and VITEK® MS Plus (bioMérieux, Marcy l 'Etoile, France) are main MALDI-TOF MS systems used for microbial identification (Jang and Kim, 2018). Many researchers compared the identification performance between the two systems (Martiny et al., 2012; Zangenah et al., 2012; Ha et al., 2018; Rocca et al., 2019). None of them compared the identification performance of different series in the same system. Since the Clinical Microbiology Laboratory at Turku University Hospital recently installed a newer MALDI-TOF instrument (MALDI Biotyper MBT Smart), we had the opportunity to compare two MALDI-TOF MS Microflex series, the newly installed MALDI Biotyper MBT Smart and the three-year-old installed Microflex LT. Both instruments are benchtop MALDI-TOF MS spectrometers from Bruker Daltonics (Bremen, Germany). According to the manufacturer's brochure, the MALDI Biotyper MBT Smart is based on the Microflex LT/SH system. MALDI Biotyper MBT Smart is more compact, lighter, and using smartbeam™ Lifetime Laser technology. The laser repetition rates are up to 200 Hz, i.e., three times faster than Microflex LT/SH with 60 Hz laser repetition rates. However, instead of improving the performance of identifying human *Capnocytophaga* species in our study, the newer technology failed to reach the percentage score of the older technology. Although there was no statistical difference between the two instruments' results, the identification quality at the species level of the Microflex LT instrument was slightly higher than that of the MALDI Biotyper MBT Smart (Fig. 1).

In the present study, the species-level cut-off and identification scores of two instruments after storage of plates for one week suggest that laser repetition rates of 60 Hz may give more accurate and reliable scores at the species level of identification than 200 Hz (Table 4). This indicates that low laser repetition rates and increased analysis time allow the instrument to create clearer spectra and identify the most suitable peak for certain species. Using a prototype MALDI TOF for examining peptides within alpha-cyano-4-hydroxycinnamic acid (CHCA) thin films, Spraggins and Caprioli (2011) found that at higher repetition rates, laser pulses overlap excessively and lead to poor spectra quality thus lowering the repetition rate for a given energy increases ion intensity detection per pixel and laser pulse.

5. Conclusion

The investigation of potential factors affecting the reproducibility and quality of MALDI-TOF MS identification for human *Capnocytophaga* species revealed that the universal protocol of the anaerobic bacteria is useful for the most members of this genus as long as they are adequately represented in the database. However, for *C. haemolytica* and *C. granulosa*, which have limited database entries, formic acid extraction is essential to enhance species-level identification.

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CRediT authorship contribution statement

Ahmed Algahawi: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Inka Harju:** Writing – review & editing, Methodology, Investigation. **Eija Könönen:** Writing – review & editing, Validation, Supervision, Methodology. **Kaisu Rantakokko-Jalava:** Writing – review & editing, Resources, Methodology. **Mervi Gürsoy:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflicts of interests.

Data availability

Data will be made available on request.

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