

# Identification of coagulase-negative staphylococci by the Bruker MALDI-TOF Biotyper compared to the Vitek 2 and MIS gas liquid chromatography

## Abstract

Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry has revolutionized clinical microbiology, enabling the rapid and accurate identification of micro-organisms; it has become the standard in clinical microbiology laboratories. We evaluated the Bruker MALDI-TOF Biotyper ability to identify 485 Coagulase-negative Staphylococci (CNS) isolated from clinical specimens and previously identified by conventional techniques. Discrepant results were resolved by 16S rRNA analysis by a reference laboratory. Overall the Bruker BioTyper performed well identifying 485 (100%) of the CNS correctly to the species level, while the Vitek 2 identified 88.6% and the MIS gas liquid chromatography 60.8% correctly. A single discrepant result resolved by 16S rRNA analysis was confirmed to be *S. xylosus*, as identified by the Bruker BioTyper. We concluded that the Bruker BioTyper is an excellent identification modality for CNS in the routine microbiology laboratory.

**Keywords:** MALDI-TOF, identification, coagulase-negative, staphylococci

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## Introduction

Coagulase-negative Staphylococci (CNS), once considered to be commensals or contaminants of clinical specimens are now recognized as potential and opportunistic pathogens. Forty different species are now recognized to be of significance in human disease.<sup>1-3</sup>

CNS are associated with many different infections and among others causes infections of foreign bodies like prosthetic joints<sup>4</sup> and they are considered to be a major cause of line associated bacteremia in hospitalized patients.<sup>5,6</sup> Coagulase-negative Staphylococci are well-known to cause other significant infections; acting as opportunistic pathogens in surgical site infections, colonization of vascular access devices or as a urinary tract pathogen such as *Staphylococcus saprophyticus*.<sup>4,5</sup> Human diseases are most frequently caused by *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus saprophyticus*. Other significant opportunistic pathogens include *Staphylococcus hominis*, *Staphylococcus warneri*, *Staphylococcus capitis*, *Staphylococcus simulans*, *Staphylococcus cohnii*, *Staphylococcus xylosus*, *Staphylococcus saccharolyticus* and *Staphylococcus lugdunensis*.<sup>2,3,7</sup> Many different factors contribute to the disease progression of CNS associated infections including the species, host-pathogen interactions, the immune status of the host and the ability of the bacteria to produce a biofilm.<sup>6,8,9</sup> Identification of CNS are important to ensure the correct interpretation of susceptibility results and distinguishing between species of CNS with differing propensity to cause disease, thereby enabling clinicians to distinguish between colonizers, contaminants and potential pathogens.<sup>4,5,10-12</sup>

Automated systems are available and have been used over the last 20 years to identify CNS. Systems such as the VITEK 2 (bioMérieux, Marcy l'Étoile, France) and the BD Phoenix Automated Microbiology System (BD Diagnostics, Sparks, MD)<sup>10,11</sup> have been evaluated as to their ability to identify these bacteria to the species and subspecies level. These automated identification and susceptibility systems are hampered by the organism's ability to express specific metabolic

activity and morphological features. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) has revolutionized clinical microbiology laboratories making rapid and accurate identification possible.<sup>13</sup> A study performed by Wragg and associates compared the identification of seventy six bacterial isolates by the Biolog GEN III MicroStation semi-automated bacterial identification system and the VITEK MS matrix-assisted laser desorption ionization-time of flight mass spectrometry.<sup>14</sup> Five isolates were *Staphylococci* that were identified correctly by both systems compared to 16S ribosomal RNA gene sequencing.<sup>14</sup> To the best of our knowledge studies comparing the Bruker MALDI-TOF BioTyper and other automated identification systems for identifying CNS are lacking in Canada.

## Objective

The objective of this study was to compare the identification capability of the Bruker MALDI-TOF BioTyper to the Vitek 2 (bioMérieux) and Microbial Identification System (Microbial ID Inc.) gas liquid chromatography of coagulase negative staphylococci to the species level using isolates previously characterized biochemically and phenotypically.

## Materials and methods

### Coagulase negative staphylococci

CNS isolates were obtained from clinical specimens at London Health Sciences, London, Ontario from specimens submitted to the microbiology laboratory. Strains isolated from significant sources such as blood, cerebrospinal fluid, peritoneal fluid and catheter tips were saved in glycerol citrate at -70°C and included in the study. Duplicate isolates from the same patient were excluded.

Four hundred and eighty five CNS were selected from a bank of organisms previously identified to the species (sp.) and subspecies (subsp.) level. The organisms were identified according to the

classification scheme of Kloos and Schleifer.<sup>12,15-17</sup> All of the CNS isolates were identified by VITEK-2 (GP #21342, BioMerieux), Microbial Identification System (MIS) (Microbial ID Inc. Newark, Del.) which uses gas liquid chromatography<sup>18</sup> and Bruker MALDI-TOF BioTyper (Bruker Daltonics, Germany) the results were compared and discrepant results were resolved by 16S rRNA sequencing.

The following strains were included to be identified by the Bruker BioTyper MALDI-TOF (Figure 1): *S. capitis* (n=100), *S. caprae* (n=50), *S. capitis*, *S. cohnii* (n=49), *S. epidermidis* (n=90), *S. haemolyticus* (n=25), *S. hominis* (n=17), *S. intermedius* (n=7), *S. lugdunensis* (n=25), *S. saprophyticus* (n=25), *S. schleiferi* (n=22), *S. simulans* (n=24), *S. warneri* (n=24) and *S. xylosus* (n=27).

### American type culture collection strains

The following American Type Culture Collection (ATCC) strains were used for quality control: *Staphylococcus cohnii* ATCC 29974, *S. epidermidis* ATCC 14990, *S. haemolyticus* ATCC 29970, *S. hominis* ATCC 27845, *S. intermedius* ATCC 29663, *S. saprophyticus* ATCC 15305, *S. warneri* ATCC 27836, and *S. xylosus* ATCC 29971.

### Bruker MALDI-TOF biotyper

The CNS were cultured from glycerol citrate onto Columbia 5% sheep blood agar plates (Oxoid, MP0351) subcultured twice and incubated at 35°C in 5% CO<sub>2</sub>. The Bruker MALDI-TOF BioTyper 48 target plate was inoculated with bacterial test standard (Bruker #8255343) and calibration was performed according to the recommendations of the manufacturer. The test organisms were applied to each target spot as a thin monolayer using the direct colony transfer method according to the manufacturer's recommendation. The target spot was allowed to dry and 1 µL of 70% formic acid was added, followed by 1 µL of matrix (Bruker, HCCA #8255344) and the target plate was allowed to dry at room temperature.

### Identification criteria for the Bruker MALDI-TOF biotyper

The identifications of the isolates were performed on a Microflex LT instrument (Bruker Daltonics, Germany) with Flex Control (version 3.0) software (Bruker Daltonics, Germany) for the automatic acquisition of mass spectra in the linear positive mode within a range of 2 to 20 kDa. Automated analysis of the raw spectral data was performed by the MALDI BioTyper automation (version 2.0)

software (Bruker Daltonics, Germany).

### Identification criteria were followed as stipulated by the manufacturer

The identification criteria included a score, a color grading and a consistency category. If identification was not considered acceptable according to the recommended criteria the identification was repeated, the result was recorded as a failed identification if all of the acceptance criteria were not met on repeat testing. The final Bruker MALDI-TOF BioTyper identification results were compared to the identification by Vitek2 and MIS gas liquid chromatography.

### Discordant results

Results were considered to be discordant if the Bruker MALDI-TOF BioTyper identification did not correlate with the anticipated species. All discordant results at the species level were sent for 16S rRNA sequencing at a reference facility.

## Results

### Bruker MALDI-TOF biotyper results

A total of 485 CNS isolates were identified by the Bruker MALDI-TOF BioTyper (B). The identifications were evaluated according to highest and lowest scores (hS, lS), best and worst consistency (bC, wC) for each species and the average (A) score for next possible identification as seen in Table 1.

The Bruker MALDI TOF BioTyper correctly identified 470 (96%) of the isolates on the initial identification run, thirteen isolates of *S. cohnii*, one *S. warneri* and one *S. xylosus* were re-identified on the Bruker BioTyper as they failed their initial identification run based on their consistency (C-) and their initial score of less than 1.7. Subsequently they all passed the identification criteria and identified as the correct species. The final results reflected that the Bruker MALDI TOF BioTyper identified 485 (100%) of the isolates correctly to the species level (score ≥ 2.0).

### Comparative data for the three identification systems

The identification results were subsequently compared to the results obtained by the VITEK 2 (V) and MIS gas liquid chromatography (M) these results are presented in Table 2.

**Table 1** Summary of the identification results by the Bruker MALDI-TOF BioTyper (Daltonics). Δ One isolate of the *S. saprophyticus* were identified as a *S. xylosus* (hS=2.216, bC=A++, next sp. score=1.648) this isolate passed the Bruker MALDI-TOF BioTyper criteria for identification and was sent for 16S rRNA sequencing. The isolate was confirmed as a *S. xylosus*, Bruker BioTyper result was there for considered to be correct.

Staphylococci	Number tested (n=485)	Bruker MALDI-TOF ID correct	Consistency		MALDI-ToF score value		Next sp. value		Average score difference with next sp.	Result
			Best	Worst	Highest	Lowest	Highest	Lowest		
<i>S. capitis</i>	100	100 (100%)	A+++	A++	2.326	2.2	2.247	2.24	0.158	Pass
<i>S. caprae</i>	50	50 (100%)	A+++	C-	2.081	1.653	1.76	1.378	0.440	Pass
<i>S. cohnii</i>	49	49 (100%)	B+	C-	1.804	1.654	1.768	1.317	0.252	Pass
<i>S. epidermidis</i>	90	90 (100%)	A+++	B+	2.32	1.857	1.69	1.69	0.382	Pass
<i>S. haemolyticus</i>	25	25 (100%)	A+++	B+	2.357	1.916	1.824	1.335	0.746	Pass
<i>S. hominis</i>	17	17 (100%)	A+++	A++	2.348	2.189	2.085	1.354	0.757	Pass
<i>S. intermedius</i>	7	7 (100%)	A++	B+	2.062	1.895	2.014	1.083	0.241	Pass
<i>S. lugdunensis</i>	25	25 (100%)	A++	B+	2.373	1.948	1.763	1.354	0.641	Pass

Table Continued..

Staphylococci	Number tested (n=485)	Bruker MALDI-TOF ID correct	Consistency		MALDI-TOF score value		Next sp. value		Average score difference with next sp.	Result
			Best	Worst	Highest	Lowest	Highest	Lowest		
<i>S. saprophyticus</i> Δ	25	24 (96%)	A++	B+	2.175	1.919	1.783	1.384	0.497	Pass
<i>S. schleiferi</i>	22	22 (100%)	A+++	B+	2.322	1.889	1.831	1.296	0.485	Pass
<i>S. simulans</i>	24	24 (100%)	A++	B+	2.332	2.227	1.525	1.295	0.848	Pass
<i>S. warneri</i>	24	24 (100%)	A++	C-	2.083	1.623	1.82	1.352	0.427	Pass
<i>S. xylosus</i> Δ	27	27 (100%)	A++	C-	2.366	1.686	1.684	1.391	0.630	Pass

**Table 2** Comparative adjusted identification results of coagulase negative staphylococci by the Bruker MALDI-TOF BioTyper (Daltronics), Vitek-2 (bioMerieux) and Microbial Identification System (Microbial ID Inc.). \*The results were adjusted in Table 2 and reflect that there were only 24 isolates of *S. saprophyticus* subsp. *saprophyticus* and 28 isolates of *S. xylosus* tested

	Number	MALDI-TOF correct-sp. level	VITEK-2 % correct	MIDI % correct
<i>S. capitis sub sp. ureolyticus</i>	100	50/50 (100%)	27/50 (54%)	36/50 (62%)
<i>S. caprae</i>	50	50/50 (100%)	45/50 (90%)	44/50 (88%)
<i>S. cohnii</i>	49	49/49 (100%)	44/49 (90%)	22/49 (45%)
<i>S. epidermidis</i>	90	90/90 (100%)	88/90 (98%)	18/90 (20%)
<i>S. haemolyticus</i>	25	25/25 (100%)	24/25 (96%)	23/25 (92%)
<i>S. hominis</i>	17	17/17 (100%)	16/17 (94%)	11/17 (64%)
<i>S. intermedius</i>	7	7/7 (100%)	5/7 (71%)	3/7 (43%)
<i>S. lugdunensis</i>	25	25/25 (100%)	19/25 (76%)	22/25 (88%)
<i>S. saprophyticus</i> *	24	24/24 (100%)	19/24 (79%)	22/24 (92%)
<i>S. schleiferi</i>	22	22/22 (100%)	22/22 (100%)	20/22 (91%)
<i>S. simulans</i>	24	24/24 (100%)	23/24 (96%)	13/24 (54%)
<i>S. warneri</i>	24	24/24 (100%)	23/24 (96%)	8/24 (33%)
<i>S. xylosus</i> *	28	28/28 (100%)	25/28 (89%)	27/28 (96%)
Totals	485	485	431	295
Percentage		100%	88.60%	60.80%

One isolate of *S. saprophyticus* was identified as *S. xylosus* (hS=2.216, bC=A++, next sp. score=1.648) by the Bruker MALDI-TOF BioTyper and by the MIS gas liquid chromatography. This isolate was confirmed by a reference laboratory with 16S rRNA analysis to be *S. xylosus*. Vitek 2 identified this isolate as *S. warneri*. The final results for *S. saprophyticus* and *S. xylosus* were adjusted and were as follows *S. saprophyticus* (n=24, B<sub>sp.</sub>=100%, B<sub>subsp.</sub>=100% V<sub>sp.</sub>=79%, V<sub>subsp.</sub>=79%, M<sub>sp.</sub>=92%, M<sub>subsp.</sub>=92%) and *S. xylosus* (n=28, B<sub>sp.</sub>=100%, V<sub>sp.</sub>=89%, M<sub>sp.</sub>=96%).

Overall the Bruker MALDI-TOF identified 485(100%) of the isolates to the species level, Vitek 2 identified 88.6% and the MIS gas liquid chromatography identified 60.8% of the coagulase staphylococcus correct to the species and subspecies where applicable.

## Discussion

This study compared the ability of the Bruker MALDI-TOF BioTyper to identify CNS to the species level. All isolates were identified with all three systems; this presented us with an excellent opportunity to evaluate the Bruker MALDI-TOF BioTyper.

CNS consists of organisms playing a complex and diverse role in infectious diseases, ranging from normal microbiota to opportunistic pathogens and accurate identification may be important to ensure effective patient care.<sup>5</sup> The ease of use of the Bruker MALDI-TOF BioTyper makes it an attractive method. Current automated systems most frequently used in diagnostic laboratories include Vitek2 (bioMerieux) and the BD Phoenix system (Becton Dickinson).<sup>19</sup> There is a lack of reports and or studies comparing these systems ability to identify CNS with the Bruker MALDI-TOF BioTyper. Ligozzi et al.,<sup>20</sup> and Spanu et al.,<sup>21</sup> respectively reported success rates of identifying CNS with the Vitek 2 to the species level of 86% and 90.5% both these studies noted that the identification of *S. hominis* was problematic and needed improvement. Funke and Funke-Kissling<sup>22</sup> used the colorimetric based VITEK 2 ID-GP identification card and concluded that the Vitek2 was reliable in identifying staphylococci correctly.<sup>22</sup> In a study performed by Wragg and associates 5 isolates of CNS were identified to the correct genus and species level by the VITEK MS giving overall comparable results to the Biolog GEN III MicroStation semi-automated bacterial identification system and 16S ribosomal RNA gene sequencing.<sup>14</sup>

In our study we evaluated the ability of the Bruker MALDI-TOF BioTyper to identify most of the clinically relevant CNS that we routinely encounter in our laboratory. During the study we included eight American Type Culture Collection (ATCC) strains in every run of identifications to act as positive controls and reference isolates. All the instruments correctly identified the ATCC strains.

The Bruker MALDI-TOF BioTyper performed exceptionally well, speciating all four hundred and eighty five (100%) of the coagulase negative staphylococci compared to Vitek-2's 88.6% and MIDI 60.8% (Table 2). Although we did not intentionally pursue or include subspeciating CNS we noted that *S. capitis* sub sp. *ureolyticus* posed some problems with 48 of 50 (96%) isolates being identified as *S. capitis* sub sp. *capitis* by the Bruker BioTyper. Retesting of *S. capitis* sub sp. *ureolyticus* using a thinner monolayer on the target plate had no impact on the final identification. All of the *S. capitis* sub sp. *ureolyticus* had excellent scores in excess of 2.00, similarly the *S. capitis* sub sp. *capitis* had equally good results. This probably is indicative of the close relationship of these two species. One isolate *S. saprophyticus* was identified as a *S. xylosus* by both the Bruker BioTyper and the MIS gas liquid chromatography and we subsequently confirmed this identification by 16S rRNA sequencing. The results were adjusted accordingly and reflected that all of the *S. saprophyticus* (n=24) were in fact identified correctly.

The Bruker BioTyper databank supports identification of relevant staphylococci to the subspecies level. Subspeciating was however not part of the initial objective of this study, it was noted however that where applicable it did perform adequately in subspeciating the isolates correctly with the exception of *S. capitis* where a 48 (96%) of subspecies *urealyticus* were identified as subspecies *capitis*. This was noted with all three systems, indicating that these two subspecies are very closely related. The clinical impact of such an error is questionable, with no anticipated change in antibiotic treatment or disease spectrum. Since this study has been completed an update of the Bruker MALDI-TOF BioTyper databank has been performed, this update may actually improve identification of these closely related subspecies. In conclusion the Bruker MALDI-TOF BioTyper presents a simple and accurate identification system for coagulase negative staphylococci in the routine diagnostic microbiology laboratory.

## Acknowledgements

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## Conflict of interest

The author declares no conflict of interest.

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