

Identification of *Neisseria gonorrhoeae* by the Bruker Biotyper Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry System Is Improved by a Database Extension

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Identification of *Neisseria gonorrhoeae* by the Bruker matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) system may be affected by “B consistency categorization.” A supplementary database of 17 *N. gonorrhoeae* main spectra was constructed. Twelve of 64 *N. gonorrhoeae* identifications were categorized with B consistency, which disappeared using the supplementary database. Database extension did not result in misidentification of *Neisseria meningitidis*.

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) yields highly reliable, rapid bacterial identifications. The Bruker MALDI-TOF MS system uses the best database match of a mass spectrum for identification. MALDI-TOF MS may misidentify species within closely related bacteria such as the *Streptococcus mitis*, *Enterobacter cloacae*, *Acinetobacter baumannii*-*Acinetobacter calcoaceticus*, *Bacillus cereus*, and *Burkholderia cepacia* complexes due to highly similar mass spectra (1–5). The Bruker Biotyper MALDI-TOF MS system adds a “category B consistency” warning when the list of top 10 database matches contains different species of the same genus with score values of ≥ 2.000 . We and others observed that B consistency categorizations of *Neisseria gonorrhoeae* isolates frequently occur with *Neisseria meningitidis* score values of ≥ 2.000 (6). B consistency categorizations indicate uncertainty about the true identification of the organisms, which is of concern because *N. gonorrhoeae* and *N. meningitidis* may be isolated from the same body sites (7). Misidentification of *N. gonorrhoeae* can have implications for diagnosis in patients and their sexual partners and for public health. An extension of the database may improve the MALDI-TOF MS identification results (8). We tested whether a database extension of 17 *N. gonorrhoeae* main spectra (MSPs) (NG17) superimposed on the MBT-BDAL-5627 MSP library (5627) resolved B consistency categorizations in *N. gonorrhoeae* identifications without affecting *N. meningitidis* identifications using the Bruker Biotyper system. Eighty-one *N. gonorrhoeae* unrelated (i.e., one isolate per individual patient) isolates were collected (see Table S1 in the supplemental material). *N. gonorrhoeae* isolates were confirmed as *N. gonorrhoeae* by the Cobas 4800 CT/NG PCR (Roche Molecular Diagnostics, Almere, The Netherlands), performed according to the manufacturer’s instructions. Quality control (QC) strains from the Gonococcal Resistance against Antibiotics Surveillance project (GRAS) QC distribution, which is a national surveillance program for *N. gonorrhoeae* resistance, were not reconfirmed by PCR. Seventeen isolates were used for construction of the supplementary NG17 MSP library (see Table S1). Sixty-four *N. gonorrhoeae* isolates were used to test the databases. Thirty *N. meningitidis* isolates were collected at the University Medical Centre Utrecht (UMCU) and isolated from either blood ($n = 18$) or cerebrospinal fluid ($n = 11$) or from a

quality control distribution ($n = 1$). *N. meningitidis* isolates from invasive meningococcal disease were confirmed by The Netherlands Reference Laboratory for Bacterial Meningitis, Amsterdam, The Netherlands (Gram-negative diplococci, gamma-glutamyl aminopeptidase positive, reacting with one of the serogrouping antibodies, typable using *fetA* and *porA* molecular typing methods). Twenty-seven of 30 *N. meningitidis* isolates were also tested and found negative in the *N. gonorrhoeae* PCR. Direct transfer preparation was performed in duplicate on polished steel target plates (Bruker, Bremen, Germany) after a 1- or 2-day incubation (depending on whether sufficient biomass was harvested from a colony) on chocolate agar (chocolate agar GC II Agar with IsoVitalX, 254060; BD, NJ, USA) under 5% CO₂, using a sterilized wooden toothpick. α -Cyano-4-hydroxycinnamic acid (HCCA) matrix (Bruker, Bremen, Germany) was applied, and the plates were air dried. Analyses were performed using the Bruker Microflex MALDI mass spectrometer, Biotyper software, and the MBT-BDAL-5627 MSP library (5627) (Bruker) according to the manufacturer’s instructions (9). Acquired spectra were reanalyzed with the NG17 database extension to the 5627 database (5627+NG17).

MSPs were generated after a 2-day incubation as described previously (8). MSPs were created using 240 shots with a maximum of 80 shots per raster spot. Eight target spots were used per isolate, and 3 spectra were acquired per target spot. Subsequently,

Received 12 January 2016 Returned for modification 13 January 2016

Accepted 13 January 2016

Accepted manuscript posted online 13 January 2016

Citation Schweitzer VA, van Dam AP, Hananta IPY, Schuurman R, Kusters JG, Rentenaar RJ. 2016. Identification of *Neisseria gonorrhoeae* by the Bruker Biotyper matrix-assisted laser desorption ionization–time of flight mass spectrometry system is improved by a database extension. J Clin Microbiol 54:1130–1132. doi:10.1128/JCM.00016-16.

Editor: E. Munson

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Supplemental material for this article may be found at <http://dx.doi.org/10.1128/JCM.00016-16>.

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TABLE 1 *N. gonorrhoeae* MALDI-TOF score values are increased and B consistency categorizations are eliminated by database extension with 17 *N. gonorrhoeae* main spectra

Measurement	Database 5627	Database 5627 + NG17	P
No. of <i>N. gonorrhoeae</i> isolates	64	64	
Score value of the top database match (with <i>N. gonorrhoeae</i> MSP) (mean [range])	2.259 (1.875–2.453)	2.436 (1.974–2.700)	<0.05 ^a
Best database match with <i>N. gonorrhoeae</i> MSP with score value of ≥ 2.000 (no. [%]) ^c	63/64 (98)	63/64 (98)	
B consistency due to any database match among the top 10 with <i>N. meningitidis</i> MSP with score value of ≥ 2.000 (no. [%])	12/63 (19)	0/63 (0)	<0.05 ^b
Rank of the best database match with <i>N. meningitidis</i> MSP (median [range])	4 (2–7)	>10	
Score value of the best database match with <i>N. meningitidis</i> (mean [range])	1.931 (1.544–2.133)	NA ^d	
B consistency due to database match among the top 2 with <i>N. meningitidis</i> MSP with score value of ≥ 2.000 (no. [%])	7/63 (11)	0/63 (0)	<0.05 ^b

^a Wilcoxon signed-rank test.^b McNemar test for paired proportions.^c One isolate was identified to the genus level only (*N. gonorrhoeae* score value of 1.875).^d NA, not applicable.

a maximum of the 3 most aberrant spectra from these 24 spectra were deselected for MSP creation. MSP dendrograms were constructed using Biotyper 3.1 software. Results obtained with the 5627 database versus those with the 5627+NG17 database were analyzed using the Wilcoxon signed-rank test for score value results (Graph Pad Prism software version 6.02) or the McNemar test for B consistency categorization results (SPSS version 21).

Using the best database match (with a score value of ≥ 2.000), MALDI-TOF MS identified 63 of 64 *N. gonorrhoeae* isolates correctly to the species level (Table 1; see also Fig. S1A in the supplemental material). The best matches were almost uniquely (62/64; 97% of tested isolates) with a single entry from the 5627 database, i.e., *N. gonorrhoeae* MSP ATCC 49226 THL. In the 2 remaining isolates, the best match was with MSP *N. gonorrhoeae* ch012 PGM. One isolate was identified to the genus level only (best match with the 5627 database: *N. gonorrhoeae* score value of 1.875) (Table 1). All 64 identifications were confirmed by *N. gonorrhoeae*-specific PCRs (see Table S1 in the supplemental material). B consistency categorization occurred in 19% of *N. gonorrhoeae* isolates using the 5627 database (Table 1). Notably, in 11% of *N. gonorrhoeae* isolates with score values of ≥ 2.000 , a *N. meningitidis* MSP with a score value of ≥ 2.000 occurred as the second-best database match (Table 1). In a MSP dendrogram, all 17 newly created *N. gonorrhoeae* main spectra clustered together with a single 5627 MSP, *N. gonorrhoeae* ATCC 49226 THL (see Fig. S2 in the supplemental material). The 6 other *N. gonorrhoeae* MSPs from the 5627 database were more distantly related to the ATCC 49226 THL entry and the NG17 MSPs. Analyses using the 5627+NG17 database resulted in significantly increased score values and complete disappearance of B consistency categorizations in *N. gonorrhoeae* isolates (Table 1; see also Fig. S1 in the supplemental data). The best database matches were with the newly created 17 *N. gonorrhoeae* MSPs and not with the MSPs from the 5627 database (data not shown). In *N. meningitidis* isolates, no B consistency categorizations were found using either the 5627 or the 5627+NG17 database. The score values of the best database matches were unaffected by addition of the NG17 MSPs (see Fig. S1B and Table S2 in the supplemental material).

Nucleic acid amplification tests (NAAT) have become the cornerstone in the diagnosis of genital as well as many extragenital *N. gonorrhoeae* infections. However, at present, NAATs alone are insufficient for accurate detection of antibiotic resistance in cases of treatment failure. Therefore, culturing of *N. gonorrhoeae* remains an important adjunct to NAATs (10–13). We found that MALDI-TOF MS *N. gonorrhoeae* identification was highly reliable, with abrogation of B consistency categorizations by extension of the database. Reliable MALDI-TOF MS identification of *N. gonorrhoeae*, using the best database match, was also demonstrated by others (6, 14–16). Nevertheless, Bruker has not yet received FDA approval for *N. gonorrhoeae* identification using the MALDI Biotyper CA system (see http://www.accessdata.fda.gov/cdrh_docs/pdf14/k142677.pdf).

Previously, B consistency categorizations were reported in 4/5 *N. gonorrhoeae* isolates tested (6). In our study, the disappearance of B consistency categorization resulted from ≥ 10 database matches with *N. gonorrhoeae* MSPs with higher score values than the matches with *N. meningitidis* MSPs from the 5627 library, effectively displacing the *N. meningitidis* MSPs from the list of the top 10 database matches. With the 5627 database only, the best matches were with *Neisseria gonorrhoeae* ATCC 49226 THL MSP in 62 of 64 identifications. Also, this single MSP was found to closely cluster with the newly constructed NG17 MSPs in an MSP dendrogram. Moreover, this MSP was distantly related to the 6 other *N. gonorrhoeae* MSPs from the 5627 database. By merging the 5627 and NG17 databases, 2 clusters of MSPs appear (see Fig. S2 in the supplemental material): one cluster of MSPs, likely from the Iliina study, representing different Por types from Russian clinical isolates and another cluster with the ATCC 49226 THL MSP that clustered together with the NG17 MSPs (16). Subtle differences in factors such as the media used, the incubation conditions, and the instrument settings might influence *N. gonorrhoeae* isolates to better match our locally produced MSPs.

In conclusion, *N. gonorrhoeae* score values are improved and B consistency categorizations of *N. gonorrhoeae* identifications can be abrogated by the NG17 database extension without affecting *N. meningitidis* identification.

ACKNOWLEDGMENTS

We thank Nuria Roca, Judith Vlooswijk, and Peter van Bodegom for technical help and L. Spanjaard from The Netherlands Reference Laboratory for Bacterial Meningitis for advice on meningococcal identification.

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