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Guide for the interpretation of MALDI-TOF (Bruker Daltonics) results

Alternatives for microorganism identification

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<u>General Rule:</u> The Top Ten must be analyzed in every microorganism identification, and in the case of having a result including several species with score levels higher than 2,0 (Category B) the following criteria must be used to validate the interpretation:

"In order to discriminate among very similar species, a 10% divergence is recommended between the first species and the next different one in the Top Ten"

Meaning of score value

Range	Description	Symbols	Color
2.300 ... 3.000	highly probable species identification	(+++)	green
2.000 ... 2.299	secure genus identification, probable species identification	(++)	green
1.700 ... 1.999	probable genus identification	(+)	yellow
0.000 ... 1.699	not reliable identification	(-)	red

Meaning of consistency category A, B, C

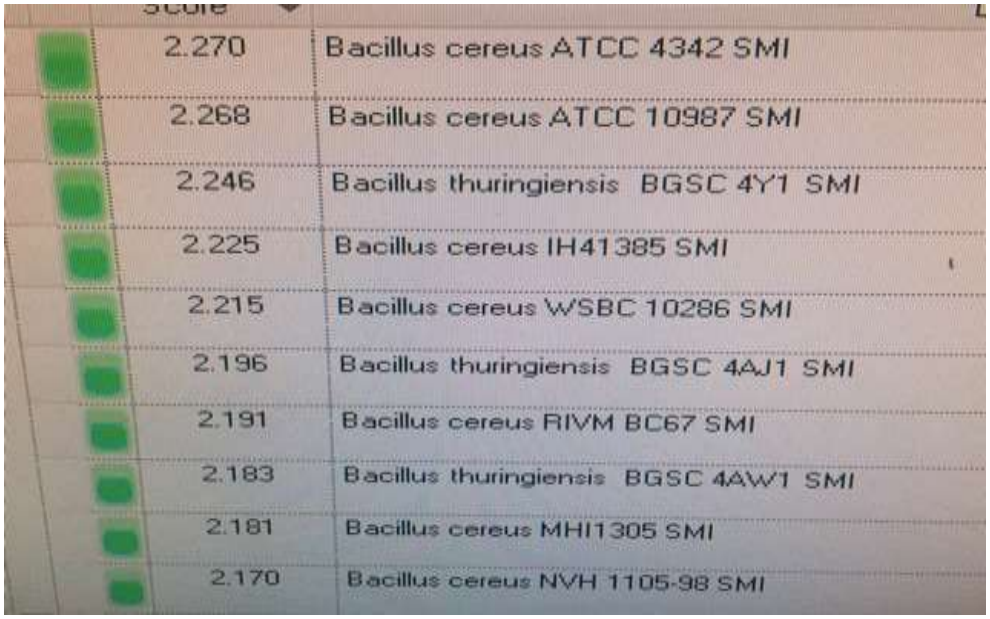
Category	Description
A	Species consistency: The best match was classified as green. Further green matches are of the same species as the first one. First yellow match are at least the same genus as the first one.
B	Genus consistency: The best match was classified as green or yellow. Further green or yellow matches are of the same genus as the first one. The conditions of species consistency are not fulfilled.
C	No consistency: Neither species nor genus consistency. Check for synonyms of names or microbial mixtures.

<u>Premise:</u> In the presence of a little-known or rare microorganism identified by mass spectrometry, the identification must be confirmed by a reference method.

Example:

Prime score: 2,270. The 10% is: 0,2270

The next different species should have a score value less than or equal to:
2,270 – 0,2270: 2,043



Score	
2.270	Bacillus cereus ATCC 4342 SMI
2.268	Bacillus cereus ATCC 10987 SMI
2.246	Bacillus thuringiensis BGSC 4Y1 SMI
2.225	Bacillus cereus IH41385 SMI
2.215	Bacillus cereus WSBC 10286 SMI
2.196	Bacillus thuringiensis BGSC 4AJ1 SMI
2.191	Bacillus cereus RIVM BC67 SMI
2.183	Bacillus thuringiensis BGSC 4AW1 SMI
2.181	Bacillus cereus MHI1305 SMI
2.170	Bacillus cereus NVH 1105-98 SMI

In this case, it is not possible to make a distinction at the species level between *Bacillus cereus* and *Bacillus thuringiensis.*

References:

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A

Abiotrophia defectiva

Organisms of genera *Abiotrophia* and *Granulicatella* are known as nutritionally variant streptococci (NVS).

The satellitism test is essential for the identification of both genera.

Abiotrophia defectiva is accurately identified by MALDI-TOF.

There are three reference strain profiles (MSPs) in the database for this microorganism.

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Note: This recommendation is based on a limited number of isolates.

References:

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Achromobacter

The taxonomy of genus *Achromobacter* is closely linked to genus *Alcaligenes.*

Most species of this genus can be differentiated by using the partial sequencing of gene *nrd*A. Alternatively, the MLST method can be used based on genes *atp*D*, icd, rec*A*, rpo*B and *gyr*B.

Partial sequencing of the 16S rRNA gene cannot differentiate among the species of the genus.

IMPORTANT: It is recommended that any species identified by MALDI-TOF should be reported as *Achromobacter* sp., since they cannot be identified to the species level using this method.

Refer to the Annex for microbial identification by phenotypic testing.

Table 1. Transcription of *Achromobacter* species of clinical origin.

Species	Present in Bruker Database (BD)	No. of MSPs in BD
A. aegrifaciens	NO	
A. animicus	NO	
A. anxifer	NO	
A. denitrificans	YES	3
A. dolens	NO	
A. insuavis	NO	
A. marplatensis	NO	
A. mucicolens	NO	
A. piechaudii	YES	2
A. pulmonis	NO	
A. ruhlandii	YES	1
A. sediminum	NO	
A. spanius	YES	2
A. xylosoxidans	YES	7
A.spiritinus	NO	
A. insolitus	YES	2

References:

- AbdulWahab A, Taj-Aldeen SJ, ibrahi, EB, Talaq E, Abu-Madi M, Fotedar R. Discrepancy in MALDI-TOF MS identification of uncommon Gram-negative bacteria from lower respiratory secretions in patients with cystic fibrosis. Infection and Drug Resistance 2015;8,83-88. doi: https://doi.org/10.2147/IDR.S80341.
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Acidovorax

Genus *Acidovorax* comprises, for the most part, environmental or pathogenic species of plants. Only *A. delafieldii*, *A. temperans*, *A facillis, A . avenae, A. oryzae,* and *A. wautersii* were isolated from clinical samples.

There is little scientific evidence to evaluate the reliability of *Acidovorax*. Given its rare occurrence in clinical isolates, and due to our limited experience with our own isolates, it is strongly suggested to only report the identification to the genus level, according to criteria recommended by the manufacturer.

That is, with **scores >1,7**, it must be reported as *Acidovorax* sp.

Refer to the Annex for microbial identification by phenotypic testing.

Table 2. Transcription of *Acidovorax* spp. species isolated from clinical samples.

Species	Present in Bruker Database (BD)	No. of MSPs in BD
A. avenae	YES	2
A. delafieldii	YES	1
A. facillis	YES	2
A. oryzae	NO	
A. temperans	YES	2
A. wautersii	NO	

References:

- Choi A, Bae J, Cha C, Chun J, Im W, Jahng KY, Jeon CO, Joh K, Kim SB, Seong CN, Yoon J, Cho J. A report of 39 unrecorded bacterial species in Korea, belonging to the *Betaproteobacteria* and *Gammaproteobacteria.* Journal of Species Research 2015;4(2):109-126. doi: 10.12651/JSR.2015.4.2.109.
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Acinetobacter

MALDI-TOF's limitations ocurr in the identification of the species within the complex *A. calcoaceticus/A. baumannii, A. junii/A. johnsonii* and *A. guillouiae,* and those species that are poorly represented or are not included in the protein spectra database, as is the case of *A. soli, A. beijerinckii, A. berenziniae, A. variabilis.*

For a complete identification to the species level, gene sequencing is required, with genes such as ***rpo*B**, *gyr*B and *rec*A.

Results will be reported following the manufacturer's recommendations, and contemplating the 10% divergence between the first species and the next different one in the Top Ten.

SCORE ≥2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE <1,7 = No identification

Table 3. Transcription of *Acinetobacter* spp species.

ID by MALDI-TOF	Present in BD	No. of MSPs in BD	Report only if score ≥ 2	Observations
A. calcoaceticus	YES	10	*Acinetobacter alcoaceticus-baumannii* Complex	
A. baumannii	YES	12	*A. baumannii*	*A. baumannii* may be identified as *A. nosocomialis.* Report *A. baumannii / nosocomialis* complex.
A. baylyi	YES	3	*Acinetobacter* sp.	Differentiate from *A. bereziniae* and *A.soli*. Confirm species with *rpo*B.
A. bereziniae	YES	1	*Acinetobacter* sp.	Confirm species with *rpo*B. Typical peaks of *A. bereziniae*: 7156, 7407, 7796 Da.

A. guillouiae	YES	2	*Acinetobacter* sp.	Confirm species using *rpo*B. Typical peaks of *A. guillouiae*: 3258, 3690, 6513, 6978, 7378, 7813 Da.
A. haemolyticus	YES	7	*A. haemolyticus*	
A. johnsonii	YES	9	*Acinetobacter* sp.	Differentiate from *A. ursingii* and *A. oleovorans* using *rpo*B.
A. junii	YES	8	*A. junii/A johnsonii*	
A. lwoffii	YES	11	*A. lwoffii*	
A. nosocomialis	YES	8	*Acinetobacter* sp.	*A. baumannii* may be identified as *A. nosocomialis.* Report *A. baumannii / nosocomialis* complex. Confirm species using *rpo*B.
A. parvus	YES	1	*Acinetobacter* sp.	Small colonies on nutritive agar. Since our experience with this species is limited, confirm species with *rpo*B.
A. pitti	YES	18	*A. pitti*	
A. radioresistens	YES	8	*A. radioresistens*	
A. schindleri	YES	1	*A. schindleri*	
A. ursingii	YES	9	*A. ursingii*	

In the cases where the identification results in the Top Ten yield *A.baumannii* and *A. nosocomialis,* they should be reported as *A. baumannii/nosocomialis* complex.

The following species are not represented in the equipment's database, so they may show as *Acinetobacter* sp., with a low score and without the 10% divergence among species. In order to confirm the identification in these cases, *rpo*B gene sequencing is recommended:

- *Acinetobacter beijerinckii*
- *Acinetobacter courvalini*
- *Acinetobacter dijkshoorniae*

- *Acinetobacter dispersus*
- *Acinetobacter gyllenbergii*
- *Acinetobacter modestus*
- *Acinetobacter proteolyticus*
- *Acinetobacter seifertii*
- *Acinetobacter soli*
- *Acinetobacter variabilis*
- *Acinetobacter venetianus*
- *Acinetobacter vivianii*

Refer to the Annex for microbial identification by phenotypic testing.

References:

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Actinobacillus

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Actinobacillus hominis is not represented in the equipment's database, and inconsistencies may arise when it is identified as other species of the genus with a high score value.

In all cases, it is recommended to confirm the identification to the species level using additional phenotypic tests (Table 5).

Table 4. Transcription of *Actinobacillus* spp. species.

ID by MALDI-TOF	Present in BD	No. of MSPs in BD	Report	Observations
A. equuli	YES	1	*Actinobacillus suis/ equuli*	
A. hominis	NO			
A. lignieresii	YES	1	*Actinobacillus lignieresii/ pleuropneumoniae*	
A. pleuropneumoniae	YES	2	*Actinobacillus lignieresii/ pleuropneumoniae*	
A. suis	YES	1	*Actinobacillus suis/ equuli*	
A. ureae	YES	1	*A. ureae*	Report with score value ≥ 2,0

Table 5. Phenotypic features of *Actinobacillus* spp species.

Assay	*Actinobacillus lignieresii*	*Actinobacillus pleuropneumoniae*	*Actinobacillus equuli*	*Actinobacillus suis*	*Actinobacillus ureae*	*Actinobacillus hominis*	*Aggregatibacter actinomycetemcomitans*
Hemolysis	-	+	V	+	-	-	-
Esculin Hydrolysis	-	-	-	+	-	V	-
Urease	+	+	+	+	+	+	-
ONPG	+	+	+	V	-	+	
Lactic acid	V	V	+	+	-	+	-
Trehalose acid	-	-	+	+	-	+	-
Melibiose acid	-	-	+	+	-	+	-

Symbols: V, variable.

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Actinobaculum/ Actinotignum

MALDI-TOF can correctly identify the species of this genus.

In the case of *Actinobaculum schaalii*, the following score values have been accepted for a reliable identification:

SCORE ≥1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

In the case of the other species (*A. urinale, A. suis, A. sanguinis*), given the scarce bibliographic data, the species should only be reported if the **score is ≥ 2,0** (as suggested by the manufacturer).

Actinobaculum masiliense species is absent in the current taxonomy, therefore, in the presence of an isolate identified as *A. massiliense* by MALDI-TOF, it should be reported as *Actinotignum* sp.

Refer to the Annex for microbial identification by phenotypic testing.

Table 6. Transcription of *Actinobaculum/Actinotignum* spp. species.

ID by MALDI-TOF	Present in BD	No of MSPs in BD	Report
A. massiliense	YES	1	*Actinotignum* sp.
A. sanguinis	YES	2	*A. sanguinis*
A. schaalii	YES	3	*A. schaalii*
A. suis	YES	2	*A. suis*
A. urinale	YES	1	*A. urinale*

References:

- Lotte R, Lotte L, Ruimy R. Actinotignum schaalii (formerly Actinobaculum schaalii): a newly recognized pathogen-review of the literature. Clin Microbiol Infect 2016;22(1):28-36. doi: 10.1016/j.cmi.2015.10.038.
- Schmitt BH, Cunningham SA, Dailey AL, Gustafson DR, Patel R. Identification of anaerobic bacteria by Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry with on-plate formic acid preparation. J Clin Microbiol 2013;51(3):782-6. doi: 10.1128/JCM.02420-12.
- Schulthess B, Bloemberg GV, Zbinden R, Böttger EC, Hombach M. Evaluation of the Bruker MALDI Biotyper for identification of Gram-

positive rods: development of a diagnostic algorithm for the clinical laboratory. J Clin Microbiol 2014;52(4): 1089-97. doi: 10.1128/JCM.02399-13.

- Yassin AF, Spröer C, Pukall R, Sylvester M, Siering C, Schumann P. Dissection of the genus Actinobaculum: Reclassification of Actinobaculum schaalii Lawson et al. 1997 and Actinobaculum urinale Hall et al. 2003 as Actinotignum schaalii gen. nov., comb. nov. and Actinotignum urinale comb. nov., description of Actinotignum sanguinis sp. nov. and emended descriptions of the genus Actinobaculum and Actinobaculum suis; and re-examination of the culture deposited as Actinobaculum massiliense CCUG 47753T (5DSM 19118T), revealing that it does not represent a strain of this species. Int J Syst Evol Microbiol 2015;65, 615–624. doi: 10.1099/ijs.0.069294-0.

Actinomyces

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Species *A. oris, A. naeslundii, A. viscosus* and *A. johnsonii* belong to a heterogeneous group that cannot be differentiated; therefore, they should be reported as *Actinomyces naeslundii* Group.

It is recommended to confirm the identification using 16S rRNA gene sequencing.

Table 7. Transcription of *Actinomyces* spp. species found in clinical samples.

ID by MALDI-TOF	Present in BD	No. of MSPs in BD	Report	Observations
A. cardiffensis	YES	1	*A. cardiffensis*	Confirm using 16S rRNA
A. dentalis	YES	1	*A. dentalis*	Confirm using 16S rRNA
A. europaeus (Funke *et al*., 1997)	YES	8	*Gleimia europea* (Nouioui *et al*., 2018)	Confirm using 16S rRNA
A. funkei	YES	3	*A. funkei*	
A. georgiae	YES	2	*A. georgiae*	Confirm using 16S rRNA
A. gerencseriae	YES	1	*A. gerencseriae*	Confirm using 16S rRNA
A. graevenitzii	YES	8	*A. graevenitzii*	Confirm using 16S rRNA In Blood Agar, it shows red fluorescence under UV light.
A. hominis	YES	1	*A. hominis*	Confirm using 16S rRNA
A. israelii	YES	3	*A. israelii*	
A. johnsonii	NO		*A. naeslundii* group	
A. meyeri	YES	11	*A. meyeri*	
A. naeslundii	YES	4	*A. naeslundii* group	
A. neuii	YES	15	*A. neuii*	
A. odontolyticus	YES	19	*A. odontolyticus*	
A. oris	YES	10	*A. naeslundii* group	
A. radicidentis	YES	1	*A. radicidentis*	Confirm using 16S rRNA
A. radingae	YES	7	*A. radingae*	Confirm using 16S rRNA
A. turicensis	YES	7	*A. turicensis*	Confirm using 16S rRNA
A. urogenitalis	YES	6	*A. urogenitalis*	
A. viscosus	YES	1	*A. naeslundii* group	

The following species are not represented in the equipment's database; therefore, they may show as *Actinomyces* sp., with a low score and without the 10% divergence among species. In order to confirm the identification in these cases, 16S rRNA gene sequencing is recommended:

- *A. hongkongensis*
- *A. johnsonii*
- *A. massiliensis*
- *A. oricola*
- *A. timonensis*

Refer to the Annex for microbial identification using additional phenotypic testing.

References:

- Barberis C, Almuzara M, Join-Lambert O, Ramırez MS, Famiglietti A, Vay C. Comparison of the Bruker MALDI-TOF mass spectrometry system and conventional phenotypic methods for identification of Gram-positive rods. PLoS One 2014;9(9):e106303. doi: 10.1371/journal.pone.0106303.
- Funke G, Alvarez N, Pascual C, Falsen E, Akervall E, Sabbe L, Schouls L, Weiss N, Collins MD. Actinomyces europaeus sp. nov., isolated from human clinical specimens. Int J Syst Bacteriol 1997;47(3):687-692. doi: 10.1099/00207713-47-3-687.
- Hall V, Collins MD, Lawson PA, Falsen E, Duerden BI. Actinomyces dentalis sp. nov., from a human dental abscess. Int J Syst Evol Microbiol 2005;55, 427–431. doi: 10.1099/ijs.0.63376-0.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Nouioui I, Carro L, García-López M, Meier-Kolthoff JP, Woyke T, Kyrpides NC, Pukall R, Klenk HP, Goodfellow M, Göker M. Genome-Based Taxonomic Classification of the Phylum *Actinobacteria*. Front Microbiol 2018;22,9:2007. doi: 10.3389/fmicb.2018.02007.
- Ramos CP, Falsen E, Alvarez N, Akervall E, Sjodén B, Collins MD. Actinomyces graevenitzii sp. nov., isolated from human clinical specimens. Int J Syst Bacteriol 1997; 47(3): 885-888. doi: 10.1099/00207713-47-3-885.
- Stingu CS, Borgmann T, Rodloff AC, Vielkind P, Jentsch H, Schellenberger W, Eschrich K. Rapid identification of oral *Actinomyces* species cultivated from subgingival biofilm by MALDI-TOF-MS. J Oral Microbiol 2015;7: 10.3402/jom.v7.26110. doi: 10.3402/jom.v7.26110.
- UK Standards for Microbiology Investigations ID 15: Identification of Anaerobic *Actinomyces* species. Public Health England 2015.

Advenella

Identification is performed according to manufacturer's instructions. There is limited experience both with our own data and with bibliographic data.

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE <1,7 = No identification

Table 8. Transcription of *Advenella* spp. species

Species	**Present in BD**	**No. of MSPs in BD**
A. incenata	YES	1
A. kashmirensis	YES	3

Aerococcus

The following score values have been accepted for a reliable identification of *Aerococcus urinae* and *Aerococcus viridans*:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Clarification: In most cases, *A. viridans* yields low score values.

Owing to limited experience, in the case of the following species it is recommended to report the identification to the species level with a **score value ≥ 2,0**:

- *A. christensenii*
- *A. sanguinicola*
- *A. urinaehominis*

Table 9. Transcription of *Aerococcus* spp. species

Species	Present in BD	No. of MSPs in BD
A. christensenii	YES	2
A. sanguinicola	YES	3
A. urinae	YES	7
A. urinaehominis	YES	1
A. viridans	YES	19

Refer to the Annex for microbial identification using additional phenotypic testing.

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Senneby E, Nilson B, Petersson AC, Rasmussen M. Matrix-assisted laser desorption ionization–time of flight mass spectrometry is a sensitive and specific method for identification of aerococci. J Clin Microbiol 2013;51(4): 1303–1304. doi: 10.1128/JCM.02637-12.

Aeromonas

Identification is performed according to manufacturer's instructions:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE <1,7 = No identification

It is not posible to make an accurate distinction among the species of the genus; it is recommended to report their identification as *Aeromonas* sp.

For an identification to the species level, *rpo*D gene sequencing needs to be carried out.

However, if the results yielded by MALDI-TOF are *Aeromonas caviae / hydrophila*, the user can perform a manual search of typical peaks and/or complete the identification using phenotypic testing (Table 11).

Aeromonas hydrophila: 2222Da, 4322Da, 4450Da, 6026Da.

Report as ***Aeromonas hydrophila* Complex***: A. hydrophila, A. bestiarum, A. salmonicida.*

Aeromonas caviae: 2942Da, 3852Da, 4305Da, 4976Da, 5886Da, 7701Da.

Report as ***Aeromonas caviae* Complex***: A. caviae, A. media, A. eucrenophila.*

Report as ***Aeromonas veronii* Complex*:*** includes species *A. veronii, A. jandaei, A. schubertii, A. trota.*

Table 10. Transcription of *Aeromonas* spp. species

Species	**Present in BD**	**No. of MSPs in BD**	**Report**
A. bestiarum	YES	2	*Aeromonas hydrophila* Complex
A. caviae	YES	3	*Aeromonas caviae* Complex
A. dhakensis	NO		
A. eucrenophila	YES	2	*Aeromonas caviae* Complex
A. hydrophila	YES	5	*Aeromonas hydrophila* Complex
A. ichthiosmia	YES	1	In today's taxonomy, it is equivalent to *Aeromonas veronii*
A. jandaei	YES	2	*Aeromonas veronii* Complex
A. media	YES	4	*Aeromonas caviae* Complex
A. punctata	YES	1	In today's taxonomy, it is equivalent to *Aeromonas caviae*
A. salmonicida	YES	5	*Aeromonas hydrophila* Complex
A. schubertii	YES	2	*Aeromonas veronii* Complex
A. sobria	YES	2	*A. sobria*
A. trota	NO		*Aeromonas veronii* Complex
A. veronii	YES	7	*Aeromonas veronii* Complex

If it is not posible to get a reliable identification, and if the criteria of the 10% divergence among the species is used, it may be equivalent to *Aeromonas dhakensis,* this species profile is not included in the commercial database; in these cases, it is recommended to carry out the biochemical test profile (Table 11).

Table 11. Phenotypic testing for related species within genus *Aeromonas* spp.

Assay	*A. caviae*	*A. hydrophila*	*A. dhakensis*
Glucose gas	-	+	+
VP	-	+	+
LDC	-	+	+
Arabinose	+	+	-

References:

- Abbott SL, Cheung WK, Janda JM. The genus Aeromonas: biochemical characteristics, atypical reactions, and phenotypic identification schemes. J Clin Microbiol 2003; 41(6):2348–2357.
- *Aeromonas punctata* (Zimmermann 1890) Snieszko 1957 (Approved Lists 1980) is an earlier homotypic synonym of *Aeromonas caviae* (*ex* Eddy 1962) Popoff 1984.
- Beaz-Hidalgo R, Martínez-Murcia A, Figueras MJ. Reclassification of *Aeromonas hydrophila* subsp. *dhakensis* Huys et al. 2002 and *Aeromonas aquariorum* Martinez-Murcia et al. 2008 as *Aeromonas dhakensis* sp. nov. comb nov. and emendation of the species *Aeromonas hydrophila*. Syst Appl Microbiol 2013;36(3): 171-176. doi: 10.1016/j.syapm.2012.12.007.
- Huys G, Kämpfer P, Swings J. New DNA-DNA hybridization and phenotypic data on the species *Aeromonas ichthiosmia* and *Aeromonas allosaccharophila*: *A. ichthiosmia* Schubert *et al.* 1990 is a later synonym of *A. veronii* Hickman-Brenner *et al.* 1987. Syst Appl Microbiol 2001;24(2): 177-182. doi: 10.1078/0723-2020-00038.
- Janda JM, Abbott SL. The Genus *Aeromonas:* Taxonomy, Pathogenicity and Infection. Clin Microbiol Rev 2010;23(1):35-73. doi: 10.1128/CMR.00039-09.
- Puthucheary SD, Puah SM, Chua KH. Molecular characterization of clinical isolates of Aeromonas species from Malaysia. PLoS One 2012;7(2):e30205. doi: 10.1371/journal.pone.0030205.

Aggregatibacter

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

The main species of the genus are accurately identified by MALDI-TOF.

Score values increase when the microorganism is under optimal growth conditions.

Refer to the Annex for microbial identification using additional phenotypic testing

Table 12. Transcription of *Aggregatibacter* spp. species

Species	Present in BD	No. of MSPs in BD
A. actinomycetemcomitans	YES	6
A. aphrophilus	YES	5
A. segnis	YES	2

References:

- Couturier MR, Mehinovic E, Croft AC, Fisher MA. Identification of HACEK Clinical Isolates by matrix-assisted laser desorption ionization–time of flight mass spectrometry. J Clin Microbiol 2011;49(3):1104–1106. doi: 10.1128/JCM.01777-10.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Khot PD, Couturier MR, Wilson A, Croft A, Fisher MA. Optimization of matrix-assisted laser desorption ionization–time of flight mass spectrometry analysis for bacterial identification. J Clin Microbiol 2012;50(12):3845–3852. doi: 10.1128/JCM.00626-12.
- Nørskov-Lauritsen N. Classification, identification, and clinical significance of Haemophilus and Aggregatibacter species with host specificity for humans. Clin Microbiol Rev 2014; 27(2):214–240. doi: 10.1128/CMR.00103-13.

Alcaligenes

Alcaligenes faecalis: There are 11 MSPs in the database for this microorganism.

Report to the species level with a **score value ≥ 1,7.**

Do not report to the subspecies level .

Alishewanella

Alishewanella fetalis: identification is performed according to manufacturer's recommendations. In our experience, there is no molecular validation.

It is represented by a single reference profile or MSP in the database. The following score values have been accepted for a reliable identification:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE <1,7 = No identification

Alloiococcus

Identification is performed according to manufacturer's recommendations. In our experience, there is no molecular validation.

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE <1,7 = No identification

Alloiococcus otitis, is represented by 6 MSPs in the database.

Anaerobiospirillum

Identification is performed according to manufacturer's recommendations. In our experience, there is no molecular validation.

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE <1,7 = No identification

Table 13. Transcription of *Anaerobiospirillum* spp. species

Species	Present in BD	No. of MSPs in BD
A. succiniciproducens	YES	3
A. thomasii	NO	

Refer to the Annex for microbial identification using additional phenotypic testing.

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Malnick H. *Anaerobiospirillum thomasii* sp. nov., an anaerobic spiral bacterium isolated from the feces of cats and dogs and from diarrheal feces of humans, and emendation of the genus *Anaerobiospirillum*. Int J Syst Bacteriol 1997;47(2):381-4. doi: 10.1099/00207713-47-2-381.

Anaerococcus

According to the scientific literature on this subject, this genus often shows score values lower than 2,0, and it is necessary to expand the original database.

In our experience, there is no molecular validation. Based on the available literature, the following score values have been accepted for a reliable identification:

SCORE ≥1,8 = Identification to the species level

SCORE 1,6-1,79 = Identification to the genus level

SCORE <1,6 = No identification

Table 14. Transcription of *Anaerococcus* spp. species found in clinical samples.

Species	Present in BD	No. of MSPs in BD
A. degenerii	NO	
A. hydrogenalis	YES	1
A. lactolyticus	YES	4
A. meditarraneensis	NO	
A. murdochii	YES	7
A. nayae	NO	
A. octavius	YES	2
A. prevotii	YES	2
A. provenciensis	NO	
A. senegalensis	NO	
A. tetradius	YES	2
A. vaginalis	YES	7

Refer to the Annex for microbial identification using additional phenotypic testing.

References:

- Diop K, Bretelle F, Fournier PE, Fenollar F. *"Anaerococcus mediterraneensis"* sp. nov., a new species isolated from human female genital tract. New Microbes New Infect 2017;17:75-76. doi: 10.1016/j.nmni.2017.02.007.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Lagier JC, Karkouri KE, Nguyen TT, Armougom F, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of *Anaerococcus senegalensis* sp. nov. Stand Genomic Sci 2012;6(1):116–125. doi: 10.4056/sigs.2415480.
- Murphy EC, Frick IM. Gram-positive anaerobic cocci – commensals and opportunistic pathogens. FEMS Microbiol Rev 2013;37(4):520-53. doi: 10.1111/1574-6976.12005.
- Rodríguez-Sánchez B, Alcalá L, Marín M, Ruiz A, Alonso E, Bouza E. Evaluation of MALDI-TOF MS (Matrix-Assisted Laser Desorption-Ionization

Time-of- Flight Mass Spectrometry) for routine identification of anaerobic bacteria. Anaerobe 2016;42:101-107. doi: 10.1016/j.anaerobe.2016.09.009.

Arcanobacterium

Arcanobacterium haemolyticum: report to the species level with a **score value ≥1,7.** There are 6 MSPs in the database that belong to this microorganism.

Suggested additional assays:

Catalase (-), Esculin (-), Urease (-), Gelatinase (-)

Hemolysis (+), Pyrazinamidase (+), DNAse (+), reverse CAMP (+)

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Miyamoto H, Suzuki T, Murakami S, Fukuoka M, Tanaka Y, Kondo T, Nishimiya T, Suemori K, Tauchi H, Osawa H. Bacteriological characteristics of Arcanobacterium haemolyticum isolated from seven patients with skin and soft-tissue infections. J Med Microbiol 2015;64, 369–374. doi: 10.1099/jmm.0.000038.

Arcobacter

Identification is performed according to manufacturer's recommendations. In our experience, there is no molecular validation.

SCORE ≥2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE <1,7 = No identification

Table 15. Transcription of *Arcobacter* sp. species more frequently found in clinical samples.

Species	Present in BD	No. of MSPs in BD
A. butzleri	YES	4
A. cibarius	YES	1
A. cryaerophilus	YES	6
A. mytili	NO	
A. nitrofigilis	YES	1
A. skirrowii	YES	2
A. thereius	NO	

Refer to the Annex for microbial identification using additional phenotypic testing.

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- WEBSITE: *LPS bacterio.net*

Arthrobacter

Due to limited experience, it is recommended to only report to the genus level: *Arthrobacter* sp. with a **score value ≥1,5.**

Partial sequencing of the 16S rRNA gene is necessary for a complete identification.

Refer to the Annex for microbial identification using additional phenotypic testing.

Table 16. Transcription of *Arthrobacter* spp. species

Species	**Present in BD**	**No. of MSPs in BD**
A. albus	NO	
A. citreus	YES	2
A. creatinolyticus	YES	3
A. cumminsii	YES	5
A. luteolus	YES	1
A. oxydans	YES	2
A. scleromae	YES	2
A. woluwensis	YES	1

References:

- Funke G, Hutson RA, Bernard KA, Pfyffer GE, Wauters G, Collins MD. Isolation of *Arthrobacter* spp. from clinical specimens and description of *Arthrobacter cumminsii* sp. nov. and *Arthrobacter woluwensis* sp. nov. J Clin Microbiol 1996;34(10):2356-2363.
- Hou XG, Kawamura Y, Sultana F, Shu S, Hirose K, Goto K, Ezaki T. Description of *Arthrobacter creatinolyticus* sp.nov., isolated from human urine. Int J Syst Bacteriol 1998;48,423-429. doi: 10.1099/00207713-48-2-423.
- Huang Y, Zhao N, He L, Wang L, Liu Z, You M, Guan F. *Arthrobacter scleromae* sp. nov. isolated from human clinical specimens. J Clin Microbiol 2005;43(3):1451-1455. doi: 10.1128/JCM.43.3.1451-1455.2005.
- Koch C, Rainey FA, Stackebrandt E. 16S rDNA studies on members of *Arthrobacter* and *Micrococcus*: An aid for their future taxonomic restructing. FEMS Microbiol Lett 1994;123(1-2)167-171. doi: https://doi.org/10.1111/j.1574-6968.1994.tb07217.x.
- Vargha M, Takáts Z, Konopka A, Nakatsu CH. Optimization of MALDI-TOF MS for strain level differentiation of Arthrobacter isolates. J Microbiol Methods 2006;66(3):399-409. doi: 10.1016/j.mimet.2006.01.006.
- Wauters G, Charlier J, Janssens M, Delmée M. Identification of *Arthrobacter oxydans*, *Arthrobacter luteolus* sp. nov., and *Arthrobacter albus* sp. nov., isolated from human clinical specimens. J Clin Microbiol 2000;38(6): 2412-15.

B

Bacillus

The following criteria recommended by the manufacturer is applied:

SCORE ≥2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE <1,7 = No identification

Clarification: Errors may occurr in the identification, depending on the degree of sporulation; fresh cultures should be used.

Report as ***Bacillus cereus* group** (it includes species *B. cereus, B. thuringiensis, B. mycoides, B. pseudomycoides, B. cytotoxicus, B. weihenstephanensis* and *B. toyonensis*).

Bacillus anthracis (*Bacillus cereus* Group) is not included in the database because it is a bioterrorism agent. Therefore, if there is a lecithinase-positive, gamma-hemolytic and immobile isolate, the suspicion of *Bacillus anthracis* should be confirmed with molecular biology.

It is recommended to complete the identification of the species within group *Bacillus cereus,* using the biochemical test profile (refer to Annex)

This can also be accomplished using 16S rRNA gene sequencing, and if it is impossible to discriminate among species, it should be completed using other genes: 23S rRNA, *gyr*B, *rpo*B and *gro*EL; as with the rest of the groups within the genus.

Table 17. Transcription of species within genus *Bacillus* spp.

ID by MALDI-TOF	Present in BD	No. of MSPs in BD	Report	Observations
B. cereus	YES	3	*Bacillus cereus* group	
B. thuringiensis	YES	1	*Bacillus cereus* group	
B. mycoides	YES	1	*Bacillus cereus* group	
B. pseudomycoides	YES	1	*Bacillus cereus* group	
B. weihenstephanensis	YES	1	*Bacillus cereus* group	
B. subtilis	YES	9	*Bacillus subtilis* group	
B. atrophaeus	YES	4	*Bacillus subtilis* group	
B. mojavensis	YES	1	*Bacillus subtilis* group	
B. vallismortis	YES	1	*Bacillus subtilis* group	
B. sonorensis	YES	1	*Bacillus subtilis* group	
B. amyloliquefaciens	YES	1	*Bacillus subtilis* group	*B.amyloliquefaciens* should be reported as "Operational Group *B. amyloliquefaciens"* which includes *B. amyloliquefaciens*, *B. siamensis and B. velezensis.* *
B. licheniformis	YES	3	*B. licheniformis*	Report to the species level if anaerobiosis +
B. pumilus	YES	6	*Bacillus pumilus* group	
B. safensis	YES	1	*Bacillus pumilus* group	
B. altitudinis	YES	2	*Bacillus pumilus* group	
B. megaterium	YES	3	*B. megaterium*	
B. circulans	YES	2	*Bacillus circulans* group	

B. firmus	YES	1	*Bacillus circulans* group	
B. lentus	YES	2	*Bacillus circulans* group	
B. coagulans	YES	2	*Bacillus circulans* group	
B. clausii	YES	3	*B. clausii*	Confirm with 16S rRNA or *gyr*B
B. halmapalus	YES	1	*Bacillus* sp.	Confirm with *gyr*B
B. horikoshii	YES	1	*Bacillus* sp.	Confirm with *gyr*B
B. jeotgali	YES	1	*Bacillus sp.*	Confirm with *gyr*B
B. simplex	YES	2	*Bacillus sp.*	Confirm with *gyr*B

*It is recommended to refer to the following quote in the laboratory report: Fan B, Blom J, Klenk HP, Borriss R. *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* Form an "Operational Group B. amyloliquefaciens" within the *B. subtilis* Species Complex. Front Microbiol 2017;8:22. doi: 10.3389/fmicb.2017.00022.

The following species are not represented in the equipment's database:

- *B. cytotoxicus (B. cereus* group*)*
- *B. rhizosphareae*
- *B. toyonensis (B. cereus* group*)*
- *B. velezensis (B. subtilis* group*)*
- *Bacillus beringensis / kortensis*
- *Bacillus borboni / carboniphilus*
- *Bacillus stratosphaericus / B. aerophilus (B. pumilus* group*)*

These species have been confirmed by molecular biology.

References:

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- Fan B, Blom J, Klenk HP, Borriss R. *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* Form an "Operational Group B. amyloliquefaciens" within the *B. subtilis* Species Complex. Front Microbiol 2017;8:22. doi: 10.3389/fmicb.2017.00022.
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Bacteroides

It is recommended to use the following criteria accepted in the scientific literature on this subject:

SCORE ≥1,8 = Identification to the species level

SCORE 1,6-1,79 = Identification to the genus level

SCORE <1,6 = No identification

According to Jorgensen *et al.* (2015), identification to the species level is accurate.

However, we suggest that, in some cases, the identification should be differentiated:

When MALDI-TOF yields a *Bacteroides ovatus* result, it should be reported as *Bacteriodes ovatus/ xylanisolvens* because of their close similarity and due to the absence of the latter in the equipment's database; manual tests that are useful for their differentiation could also be used (see Table 18).

On the other hand, species *Bacteroides fragilis/clarus* could show as *Bacteroides stercoris* in MALDI-TOF (see Table 18).

When MALDI-TOF yields a *Bacteroides vulgatus* result, it should be reported as *Bacteroides vulgatus/dorei* because of their close similarity and due to the absence of the latter in the equipment's database. Similarly, when the equipment yields a *Bacteroides thetaiotaomicron* result, it should be reported as *B. thetaiotamicron/faecis* since they are similar, but the number of spectra of *B. faecis* is lower than that of *B. thetaiotaomicron.*

Table 18. Phenotypic differences of *Bacteroides* spp. species that may be difficult to identify by MALDI-TOF.

Species	Indole	Catalase	α-Fucosidase	Arabinose	Trehalose	Xylose
B. stercoris	+	V	V	-	-	+
B. fragilis	-	+	+	-	-	+
B. clarus	+	-	-	-	W	+
B. ovatus	+	+	+	+	+	+
B. xylanisolvens	-	-	+	+	+	+

Symbols: V, variable; W, weak.

Table 19. Transcription of species within genus *Bacteroides* spp.

Species	Present in BD	No. of MSPs in BD
B. caccae	YES	5
B. cellulosilyticus	YES	3
B. clarus	YES	1
B. coagulans	YES	1
B. coprocola	YES	1
B. coprophilis	YES	1
B. dorei	NO	
B. eggerthii	YES	3
B. faecis	YES	2
B. finegoldii	YES	3
B. fluxus	YES	1
B. fragilis	YES	14

B. galacturonicus	NO	
B. intestinalis	YES	4
B. massiliensis	YES	6
B. nordii	YES	6
B. oleiciplenus	YES	1
B. ovatus	YES	6
B. pectinophilus	NO	
B. plebeius	YES	1
B. pyogenes	YES	7
B. salyersae	YES	9
B. stercoris	YES	5
B. thetaiotaomicron	YES	8
B. uniformis	YES	10
B. vulgatus	YES	8
B. xylanisolvens	NO	

References:

- Johnson JL, Moore WEC, Moore LVH. *Bacteroides caccae* sp. nov., *Bacteroides merdae* sp. nov., and *Bacteroides stercoris* sp. nov. isolated from human feces. Int J Syst Bacteriol 1986;36, 499-501. doi: 10.1099/00207713-36-4-499.
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from human faeces. Int J Syst Evol Microbiol 2010;60(8):1864-1869. doi: 10.1099/ijs.0.015107-0.

Bartonella

Species within genus *Bartonella* are considered emerging pathogens; their natural cycle includes long-lasting intra-erythrocyte infection in a host acting as a reservoir. Bacteria are transmited from the reservoir to the susceptible host (including humans) by arthropod vectors.

Out of the 19 species officially recognized, the most common human pathogens are: *Bartonella bacilliformis, Bartonella quintana* and *Bartonella henselae*.

Bartonella species grow very slowly, requiring from 7 days to 6 weeks of incubation.

They are usually identified using molecular techniques (target genes: 16S rRNA, *glt*A, *rpo*B, *rib*C, *fts*Z, *gro*EL).

According to the scientific literature on this subject, species within this genus are accurately identified by MALDI-TOF after having included protein profiles (MSP) of reference strains in the commercial database.

The only reference spectrum in the equipment's database belongs to *Bartonella japonica.*

Due to limited experience, it is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE <1,7 = No identification

References:

- Fournier PE, Couderc C, Buffet S, Flaudrops C, Raoult D. Rapid and cost-effective identification of Bartonella species using mass spectrometry. J Med Microbiol 2009;58(9):1154-9. doi: 10.1099/jmm.0.009647-0.
- WEBSITE: *http://www.bacterio.cict.fr/b/bartonella.html*

Bergeyella

The only reference spectrum in the equipment's database belongs to *Bergeyella zoohelcum.*

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE <1,7 = No identification

Bifidobacterium

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE <1,7 = No identification

According to data obtained from our limited experience with sequenced isolates, *Bifidobacterium scardovii* can be reported to the species level with **score values ≥ 1,7.**

Table 20. Transcription of species within genus *Bifidobacterium* spp.

Species	Present in BD	No. of MSPs in BD
B. adolescentis (including *B. angulatum* and *B. merycicum)*	YES	4
B. animalis	YES	3
B. asteroides	YES	2
B. bifidum	YES	5
B. breve	YES	5
B. catenulatum	YES	2
B. dentium	YES	4
B. infantis	NO	
B. longum	YES	6
B. magnum	YES	2
B. pseudocatenulatum	YES	2
B. scardovii	YES	3
B. suis	NO	

References:

- Barberis C, Almuzara M, Join-Lambert O, Ramırez MS, Famiglietti A, Vay C. Comparison of the Bruker MALDI-TOF mass spectrometry system and conventional phenotypic methods for identification of Gram-positive rods. PLoS One 2014;9(9):e106303. doi: 10.1371/journal.pone.0106303.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
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- Turroni F, Duranti S, Bottacini F, Guglielmetti S, Van Sinderen D, Ventura M. *Bifidobacterium bifidum* as an example of a specialized human gut commensal. Front Microbiol 2014;5:437. doi: 10.3389/fmicb.2014.00437

Bordetella

It is recommended to apply the criteria recommended by the manufacturer:

SCORE ≥ 2,0 = species level identification

SCORE 1,7-1,99 = gender level identification

SCORE <1,7 = does not identify

B. bronchiseptica, B. pertussis and ***B. parapertussis*** are not correctly differentiated by mass spectrometry applying the manufacturer's criteria.

It is recommended to use the following criteria:

i. Score ≥2 + Consistency A Category = identification at **species** level.
ii. Score ≥2 + Consistency B Category = apply 5% divergence to inform the species, otherwise report only the gender.
iii. Score 1,7-1,9 and top ten with a single species = identification at the **species** level.
iv. Score 1,7-1,9 and top ten with different species = apply 5% divergence to inform the species, otherwise report only the genus.

Consistency A: the first species identified appears in green, the rest of the wells in green correspond to that same species. If yellow results appear, they correspond to at least the same gender as the first one.

Consistency B: the first species identified appears in green or yellow; other species of the genus may appear in green or yellow. The identification criteria at the species level is not met.

Table 21. Transcription of *Bordetella* spp. species isolated from clinical samples.

Species	Present in BD	No. of MSPs in BD
B. avium	YES	2
B. bronchialis	NO	
B. bronchiseptica	YES	9
B. flavilis	NO	
B. hinzii	YES	5
B. homesii	YES	3
B. parapertussis	YES	11
B. pertussis	YES	10
B. petrii	YES	7
B. sputigena	NO	
B. trematum	YES	4

Table 22. Phenotypic testing of *Bordetella* spp. species.

Assay	*B. pertussis*	*B. parapertussis*	*B. bronchiseptica*	*B. avium*	*B. hinzii*	*B. holmesii*	*B. petrii*	*B. trematum*	*B. bronchialis*	*B. flabilis*	*B. sputigena*
Oxidase	+	-	+	+	+	-	+	-	+	+	+
Catalase	+	+	+	+	-	+	+	+	+	+	+
Motility	-	-	+	-	+	-	-	+	+	+	+
Pigment	-	brown	-	-	-	brown	yellow	-	-	-	-
Development in MacConkey medium	nd	+	+	+	+	+	+	+	+	+	+
Urea	-	+	+	-	-	-	-	-	nd	nd	nd

Symbols: w, weak; nd, not determined

PCR algorithm proposed by the NATIONAL REFERENCE LABORATORY (Clinical Bacteriology Service) for the identification and confirmation of *Bordetella* species related to whooping cough:

- ***B. pertussis:***

  Amplification targets used: IS*481* + *ptx*S1 or IS*481* + *ptx*P

- ***B. parapertussis:***

  Amplification targets used: pIS*1001* + *ptx*S1

- ***B. holmesii:***

  Amplification targets used: IS*481* + hIS*1001*

For the confirmation of these species, it is necessary to use **at least two different amplification targets**.

References:

- Gentry-Weeks, Hultsch AL, Kelly SM, Keith JM, Curtiss R. Cloning and sequencing of a gene encoding a 21-kilodalton outer membrane protein from Bordetella avium and expression of the gene in Salmonella typhimurium. J Bacteriol 1992;174 (23): 7729-42.
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- Zintgraff J, Irazu L, Lara CS, Rodriguez M, Santos M. The classical Bordetella species and MALDI-TOF technology: a brief experience. J Med Microbiol 2018;67(12):1737-1742. doi: 10.1099/jmm.0.000860.
- WEBSITE: *www.uniprot.org*

Brevibacillus

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE <1,7 = No identification

Table 23. Transcription of *Brevibacillus* spp. species isolated from clinical samples.

Species	Present in BD	No. of MSPs in BD
B. agri	YES	1
B. borstelensis	YES	2
B. brevis	YES	2
B. centrosporus	YES	1
B. invocatus	YES	1
B. laterosporus	YES	2
B. massiliensis	NO	
B. parabrevis	YES	2

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- WEBSITE: *www.bacterio.net*

Brevibacterium

Overall, MALDI-TOF has been successfully used for this type of microorganisms; but it may not achieve an identification, depending on the condition of the culture.

Owing to limited experience, it is recommended to only report to the genus level: *Brevibacterium* sp. with a **score value ≥1,5**, with the exception of *Brevibacterium casei* that can be identified to the species level with a **score value ≥1,7** (Barberis *et al*., 2014).

16S rRNA gene sequencing is necessary for a complete identification.

Table 24. Transcription of *Brevibacterium* spp. species

Species	Present in BD	No. of MSPs in BD
B. album	NO	
B. casei	YES	7
B. epidermidis	NO	
B. luteolum	NO	
B. masiliense	NO	
B. mcbrellneri	NO	
B. otitidis	NO	
B. paucivorans	YES	2
B. ravenspurgense	YES	3
B. sanguinis	YES	2

References:

- Barberis C, Almuzara M, Join-Lambert O, Ramırez MS, Famiglietti A, Vay C. Comparison of the Bruker MALDI-TOF mass spectrometry system and conventional phenotypic methods for identification of Gram-positive rods. PLoS One 2014;9(9):e106303. doi: 10.1371/journal.pone.0106303.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Brevundimonas

Results should be reported using the following criteria:

SCORE ≥2,0 = Identification to the species level

SCORE 1,7-1,9 = Identification to the genus level

SCORE <1,7 = No identification

Table 25. Transcription of *Brevundimonas* spp. species

Species	Present in BD	No. of MSPs in BD	Observations
B. bullata	NO		Maltose acid: -
B. diminuta (Brown pigment)	YES	8	Esculin: - Maltose acid: -
B. nasdae	YES	1	
B. vancanneytii	NO		Esculin: - Maltose acid: +
B. vesicularis (yellow-orange pigment)	YES	1	Esculin: + Maltose acid: +

References:

- Almuzara M, Barberis C, Traglia G, Famiglietti A, Ramirez MS, Vay C. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for species identification of nonfermenting Gram-negative bacilli. J Microbiol Methods 2015;112:24-7. doi: 10.1016/j.mimet.2015.03.004.
- Estrela AB, Abraham WR. *Brevundimonas vancanneytii* sp. nov., isolated from blood of a patient with endocarditis. Int J Syst Evol Microbiol 2010;60(9):2129-2134. doi: 10.1099/ijs.0.015651-0.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Brucella

There are no reference patterns in the commercial database, since it is equivalent to a bioterrorism agent.

The Brucellosis Laboratory within NRL will transfer their own database along with their protocols for extraction and INACTIVATION.

Burkholderia

IMPORTANT: When a score value ≥ 2,0 is achieved, it should also be considered that the divergence should be greater than the 10% between the first species and the next different one in the Top Ten.

MALDI-TOF correctly distinguishes *Burkholderia vietnamensis, Burkholderia seminalis* and *Burkholderia gladiolii*.

If it is not posible to achieve the 10% divergence among the species within the complex, it should be reported as *Burkholderia cepacia* complex, since these species are properly differentiated from other microorganisms with similar phenotypes (*Ralstonia, Cupriavidus, Pandoraea* spp.).

Additional phenotypic testing should be used to identify the species within this genus (Tables 26 and 27).

Moreover, sequencing of the *rec*A gene is needed for a complete identification to the species level.

Table 26. Features of *Burkholderia cepacia* complex (BCC).

Species	Yellow pigment (Pig)	Brown pigment	β-hemolysis on sheep blood agar	ODC	NO_3 reduction
B. cenocepacia	-	V	-	V	V
B. cepacia	V	-	-	V	-
B. contaminans	V	-	V	-	V
BCC showing beta-hemolysis			*B. ambifaria* (Pig V) *B. arboris* (Pig V) *B. contaminans* (Pig V) *B. pyrrocinia* (Pig V) *B. vietnamensis* (Pig -)		

Table 27. Phenotypic testing of *Burkholderia* spp. species.

Species	Growth at 42°C	Yellow pigment	Beta-hemolysis	Sucrose	NO_3 reduction	Esculin	Gelatin
B. ambifaria	V	V	V	+	V	V	+
B. anthina	V	-	-	V	V	-	-
B. arboris	V	V	V	V	V	-	+
B. cenocepacia	V	-	-	+	V	V	V
B. cepacia	V	V	-	V	-	V	V
B. contaminans	V	V	V	+	V	V	+
B. diffusa	V	-	-	V	+	-	V
B. dolosa	+	-	-	-	+	-	-
B. gladiolii	-	V		-	V	V	V
B. lata	-	V	-	V	V	V	V
B. latens	+	-	-	+	-	-	V
B. metallica	+	V	-	V	-	+	+
B. multivorans	+	-	-	-	+	-	-
B. pseudomultivorans	+	-	-	-	V	-	-
B. pyrrocinia	V	V	V	V	V	-	V
B. seminalis	+	V	-	V	-	V	+
B. stabilis	-	-	-	-	-	-	+
B. ubonensis	V	-	-	+	V	-	+
B. vietnamiensis	+	-	V	+	V	-	-

Symbols: V, variable.

Table 28. Transcription of species within genus *Burkholderia* spp.

Species	Present in	
	Biotyper	**Biotyper + RENAEM**
B. ambifaria	YES	YES
B.anthina	YES	YES
B.arboris	NO	YES
B.cenocepacia	YES	YES
B.cepacia	YES	YES
B.contaminans	NO	YES
B.diffusa	YES	YES
B.dolosa	YES	YES
B. gladiolii	YES	YES
B.lata	YES	YES
B.latens	YES	YES
B. metallica	YES	YES
B. multivorans	YES	YES
B. pseudomultivorans	NO	NO
B.pyrrocinia	YES	YES
B.seminalis	YES	YES
B. stabilis	YES	YES
B.ubonensis	NO	NO
B.vietnamiensis	YES	YES

References:

- Almuzara M, Barberis C, Traglia G, Famiglietti A, Ramirez MS, Vay C. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for species identification of Nonfermenting Gram-Negative Bacilli. J Microbiol Methods. 2015;112:24-27. doi:10.1016/j.mimet.2015.03.004.

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- Coutinho CP, Barreto C, Pereira L, Lito L, Melo Cristino J, Sá-Correia I. Incidence of Burkholderia contaminans at a cystic fibrosis center with an unusually high representation of Burkholderia cepacia during 15 years of epidemiological surveillance. J Med Microbiol. 2015;64:927–35.
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C

Campylobacter

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,7 = No identification

Table 29. Transcription of *Campylobacter* spp. species isolated from clinical samples.

Species	Present in BD	No. of MSPs in BD
C. coli	YES	4
C. avium	YES	1
C. canadensis	YES	1
C. concisus	YES	14
C. cuniculorum	NO	
C. curvus	YES	5
C. fetus	YES	8
C. gracilis	YES	2
C. helveticus	YES	4
C. hominis	YES	1
C. hyointestinalis	YES	3
C. insulaenigrae	NO	
C. jejuni	YES	11
C. lanienae	YES	1
C. lari	YES	5
C. mucosalis	NO	

C. peloridis	YES	1
C. rectus	YES	2
C. showae	YES	4
C. sputorum	YES	5
C. subantarcticus	NO	
C. thioglycolate	NO	
C. upsaliensis	YES	5
C. ureolyticus	YES	7
C. volucris	NO	

Capnocytophaga

The following score values have been accepted for a reliable identification, based on data gathered from our experience:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Table 30. Transcription of *Capnocytophaga* spp. species

Species	Present in BD	No. of MSPs in BD
C. canimorsus	YES	3
C. cynodegmi	YES	1
C. gingivalis	YES	6
C. granulosa	YES	2
C. haemolytica	YES	1
C. leadbetteri	NO	
C. ochracea	YES	5
C. sputigena	YES	9
Capnocytophaga genospecies AHN8471	NO	

Partial sequencing of 16S rRNA or *rpo*B genes is used for a complete identification to the species level.

Refer to the Annex for microbial identification using additional phenotypic testing.

References:

- Frandsen EV, Poulsen K, Könönen E, Kilian M. Diversity of *Capnocytophaga* species in children and description of *Capnocytophaga leadbetteri* sp. nov. and *Capnocytophaga* genospecies AHN8471. Int J Syst Evol Microbiol 2008;58(2):324-336. doi: 10.1099/ijs.0.65373-0.
- Jolivet-Gougeon A, Helsens N, Renard E, Tamanai-Shacoori Z, Bonnaure-Mallet M. Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of human oral Capnocytophaga species. Anaerobe 2017;48:89-93. doi: 10.1016/j.anaerobe.2017.07.003.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Cardiobacterium

Based on data gathered from our experience, we recommend to only report the identification to the genus level with a **score value >1,5.**

There might be no identification due to its poor representation in the commercial database.

Table 31. Transcription of *Cardiobacterium* spp. species

Species	Present in BD	No. of MSPs in BD
C. hominis	YES	1
C. valvarum	YES	1

Unlike *C. hominis, C. valvarum* grows more slowly, is non-hemolytic on sheep blood agar, and does not use sucrose, maltose nor mannitol.

References:

- Bothelo E, Gouriet F, Fournier PE, Roux V, Habib G, Thuny F, Metras D, Raoult D, Casalta JP. Endocarditis caused by *Cardiobacterium valvarum*. J Clin Microbiol 2006;44(2)657-658. doi: 10.1128/JCM.44.2.657-658.2006.

Cellulomonas

It is recommended to only report the identification to the genus level with a **score value >1,5.**

Species that cause disease in humans are not included in the commercial database, therefore, they might yield low score values in MALDI-TOF.

The definitive identification of these species is usually carried out using molecular biology.

Refer to the Annex for microbial identification using additional phenotypic testing.

Table 32. Transcription of *Cellulomonas* spp. species.

Species	Present in BD	No. of MSPs in BD
C. denverensis	NO	
C. fermentans	NO	
C. fimi	YES	1
C. flamigena	YES	1
C. gélida	YES	1
C. hominis	NO	
C. humilata	NO	
C. massiliensis	NO	
C. uda	YES	1

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Lagier JC, Ramasamy D, Rivet R, Raoult D, Fournier PE. Non contiguous-finished genome sequence and description of *Cellulomonas massiliensis* sp. nov. Stand Genomic Sci 2012;7(2)258-270. doi: 10.4056/sigs.3316719.
- WEBSITE: *www.bacterio.net/cellulomonas.html*

Cellulosimicrobium

Since they are gram-positive rods belonging to the pigmented group, MALDI-TOF can identify them to the genus level with a **score value > 1,5.**

Important: There are only two reference spectra for the whole genus, and these belong to *Cellulosimicrobium cellulans;* therefore, MALDI-TOF may mistakenly identify other species as *C. cellulans*.

Sequencing of 16S rRNA and *rpoB* genes fail to achieve species-level discrimination.

If relevant, it is recommended to use additional phenotypic tests (Table 33).

Table 33. Phenotypic features of *Cellulosimicrobium* spp. species.

Species	*C. cellulans*	*C. funkei*	*C. terreum*
Growth at 35°C	+	+	-
Growth at 42°C	+	+	-
Motility (fresh)	-	+	-
Raffinose acid	(+)	-	-
Glyc assimilation	-	+	-
D-xylose assimilation	+	-	-
MDG assimilation	-	+	+
Assimilation using API 50CH with AUX medium			

Symbols: (+), weak positive

References:

- Brown JM, Steigerwalt AG, Morey RE, Daneshvar MI, Romero LJ, McNeil MM. Characterization of clinical isolates previously identified as *Oerskovia turbata*: proposal of *Cellulosimicrobium funkei* sp. nov. and emended description of the genus *Cellulosimicrobium*. Int J Syst Evol Microbiol 2006;56(4):801-804. doi: 10.1099/ijs.0.63882-0.
- Petkar H, Li A, Bunce N, Duffy K, Malnick H, Shah JJ. *Cellulosimicrobium funkei*: First Report of Infection in a Nonimmunocompromised Patient and Useful Phenotypic Tests for Differentiation from *Cellulosimicrobium cellulans* and *Cellulosimicrobium terreum.* J Clin Microbiol 2011;49(3):1175–1178. doi: 10.1128/JCM.01103-10.
- Schumann P, Weiss N, Stackebrandt E. Reclassification of *Cellulomonas cellulans* (Stackebrandt and Keddie 1986) as *Cellulosimicrobium cellulans* gen. nov., comb. nov. Int J Syst Evol Microbiol 2001;51(3):1007-1010. doi: 10.1099/00207713-51-3-1007.

Chryseobacterium

The following score values have been accepted for a reliable identification:

SCORE >1,9 = Identification to the species level

SCORE 1,6-1,89 = Identification to the genus level

SCORE <1,6 = No identification

Species *Chryseobacterium gleum* and *Chryseobacterium indologenes* should be reported as *C. gleum / C. indologenes.*

If it is clinically relevant, 16S rRNA gene should be sequenced and supplemented with the biochemical profile (Table 35).

Table 34. Transcription of species within genus *Chryseobacterium* spp.

Species	Present in BD	No. of MSPs in BD	Observations
C. anthrophi	NO		16S rRNA may not be able to distinguish it from *C. haifense,* but the latter is not a human pathogen.
C. bernardetii	NO		
C. carnis	NO		
C. gleum	YES	6	16S rRNA cannot distinguish it from *C. indologenes*
C. hominis	YES	1	Confirmatory 16S rRNA
C. indologenes	YES	6	16S rRNA cannot distinguish it from *C. gleum*
C. lactis	NO		
C. nakagawai	NO		
C. treverense	NO		16S rRNA may not be able to distinguish it from *C. solincola,* but the latter is not a human pathogen.

Table 35. Biochemical profile of *Chryseobacterium* spp. species

Species	Flexirubin	β-hemolysis	MacConkey	Growth at 41°c	Acetate	Urease	Gelatinase
C. anthrophi	-	-	-	-	+	-	+
C. bernardetii	-	ND	+	+	ND	V	+
C. carnis	ND	ND	+	-	-	-	+
C. gleum	+	-	+	+	ND	+	+
C. hominis	-	-	-	-	+	-	+
C. indologenes	+	+	V	-	ND	-	+
C. lactis	+	ND	+	-	ND	-	+
C. nakagawai	+	ND	-	ND	-	-	+
C. treverense	-	-	-	-	-	-	-

Symbols: V, variable; ND, not determined.

References:

- Holmes B, Steigerwalt AG, Nicholson AC. DNA-DNA hybridization study of strains of *Chryseobacterium*, *Elizabethkingia* and *Empedobacter* and of other usually indole-producing non-fermenters of CDC groups IIc, IIe, IIh and IIi, mostly from human clinical sources, and proposals of *Chryseobacterium bernardetii* sp. nov., *Chryseobacterium carnis* sp. nov., *Chryseobacterium lactis* sp. nov., *Chryseobacterium nakagawai* sp. nov. and *Chryseobacterium taklimakanense* comb. nov. Int J Syst Evol Microbiol 2013;63(12):4639-4662. doi: 10.1099/ijs.0.054353-0.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- WEBSITE: *www.bacterio.net*

Citrobacter

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,7 = No identification

MALDI-TOF accurately identifies *Citrobacter koseri* to the species, but it cannot discriminate between *Citrobacter amalonaticus* and *Citrobacter farmeri;* a complete identification can be achieved using the following phenotypic tests:

Citrobacter amalonaticus: sucrose-negative and melibiose-negative

Citrobacter farmeri: sucrose-positive and melibiose-positive

Report as ***Citrobacter freundii* complex**, which includes the following species: *C. freundii, C. braakii, C. youngae, C. guillernii, C. rodentium, C. sedlackii, C. werkmanii* and *C. murliniae.*

Table 36. Transcription of species within genus *Citrobacter* spp.

Species	Present in BD	No. of MSPs in BD	Report
C. amalonaticus	YES	6	Distinguish from *C. farmeri* (sucrose and melibiose: +)
C. braakii	YES	2	*Citrobacter freundii* Complex
C. farmeri	YES	5	Distinguish from *C. amalonaticus* (sucrose and melibiose: -)
C. freundii	YES	7	*Citrobacter freundii* Complex
C. guillernii	YES	3	*Citrobacter freundii* Complex
C. koseri	YES	10	*C. koseri*
C. murliniae	YES	1	*Citrobacter freundii* Complex
C. rodentium	YES	4	*Citrobacter freundii* Complex
C. sedlackii	YES	8	*Citrobacter freundii* Complex
C. werkmanii	NO		*Citrobacter freundii* Complex
C. youngae	YES	1	*Citrobacter freundii* Complex

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- WEBSITE: *www.bacterio.net*

Clostridium

The genus comprises more than 200 anaerobic, ocasionally aerotolerant, species; however, the number of clinically relevant Clostridia in human infections is limited.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,7 = No identification

It is important to use fresh cultures, since sporulation directly affects the quality of the spectrum.

In lower score values, MALDI-TOF may wrongly identify some species. In these cases, in order to enhance the quality of the obtained spectra, perform the in-tube extraction technique with ethanol / formic acid, recommended by the manufacturer.

Clostridium argentinense is not represented in the commercial database; therefore, MALDI-TOF may identify this species as *Clostridium subterminale*.

Sequencing of the 16S rRNA gene should be used to discriminate between these species.

It is important to confirm the species for *C. septicum* (associated with gastrointestinal malignancies), *C. perfringens, C. ramosum*, *C. innocuum*, and *C. clostridioforme,* generally resistant to antibiotics (Table 37).

Table 37. Additional Phenotypic features.

Species	Gelatinase	Esculin	Lecithinase	Milk digestion	DNAse	Sucrose	Mannitol	Observations
C. clostridioforme	ND	ND	ND	ND	ND	ND	ND	Lactose-positive and b-NAG negative
C. innocuum	-	+	-	-	ND	ND	+	*C. innocuum* is saccharolytic, nonproteolytic, proline aminopeptidase negative. Hard-to-find terminal spore, mosaic internal structure, motionless.
C. perfringens	+	V	+	-	ND	ND	ND	Saccharolytic, proteolytic, double-zone beta-hemolysis around colonies, infrequent spores.
C. ramosum	-	+	-	-	ND	ND	+	Colonies with irregular borders, gram-variable, oval terminal spore, motionless. Saccharolytic, nonproteolytic
C. septicum	+	+	-	+	+	-	ND	Bacterial swarming, filamentous rods with subterminal spore, saccharolytic, proteolytic

Symbols: V, variable; ND, not determined.

Table 38. Transcription of species within genus *Clostridium* spp. with clinical significance.

Species	Present in Biotyper	Present in Biotyper + RENAEM	No. of MSPs in BD	Report / Observations
C. argentinense	NO	YES	1	*C. argentinense*
C. baratii	YES	YES	2	
C. bifermentans	YES	YES	6	

C. bolteae	YES	YES	1	*Clostridium clostridioforme* Group
C. botulinum	NO	YES		*C. botulinum*
C. butyricum	YES	YES	5	*C. butyricum*
C. cadaveris	YES	YES	4	
C. carnis	YES	YES	1	Aerobic growth
C. clostridioforme	YES	YES	5	*Clostridium clostridioforme* Group
C. difficile	YES	YES	10	*C. difficile*
C. glycolicum	YES	YES	4	
C. hastiforme	NO	NO		
C. hathewayi	YES	YES	4	*Clostridium clostridioforme* Group
C. histolyticum	YES	YES	5	Aerobic growth
C. indolis	YES	YES	1	
C. innocuum	YES	YES	5	
C. limosum	YES	YES	5	
C. novyi	YES	YES	2	
C. paraputrificum	YES	YES	5	*C. paraputrificum*
C. perfringens	YES	YES	9	*C. perfringens*
C. putrificans	NO	NO		
C. ramosum	YES	YES	8	
C. septicum	YES	YES	4	

C. sordellii	YES	YES	2	*C. sordellii*
C. sphenoides	YES	YES	4	
C. sporogenes	YES	YES	7	
C. subterminale	YES	YES	3	*Clostridium* sp. Confirm with 16S rRNA
C. symbiosum	YES	YES	2	
C. tertium	YES	YES	6	Aerobic growth
C. tetani	YES	YES	4	

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Zárate MS, Romano V, Nievas J, Smayevsky J. Utilidad de la espectrometría de masas MALDI-TOF en la identificación de bacterias anaerobias. Rev Argent Microbiol 2014;46(2):98-102. doi: 10.1016/S0325-7541(14)70055-0.

Comamonas

Species within this genus rarely cause disease in humans; among these species, the most frequent is *C. testosteroni,* which has been described in endocarditis, meningitis and catheter-related bacteremia.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,7 = No identification

In the case of *C. kerstersii,* it is accepted to report the identification to the species level with a **score value >1,7.**

If it is not posible to achieve the 10% divergence between species, 16S rRNA gene sequencing and differential phenotypic tests can be used.

References:

- Almuzara M, Barberis C, Traglia G, Famiglietti A, Ramirez MS, Vay C. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for species identification of Nonfermenting Gram-Negative Bacilli. J Microbiol Methods 2015;112:24–27. doi: 10.1016/j.mimet.2015.03.004.
- Almuzara M, Barberis C, Veiga F, Bakai R, Cittadini R, Vera Ocampo C, Alonso Serena M, Cohen E, Ramirez MS, Famiglietti A, Stecher D, del Castillo M, Vay C. Unusual presentations of *Comamonas kerstersii* infection. New Microbes New Infect 2017;19:91-95. doi: 10.1016/j.nmni.2017.07.003.
- Wauters G, De Baere T, Willems A, Falsen E, Vaneechoutte M. Description of *Comamonas aquatica* comb. nov. and *Comamonas kerstersii* sp. nov. for two subgroups of *Comamonas terrigena* and emended description of *Comamonas terrigena*. Int J Syst Evol Microbiol 2003;53,859–862. doi 10.1099/ijs.0.02450-0.

Corynebacterium

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Lipophilic species may yield lower score values. Adding 1µl of formic acid can improve the identification (score values).

Despite having a reference MSP in the commercial database, *C. pyruviciproducens* is usually not identified accurately.

The following species are correctly identified by MALDI-TOF: *C. durum* (shows adherence to agar), *C. mucifaciens*, *C. kroppenstedtii* and *C. tuberculostearicum*.

The recommended method for the complete identification of most corineiform species is the sequencing of the *rpoB* gene.

Important*: C. diphtheriae, C. ulcerans* and *C. pseudotuberculosis* must be referred to the Clinical Bacteriology Laboratory within the LNR in order to look for toxins using PCR.

Table 39. Limitations in the identification of *Corynebacterium* spp. species.

ID by MALDI-TOF	Possible errors in the ID	Confirmation	Report
C. aurimucosum	Cannot discriminate between *C. aurimucosum / C. minutissimum*	Phenotype: DNAse, Hippurate, Tyrosine *rpo*B gene sequencing	If colony is creamy, DNAse -, Hippurate +, Tyrosine -: *C. aurimucosum* (confirm with sequencing)
C. minutissimum	Cannot discriminate between *C aurimucosum / C.minutissimum/ C. singulare/ C amycolatum*	Phenotype: DNAse, Hippurate, Tyrosine *rpo*B gene sequencing	If it is a small dry colony, late + DNAse, Hippurate -, Tyrosine +: *C. minutissimum* (confirm with sequencing)
C. propinquum	Cannot discriminate between *C. pseudodiphteriticum / C. propinquum*	Urea -/+ *rpo*B gene sequencing	If urea –, it should be reported as *C. propinquum*
C. amycolatum	May be confused with *C. aurimucosum* o *C. minutissimum*	Phenotype: colony aspect, NO_3, tributyrin *rpo*B gene sequencing	If it is a dry, waxy colony, NO_3+, tributyrin +: it should be reported as *C. amycolatum*. If these tests are negative, complete phenotype and sequencing.
C. coyleae	May be confused with *C. afermentans*	LAP *rpo*B gene sequencing	LAP+: it should be reported as *C. coyleae*
C. pseudodiphteriticum	Cannot discriminate between *C. pseudodiphteriticum / C. propinquum*	Urea + *rpo*B gene sequencing	If urea +, it cannot be diferentiated from *C. propinquum*: report according to *rpo*B
C. simulans	May be confused with *C. striatum*	CAMP, ethylene glycol *rpo*B gene sequencing	If CAMP-, Etilenglicol –: It should be reported as *C. simulans*
C. striatum	May be confused with *C. simulans*	CAMP, ethylene glycol *rpo*B gene sequencing	If CAMP+, It should be reported as *C. striatum*. If CAMP-, ethylene glycol+: it shoud be reported as *C. striatum*

The following species are not represented in the commercial database:

- *C. aquatimens*
- *C. atypicum*
- *C. lowii*
- *C. masiliense*
- *C. oculi*
- *C. pilbarense*
- *C. sputi*
- *C. timonense*

Species that are generally multidrug-resistant:

- *C. afermentans ss afermentans*
- *C. amycolatum*
- *C. aurimucosum*
- *C. confusum*
- *C. coyleae*
- *C. glucuronolyticum*
- *C. jeikeium*
- *C. macginleyi*
- *C. minutissimum*
- *C. propinquum*
- *C. pseudodiphtheriticum*
- *C. resistens*
- *C. striatum*
- *C. tuberculostearicum*
- *C. urealyticum*
- *C. ureicelerivorans*

Table 40. Phenotypic markers for the differentiation of species with significant 16S rRNA gene similarities.

Species with significant 16S rRNA gene similarities	Phenotypic markers	Confirmation
C. afermentans *C. coyleae* *C. mucifaciens*	*C. afermentans sb afermentans*: fermentative metabolism, CAMP V *C. coyleae*: oxidative metabolism, CAMP + *C. mucifaciens*: yellow mucoid colonies	*rpo*B
C. aurimucosum *C. minutissimum* *C. singulare*	*C. aurimucosum*: yellow colonies, some erode agar, some have gray-black pigment. *C. minutissimum*: Tyrosine +, Urea - *C. singulare*: Tyrosine +, Urea +	*rpo*B
C. propinquum *C.pseudodiphtheriticum*	*C. pseudodiphteriticum:* Urea – *C. propinquum:* Urea +	*rpo*B
C. sundsvallense *C. thomssenii*	Phenotypically indistinguishable	*rpo*B
C. ulcerans *C. pseudotuberculosis*	Both are reverse CAMP + *C. ulcerans:* O129 susceptible *C. pseudotuberculosis*: O129 resistant Might be diphtheria toxin +	*rpo*B
C. xerosis *C. hansenii* *C. freneyi*	*C. xerosis*: PAL +, α-glu V, development at 20ºC -, Ferm Glucose 42C - *C. hansenii:* PAL - *C. freneyi*: PAL +, α-glu +, development at 20ºC +, Ferm Glucose 42C +	Partial *rpo*B No discrimination
C. ureicelerivorans *C. mucifaciens*	*C. ureicelerivorans*: Rapid urea, smooth colony *C. mucifaciens*: urea -, yellow mucoid colony	Partial *rpo*B No discrimination

Refer to the Annex for microbial identification using additional phenotypic testing.

References:

- Alatoom AA, Cazanave CJ, Cunningham SA, Ihde SM, Patel R. Identification of Non-*diphtheriae Corynebacterium* by Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. J Clin Microbiol 2012;50(1):160 – 163. doi: 10.1128/JCM.05889-11.
- Aravena-Roman M, Spröer C, Sträubler B, Inglis T, Yassin AF. *Corynebacterium pilbarense* sp. nov., a non-lipophilic corynebacterium isolated from a human ankle aspirate. Int J Syst Evol Microbiol 2010;60(7):1484-1487. doi: 10.1099/ijs.0.015966-0.
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- Vila J, Juiz P, Salas C, Almela M, García de la Fuente C, Zboromyrska Y, Navas J, Bosch J, Agüero J, Puig de la Bellacasa J, Martínez-Martínez L. Identification of Clinically Relevant *Corynebacterium* spp., *Arcanobacterium haemolyticum*, and *Rhodococcus equi* by MatrixAssisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. J Clin Microbiol 2012; 1745-1747. doi:10.1128/JCM.05821-11.
- WEBSITE: *www.bacterio.net*
- Yassin AF, Siering C. *Corynebacterium sputi* sp. nov., isolated from the sputum of a patient with pneumonia. Int J Syst Evol Microbiol 2008;58(12):2876-2879. doi: 10.1099/ijs.0.2008/000414-0.

Cronobacter (formerly *Enterobacter)*

The NRLs have not yet evaluated the performance of this genus, therefore, for the time being, it is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,7 = No identification

Table 41. Transcription of species of *Cronobacter* spp.

Species	Present in BD	No. of MSPs in BD
C. aerogenes	YES	7
*C. asburiae**	YES	3
C. bugandensis	NO	
C. cancerogenus	YES	1
*C. cloacae**	YES	15
*C. hormaechei**	YES	1
*C. kobei**	YES	1
*C. ludwigii**	YES	1
C. massiliensis	NO	
*C. nimipressuralis**	NO	

*Report as ***Cronobacter cloacae* Complex**: It comprises species *C. cloacae, C. asburiae, C. hormaechei, C. kobei, C. ludwigii* and *C. nimipressuralis.*

The complete identification of the species within this genus is achieved by sequencing specific gene targets: Elongation factor Tt, F-ATPase β-subunit, DNA gyrase *gyr*B.

Until now, the taxonomic classification of the genus continues to evolve due to the fact that:

- *C. aerogenes* and *Pantoea agglomerans* share > 99.5% identity.
- *C. aerogenes* shares 99.0% identity with the Group *Raoultella terrígena.*
- *C. aerogenes* and *Pantoea agglomerans* share >99.0% identity with *Citrobacter freundii*.

References:

- Doijad S, Imirzalioglu C, Yao Y, Pati NB, Falgenhauer L, Hain T, Foesel BU, Abt B, Overmann J, Mirambo MM, Mshana SE, Chakraborty T. *Enterobacter bugandensis* sp. nov., isolated from neonatal blood. Int J Syst Evol Microbiol 2016;66(2):968-974. doi: 10.1099/ijsem.0.000821.
- Lagier JC, El Karkouri K, Mishra AK, Robert C, Raoult D, Fournier PE. Non contiguous-finished genome sequence and description of Enterobacter massiliensis sp. nov. Stand Genomic Sci 2013; 7(3):399-412. doi: 10.4056/sigs.3396830.
- MM18A Standards from CLSI.

Cupriavidus

Limited experience with this genus.

It is phenotypically similar to *Ralstonia* sp. (refer to the Annex). For the most part, the species of this genus that cause disease in humans have been isolated from sputum cultures from fibrocystic patients and from patients with catheter-related bacteremia.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,7 = No identification

Table 42. Transcription of *Cupriavidus* spp. species isolated from clinical samples.

Species	Present in BD	No. of MSPs in BD
C. gilardii	YES	2
C. metallidurans	YES	4
C. necator	YES	7
C. pauculus	YES	7
C. respiraculi	YES	2
C. taiwanensis	NO	

References:

- D'Inzeo T, Santangelo R, Fiori B, De Angelis G, Conte V, Giaquinto A, Palucci I, Scoppettuolo G, Di Florio V, Giani T, Sanguinetti M, Rossolini GM, Spanu T. Catheter-related bacteremia by Cupriavidus metallidurans. Diagn Microbiol Infect Dis 2015;81(1):9-12. doi: 10.1016/j.diagmicrobio.2014.09.015.
- Ford BA, Burnham CA. Optimization of Routine Identification of Clinically Relevant Gram-Negative Bacteria by Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry and the Bruker Biotyper. J Clin Microbiol 2013;51(5):1412-20. doi: 10.1128/JCM.01803-12.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Vandamme P, Coenye T. Taxonomy of the genus *Cupriavidus*: a tale of lost and found. Int J Syst Evol Microbiol 2004;54(6):2285-2289. doi: 10.1099/ijs.0.63247-0.
- WEBSITE: *www.bacterio.net*

D

Delftia

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,7 = No identification

It is recommended to report as: *Delftia acidovorans* sensu lato (including species *D. acidovorans* and *D. lacustris*, which are indistinguishable by 16S rRNA gene sequencing).

There are 9 reference profiles (MSP) in the commercial database that belong to *Delftia acidovorans*.

References:

- Almuzara M, Barberis C, Traglia G, Famiglietti A, Ramirez MS, Vay C. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for species identification of Nonfermenting Gram-Negative Bacilli. J Microbiol Methods 2015;112:24–27. doi: 10.1016/j.mimet.2015.03.004.

Dermabacter

Dermabacter hominis:

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Table 43. Transcription of *Dermabacter* spp. species.

Species	Present in BD	No. of MSPs in BD
D. hominis	YES	5
D. jinjuensis	NO	
D. vaginalis	NO	

Table 44. Differential phenotypic features of *Dermabacter* spp. species.

Species	FAL	αGal	Trypsin	Glycerol
D. hominis	+	-	-	-
D. jinjuensis	+	+	ND	-
D. vaginalis	-	W	+	+

Symbols: W, weak positive; ND, not determined

References:

- Barberis C, Almuzara M, Join-Lambert O, Ramırez MS, Famiglietti A, Vay C. Comparison of the Bruker MALDI-TOF mass spectrometry system and conventional phenotypic methods for identification of Gram-positive rods. PLoS One 2014;9(9):e106303. doi: 10.1371/journal.pone.0106303.
- Bertona E, De Paulis AN, Gutiérrez MA, Santa María V, Vay CA, Predari SC. Un caso inusual de quiste sebáceo infectado por *Dermabacter hominis*. Rev Argent Microbiol 2016;48(4):303-307. https://doi.org/10.1016/j.ram.2016.09.003.
- Chang DH, Rhee MS, Kim BC. *Dermabacter vaginalis* sp. nov., isolated from human vaginal fluid. Int J Syst Evol Microbiol 2016;66(4):1881-1886. doi: 10.1099/ijsem.0.000960.
- Park YK, Lee KM, Lee WK, Cho MJ, Lee HS, Cho YG, Lee YC, Lee WK, Seong WK, Hwang KJ. *Dermabacter jinjuensis* sp. nov., a novel species of the genus *Dermabacter* isolated from a clinical specimen. Int J Syst Evol Microbiol 2016;66(7):2573-2577. doi: 10.1099/ijsem.0.001092.

Desulfovibrio

These are sulfate-reducing microorganisms, that reside in the gastrointestinal tract, but are also sporadically found in clinical specimens; bacteremia and abdominal infections in immunocompromised patients.

The low recovery rate of the constituents of this genus in clinical samples could be related to their slow growth and to the fact that in order to achieve their identification, molecular tools are needed.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,7 = No identification

Limitations in the identification are due to the absence of or to the poor representation (e.g. *D. desulfuricans*) of the microorganism's protein profile in the commercial database.

Table 45. Transcription of *Desulfovibrio* spp. species

Species	Present in BD	No. of MSPs in BD
D. desulfuricans	YES	1
D. fairfieldensis	NO	
D. piger	YES	4
D. vulgaris	NO	

Table 46. Phenotypic differentiation of clinical isolates of *Desulfovibrio* spp.

Species	NO_3 reduction	Catalase	Indole	Urease
D. desulfuricans	+	-	-	+
D. fairfieldensis	+	+	-	-
D. piger	-	-	-	-
D. vulgaris	-	-	+	-

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Manual de Microbiología Clínica de la Asociación Argentina de Microbiología. Vol I: Bacterias de importancia clínica. Parte III: Microorganismos Anaerobios. Editores: Lopardo HA, Predari SC, Vay C.

Dolosicoccus

There is no reference profile in the commercial database.

Dolosigranulum

There is a single reference profile in the commercial database, and it belongs to *Dolosigranulum pigrum.*

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,7 = No identification

It is recommended to confirm the identification of this rare or infrequent pathogen with Molecular Biology, according to CLSI standards.

References:

- Lécuyer H, Audibert J, Bobigny A, Eckert C, Jannière-Nartey C, Buu-Hoï A, Mainardi JL, Podglajen I. Dolosigranulum pigrum causing nosocomial pneumonia and septicemia. J Clin Microbiol 2007;45(10):3474-5. doi: 10.1128/JCM.01373-07.

Dysgonomonas

It is recommended to report the identification to the genus level with a **score value >1,5.**

Table 47. Transcription of *Dysgonomonas* spp. species.

Species	**Present in BD**	**No. of MSPs in BD**
D. capnocytophagoides	NO	
D. gadei	YES	1
D. hofstadii	NO	
D. mossii	NO	

There are three species that are not represented in the commercial database. Besides, it might incorrectly identify *Dysgonomonas gadei* since there is only one MSP.

Phenotypic differentiation among species is not possible.

It is recommended to use sequencing of the 16S rRNA gene for a complete identification.

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

E

Eggerthella

Species of the genera *Eggerthella* and *Paraeggerthella* have been isolated from a wide range of human infections. *E. lenta* (formerly *Eubacterium lentum*) is a very frequent intraabdominal pathogen. *E. lenta*, *E. sinensis* y *P. hongkongensis* have been found in blood in association with clinically important infections of high mortality rates.

They are gram positive cocci or short rods, non-spore-forming, anaerobic, arranged in pairs or short chains.

Table 48. Phenotypic characteristics and traduction for *Eggerthella* spp.

Species	**Glucose fermentation**	**Catalase**	**Indole**	**Nitrate reduction**	**Esculin hydrolysis**	**Arginine hydrolysis**	**Presence in BD**
E. lenta	-	+	-	+	-	+	YES (5 MSPs)
E. sinensis	-	+	-	-	ND	+	NO

Referencias:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- SITIO WEB: *www.bacterio.net*

Eikenella corrodens

The following score values have been accepted for a reliable identification, based on data gathered from our experience (not published):

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

There are 6 reference profiles in the commercial database.

References:

- Couturier MR, Mehinovic E, Croft AC, Fisher MA. Identification of HACEK Clinical Isolates by matrix-assisted laser desorption ionization–time of flight mass spectrometry. J Clin Microbiol 2011;49(3):1104–1106. doi: 10.1128/JCM.01777-10.
- Powell EA, Blecker-Shelly D, Montgomery S, Mortensen JE. Application of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Identification of the Fastidious Pediatric Pathogens *Aggregatibacter, Eikenella, Haemophilus,* and *Kingella*. J Clin Microbiol 2013;51(11):3862–3864. doi: 10.1128/JCM.02233-13.

Elizabethkingia

It is recommended to report the identification to the genus level with a **score value >1,7.**

Elizabethkingia anophelis is not included in the database and could be identified as *Elizabethkingia meningoseptica*. It is important to discriminate them with biochemical tests (Table 47).

Table 49. Phenotypic features of *Elizabethkingia* spp. species isolated from clinical samples.

Species	**Present in BD**	**No. of MSPs**	**Citrate**	**MConkey**	**Urease**	**Cellobiose acid**	**Melibiose acid**
E. meningoseptica	YES	3	V	-/v	-	-/v	+
E. miricola	YES	2	+	-	+	-	-
E. anophelis	NO		-	+	-	+	-

Symbols: V, variable.

References:

- Kämpfer P, Matthews H, Glaeser SP, Martin K, Lodders N, Faye I. Elizabethkingia anophelis sp. nov., isolated from the midgut of the mosquito Anopheles gambiae. Int J Syst Evol Microbiol 2011 Nov;61(11):2670-5. doi: 10.1099/ijs.0.026393-0.

Empedobacter brevis

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,7 = No identification

There are 2 MSPs in the commercial database that belong to *Empedobacter brevis.*

Enterococcus

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE < 1,5 = No identification

Table 50. Transcription of species of *Enterococcus* spp.

Species	Present in BD	No. of MSPs in BD
E. avium	YES	8
E. caccae	YES	1
E. casseliflavus	YES	6
E. cecorum	YES	4
E. columbae	YES	1
E. devriesei	YES	1
E. dispar	YES	1
E. durans	YES	9
E. faecalis	YES	11
E. faecium	YES	10
E. gallinarum	YES	3
E. gilvus	YES	3
E. hirae	YES	8
E. italicus	YES	2
E. malodoratus	YES	2
E. mundtii	YES	4
E. pallens	YES	1
E. raffinosus	YES	3

References:

- Almuzara M, Barberis C, Velázquez VR, Ramirez MS, Famiglietti A, Vay C. Matrix-assisted Laser Desorption Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) as a Reliable Tool to Identify Species of Catalase-negative Gram-positive Cocci not Belonging to the *Streptococcus* Genus. Open Microbiol J 2016;10,202–208. doi: 10.2174/1874285801610010202.

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- WEBSITE: *www.bacterio.net*

Erysipelothrix rhusiopathiae

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,7 = No identification

Accurate identification to the species level is achieved with a **score value >2,0.**

There are 10 representative MSPs in the commercial database.

Escherichia

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,7 = No identification

MALDI-TOF identifies *Shigella* spp. (which is not included in the commercial database) as *E.coli.* The need to complete the identification with biochemical tests will depend on the type of sample and on local epidemiology (refer to Annex).

Table 51. Transcription of *Escherichia* spp. species.

Species	Present in BD	No. of MSPs in BD
E. albertii	YES	1
E. blattae	NO	
E. coli	YES	14
E. fergusonii	YES	1
E. hermannii	YES	3
E. vulneris	YES	1

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Eubacterium

This genus continues to be poorly defined, but the species it comprises are typically isolated from infections in the oral cavity. It particularly grows on rich media and when prolonged incubation periods are used.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,7 = No identification

The equipment's limitations are mostly due to the fact that there is no protein profile of this microorganism in the commercial database.

Table 52. Transcription of species within genus *Eubacterium* spp.

Species	Present in BD	No. of MSPs in BD
E. barkeri	NO	
E. biforme	NO	
E. brachy	YES	1
E. budayi	NO	
E. callanderi	YES	1
E. contortum	NO	
E. cylindroides	NO	
E. dolichum	NO	
E. eligens	NO	
E. hallii	NO	
E. infirmum	NO	
E. limosum	YES	5
E. minutum	NO	
E. moniliforme	NO	
E. nitritogenes	NO	
E. nodatum	NO	
E. plautii	NO	
E. ramulus	NO	
E. rectale	NO	
E. saphenum	NO	
E. sulci	NO	

E. tardum	NO	
E. tenue	YES	1
E. timidum	NO	
E. ventriosum	NO	
E. yurii	YES	3

References:

- Cato EP, Holdeman LV, Moore WEC. Designation of *Eubacterium limosum* (Eggerth) Prévot as the Type Species of *Eubacterium*. Int J Syst Bacteriol 1981;31,209-210. doi: 10.1099/00207713-31-2-209.
- Cheeseman SL, Hiom SJ, Weightman AJ, Wade WG. Phylogeny of oral asaccharolytic *Eubacterium* species determined by 16S ribosomal DNA sequence comparison and proposal of *Eubacterium infirmum* sp. nov. and *Eubacterium tardum* sp. nov. Int J Syst Bacteriol 1996;46(4):957-959. doi: 10.1099/00207713-46-4-957.
- Holdeman LV, Cato EP, Burmeister JA, Moore WEC. Descriptions of *Eubacterium timidum* sp. nov., *Eubacterium brachy* sp. nov., and *Eubacterium nodatum* sp. nov. Isolated from Human Periodontitis. Int J Syst Bacteriol 1980;30(1):163-169. doi: 10.1099/00207713-30-1-163.
- Margaret BS, Krywolap GN. *Eubacterium yurii* subsp. *yurii* sp. nov. and *Eubacterium yurii* subsp. *margaretiae* subsp. nov.: Test Tube Brush Bacteria from Subgingival Dental Plaque. Int J Syst Bacteriol 1986;36,145-149. doi: 10.1099/00207713-36-2-145.
- Poco SE Jr, Nakazawa F, Sato M, Hoshino E. *Eubacterium minutum* sp. nov., isolated from human periodontal pockets. Int J Syst Bacteriol 1996;46(1):31-34. doi: 10.1099/00207713-46-1-31.
- Uematsu H, Nakazawa F, Ikeda T, Hoshino E. *Eubacterium saphenus* sp. nov., Isolated from Human Periodontal Pockets. Int J Syst Bacteriol 1993;43(2):302-304. doi: 10.1099/00207713-43-2-302.

Exiguobacterium

Since they are Gram-positive rods belonging to the pigmented group, MALDI-TOF can identify them to the genus level with a **score value > 1,5.**

Exiguobacterium acetylicum is the most frequent species.

In 10 years, *Exiguobacterium aurantiacum* has only been isolated six times by reference centers.

Table 53. Transcription of species within genus *Exiguobacterium* spp.

Species	**Present in BD**	**No. of MSPs in BD**
Exiguobacterium acetylicum	NO	
Exiguobacterium aurantiacum	YES	1
Exiguobacterium sp.	YES	3

Table 54. Useful phenotypic tests for the differentiation of species within genus *Exiguobacterium* spp.

Species	**Oxidase**	**DNAse**	**Xylose**	**Observations**
E. acetylicum	+	-	-	Yellow-gold pigment
E. aurantiacum	-	+	+	Susceptible to all drugs

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

F

Facklamia

This genus is closely related to *Globicatella*, but it is phenotypically and phylogenetically different from it. Isolates from the four species of *Facklamia* that affect humans have been recovered from blood, wounds, genitourinary tract and from a case of chorioamnionitis.

The following criteria have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Table 55. Transcription of species within genus *Facklamia* spp. isolated from clinical samples.

Species	Present in BD	No. of MSPs in BD
F. hominis	YES	3
F. ignava	NO	
F. languida	YES	1
F. sourekii	NO	

Refer to the Annex for microbial identification using additional phenotypic testing.

References:

- Almuzara M, Barberis C, Velázquez VR, Ramirez MS, Famiglietti A, Vay C. Matrix-assisted Laser Desorption Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) as a Reliable Tool to Identify Species of Catalase-negative Gram-positive Cocci not Belonging to the *Streptococcus* Genus. Open Microbiol J 2016;10,202–208. doi: 10.2174/1874285801610010202.
- Collins MD, Hutson RA, Falsen E, Sjödén B. *Facklamia sourekii* sp. nov., isolated from human sources. Int J Syst Bacteriol 1999;49(2):635-638. doi: 10.1099/00207713-49-2-635.

- Collins MD, Lawson PA, Monasterio R, Falsen E, Sjöden B, Facklam RR. *Facklamia ignava* sp. nov., isolated from human clinical specimens. J Clin Microbiol 1998;36(7):2146-2148.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Lawson PA, Collins MD, Falsen E, Sjöden B, Facklam RR. Facklamia languida sp. nov., Isolated from Human Clinical Specimens. J Clin Microbiol 1999;37(4):1161–1164.
- WEBSITE: *www.bacterionet.com*

Finegoldia magna

Among anaerobic gram-positive cocci, it is considered the most pathogenic species and it has been isolated from a wide variety of sites of infection (skin, bone tissue, ulcers, abscesses, prosthetic infections). These multiple findings suggest that the clinical relevance of *Finegoldia magna* has been underestimated.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,7 = No identification

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Flavobacterium

Flavobacterium mizutaii is not included in the commercial database; other species within these genus have been renamed as *Sphingobacterium (S. multivorum* and *S. spiritivorum).*

Francisella

Francisella tularensis is the causative agent of tularemia: an acute and fatal disease in animals and humans. Human infection may occur from being bitten or stung by an

arthropod, from being in contact with an infected animal, or by ingesting contaminated food or water.

This genus also comprises other rarely-known species that are considered environmental and / or opportunistic pathogens. While *F. noatunensis* and *F. halioticida* cause infection and death in fish; *F. novicida* and *F. philomiragia* are associated with salt water and only appear in infrequent, opportunistic infections in immunocompromised individuals.

As for *F. hispaniensis*, there is only one case of human infection described in literature.

Human infections caused by *F. philomiragia* are very rare; and they affect patients with underlying diseases. Less than 20 cases of infection caused by *F. philomiragia* and less than 10 cases by *F. novicida* have been described since the appearance of the species in 1974.

Tularemia is caused by two subspecies: *F. tularensis* subsp. *tularensis* (type A) and *F. tularensis* subsp. *holarctica* (type B).

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,0 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,7 = No identification

There are only 6 spectra in the equipment's database that belong to *Francisella philomiragia.*

The MLST technique is necessary for a complete characterization (genes: *ISFtu2, igl*C, *tul*4, *fop*A).

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Fusobacterium

It is recommended to report the identification to the genus level, except for *F. nucleatum* and *F. naviforme,* which should be reported as *F. nucleatum/naviforme*. Based on our experience, identification of species *F. necrophorum* and *F. mortiferum* is accurate.

Table 56. Transcription of species within genus *Fusobacterium* spp.

Species	Present in BD	No. of MSPs in BD
F. canifelinum	YES	7
F. equinum	YES	1
F. fastidiosum	NO	
F. gonidiaformans	YES	6
F. mortiferum	YES	2
F. naviforme	YES	2
F. necrophorum	YES	13
F. nucleatum	YES	14
F. perfoetens	NO	
F. periodonticum	YES	1
F. russii	NO	
F. simiae	NO	
F. ulcerans	YES	2
F. varium	YES	2

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Veloo ACM, Jean-Pierre H, Justesen US, Morris T, Urban E, Wybo I, Kostrzewa M, Friedrich AW. Validation of MALDI-TOF MS Biotyper database optimized for anaerobic bacteria: The ENRIA project. Anaerobe 2018;54:224-230. https://doi.org/10.1016/j.anaerobe.2018.03.007.

G

Gardnerella vaginalis

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE < 1,5 = No identification

All *G. vaginalis* isolates are consistently susceptible to SPS (Sodium polyanethole sulfonate) (zone diameter > 10mm). It is suggested to perform this as a confirmatory test.

References:

- Benagli C, Rossi V, Dolina M, Tonolla M, Petrini O. Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for the Identification of Clinically Relevant Bacteria. PLoS One 2011;6(1):e16424. doi: 10.1371/journal.pone.0016424.
- Barberis C. Tesis doctoral: Identificación e impacto clínico de Bacilos Gram positivos aerobios no esporulados oportunistas. 2013 Cátedra de Microbiología Clínica. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires.
- Greenwood JR, Pickett MJ. Transfer of *Haemophilus vaginalis* Gardner and Dukes to a new genus, *Gardnerella: G. vaginalis* (Gardner and Dukes) comb. nov. Int J Syst Evol Microbiol 1980;30,170-178. doi: 10.1099/00207713-30-1-170.

Gemella

The following score values have been accepted for a reliable identification:

SCORE >1,70 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,50 = No identification

Table 57. Transcription of species within genus *Gemella* spp. isolated from clinical samples.

Species	Present in BD	No. of MSPs in BD
G. asaccharolytica	NO	
G. bergeri	YES	1
G. haemolysans	YES	7
G. morbillorum	YES	5
G. parahaemolysans	NO	
G. sanguinis	YES	8
G. taiwanensis	NO	

Table 58. Biochemical tests for the differentiation of species of *Gemella* spp.

Species	PYR	Hippurate	FAL	Maltose acid	Mannitol acid	Sorbitol acid
G. asaccharolytica	-	+	-	-	-	-
G. bergeri	+	-	-	-	+	-
G. haemolysans	+	-	+	+	-	-
G. morbillorum	+	-	-	+	+	+
G. parahaemolysans	+	-	+	+	-	-
G. sanguinis	+	-	+	+	+	+
G. taiwanensis	+	-	+	+	+	+

References:

- Almuzara M, Barberis C, Velázquez VR, Ramirez MS, Famiglietti A, Vay C. Matrix-assisted Laser Desorption Ionization-Time-of-Flight Mass Spectrometry

(MALDI-TOF MS) as a Reliable Tool to Identify Species of Catalase-negative Gram-positive Cocci not Belonging to the *Streptococcus* Genus. Open Microbiol J 2016;10,202–208. doi: 10.2174/1874285801610010202.

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- Collins MD, Hutson RA, Falsen E, Sjöden B, Facklam RR. *Gemella bergeriae* sp. nov., isolated from human clinical specimens. J Clin Microbiol 1998;36(5):1290-1293.
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- Thjötta T, Böe J. *Neisseria hemolysans*. A hemolytie species of *Neisseria trevisan*. Acta pathologica et Microbiologica Scandinavica 1938;37,527-531.
- Ulger-Toprak N, Summanen PH, Liu C, Rowlinson MC, Finegold SM. Gemella asaccharolytica sp. nov., isolated from human clinical specimens. Int J Syst Evol Microbiol 2010;60(5):1023-1026. doi: 10.1099/ijs.0.001966-0.

Globicatella

Globicatella sanguinis has been isolated from clinical specimens, it is involved in cases of bacteremia, urinary tract infections and meningitis. The second species of this genus, *Globicatella sulfidifaciens*, has been recovered from purulent infections in domestic animals.

There are three reference profiles (MSP) for *Globicatella sanguinis* in the database, and 1 MSP for *Globicatella sulfidifaciens*.

Both species can be differentiated by biochemical tests (Table 58).

Table 59. Phenotypic features of the species within genus *Globicatella* spp.

Especie	PYR	βGal	βGur	Mannitol	Ribose
G. sulfidifaciens	-	-	+	-	-
G. sanguinis	+	+	-	+	+

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

References:

- Almuzara M, Barberis C, Velázquez VR, Ramirez MS, Famiglietti A, Vay C. Matrix-assisted Laser Desorption Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) as a Reliable Tool to Identify Species of Catalase-negative Gram-positive Cocci not Belonging to the *Streptococcus* Genus. Open Microbiol J 2016;10,202–208. doi: 10.2174/1874285801610010202.
- Vandamme P, Hommez J, Snauwaert C, Hoste B, Cleenwerck I, Lefebvre K, Vancanneyt M, Swings J, Devriese LA, Haesebrouc F. Globicatella sulfidifaciens sp. nov., isolated from purulent infections in domestic animals. Int J Syst Evol Microbiol 2001 Sep;51(5):1745-9. doi: 10.1099/00207713-51-5-1745.

Gordonia

MALDI-TOF achieves a correct identification to the genus level. It is recommended to report *Gordonia* sp. with a **score value >1,5.**

MALDI-TOF may identify other species as *Gordonia rubripertincta*, e.g.: *Gordonia otitidis /sputi /aichinensis /jacobea,* and these cannot be discriminated with sequencing of the 16S rRNA gene.

Limitations in the identification are due to the absence of or to the poor representation of the microorganism's protein profile in the commercial database, which makes it necessary to expand the commercial database.

It is recommended to confirm the identification to the species level with Molecular Biology (genes *hsp*65, *gyr*B, *sec*A).

Table 60. Transcription of species of *Gordonia* spp. isolated from clinical samples.

Species	Present in BD	No. of MSPs in BD
G. aichiensis	YES	1
G. araii	NO	
G. bronchialis	YES	2
G. effusa	NO	
G. hongkongensis	NO	
G. iterans	NO	
G. otitidis	NO	
G. polyisoprenivorans	NO	
G. rubripertincta	YES	11
G. sputi	YES	5
G. terrae	YES	2

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
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- Tsang CC, Xiong L, Poon RW, Chen JH, Leung KW, Lam JY, Wu AK, Chan JF, Lau SK, Woo PC. *Gordonia hongkongensis* sp. nov., isolated from blood culture and peritoneal dialysis effluent of patients in Hong Kong. Int J Syst Evol Microbiol 2016;66(10):3942-3950. doi: 10.1099/ijsem.0.001292.
- Verroken A, Janssens M, Berhin C, Bogaerts P, Huang TD, Wauters G, Glupczynski Y. Evaluation of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Identification of *Nocardia* Species. J Clin Microbiol 2010;48(11):4015-21. doi: 10.1128/JCM.01234-10.
- WEBSITE: *www.bacterio.net*

Granulicatella

Organisms within genus *Abiotrophia* and *Granulicatella* are known as nutritional variants of *Streptococcus* (NVS).

The satellitism test is essential for the identification of both genera.

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Table 61. Transcription of species within genus *Granulicatella* spp.

Species	Present in BD	No. of MSPs in BD
G. adiacens	YES	6
G. balaenopterae	YES	1
G. elegans	YES	1

Table 62. Phenotypic features of species within genus *Granulicatella* spp.

Species	βGur	ADH	Hippurate	Sucrose acid	Trehalose acid
G. adiacens	+	-	-	+	-
G. balaenopterae	-	+	-	-	+
G. elegans	-	+	V	+	-

Symbols: V, variable.

References:

- Almuzara M, Barberis C, Velázquez VR, Ramirez MS, Famiglietti A, Vay C. Matrix-assisted Laser Desorption Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) as a Reliable Tool to Identify Species of Catalase-negative Gram-positive Cocci not Belonging to the *Streptococcus* Genus. Open Microbiol J 2016;10,202–208. doi: 10.2174/1874285801610010202.
- Collins MD, Lawson PA. The genus Abiotrophia (Kawamura et al.) is not monophyletic: proposal of Granulicatella gen. nov., Granulicatella adiacens

comb. nov., Granulicatella elegans comb. nov. And Granulicatella balaenopterae comb. nov. Int J Syst Evol Microbiol 2000;50(1):365-9. doi: 10.1099/00207713-50-1-365.

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

H

Haemophilus

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 63. Transcription of *Haemophilus* spp. species.

Species	**Present in BD**	**No. of MSPs in BD**
H. aegyptius	NO	
H. ducreyi	YES	1
H. haemolyticus	YES	21
H. influenzae	YES	27
H. parahaemolyticus	YES	7
H. parainfluenzae	YES	11
H. paraphrohaemolyticus	YES	1
H. pittmaniae	YES	2
H. sputorum	YES	2

Refer to the Annex for microbial identification by additional phenotypic testing.

References:

- CLSI. *Methods for the Identification of Cultured Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry*. 1st ed. CLSI guideline M58. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Hafnia

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

There are 7 MSPs for *Hafnia alvei* and none for *Hafnia paralvei* in the commercial database.

Both species can be frequently isolated from clinical samples, since they produce a Vero cytolytic toxin, although *H. alvei* are more likely to be toxigenic than *H. paralvei*.

Hafnia isolates can be unequivocally assigned to the correct species (*H. alvei* or *H. paralvei*) based on the biochemical tests detailed below.

Table 64. Identification of *Hafnia* spp. species

Species	Use of Malonate	Citrate
H. alvei	+	-
H. paralvei	-	V

Symbols: V, variable.

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Helcococcus

Helcococcus kunzii can be isolated from skin and bone infections, especially from the lower extremities, such as feet. Its clinical significance is difficult to analyse since it is usually a colonization agent.

The ability of *Helcococcus kunzii* to become an opportunistic pathogen is suggested when it is recovered as either the only or the predominant organism in breast infections, sebaceous cysts, prosthetic infections, bacteremia and empyema.

Helcococcus sueciensis and *Helcococcus pyogenes* have only been isolated from a bone infection and from a prosthetic infection, respectively.

Helcococcus seattlensis has been recovered from a patient with urosepsis.

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Score values can be increased when the formic acid method and protein extraction technique are performed.

Refer to the Annex for microbial identification by additional phenotypic testing.

Table 65. Transcription of species of *Helcococcus* spp.

Species	Present in BD	No. of MSPs in BD
H. kunzii	YES	5
H. pyogenes	NO	
H. seattlensis	NO	
H. sueciensis	YES	1

References:

- Almuzara M, Barberis C, Rojas Velázquez V, Ramirez MS, Famiglietti A, Vay C. Matrix-assisted Laser Desorption Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) as a Reliable Tool to Identify Species of Catalase-negative Gram-positive Cocci not Belonging to *the Streptococcus* Genus. Open Microbiol J 2016; 10:202–208. doi: 10.2174/1874285801610010202.
- Chow SK, Clarridge JE 3erd. Identification and Clinical Significance of Helcococcus species, with Description of Helcococcus seattlensis sp. nov. from a Patient with Urosepsis. J Clin Microbiol 2014;52(3):854-8. doi: 10.1128/JCM.03076-13.

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Panackal AA, Houze YB, Prentice J, Leopold SS, Cookson BT, Liles WC, Limaye AP. Prosthetic Joint Infection Due to "*Helcococcus pyogenica*". J Clin Microbiol 2004;42(6):2872–2874. doi: 10.1128/JCM.42.6.2872-2874.2004.

Helicobacter

It is recommended to report the identification to the genus level with a **score value ≥1,7.**

The expansion of the commercial Database is necessary for a complete identification to the species level; sequencing of the specific gene *hsp*60 can also be carried out for this purpouse.

Refer to the Annex for microbial identification by additional phenotypic testing.

There are numerous studies with MALDI-TOF in which it is possible to achieve the differentiation between *Helicobacter* and *Campylobacter*, as well as the subtyping of both animal and human species of *Helicobacter,* based on the evaluation of biomarker peaks (See References).

Table 66. Transcription of *Helicobacter* spp. species.

Species	Present in BD	No. of MSPs in BD
H. acinonychis	NO	
H. anseris	NO	
H. aurati	NO	
H. baculiformis	NO	
H. bilis	NO	
H. bizzozeronii	NO	
H. bovis	NO	
H. brantae	NO	
H. canadensis	YES	1

H. canis	YES	2
H. cetorum	NO	
H. cholecystus	YES	1
H. cinaedi	YES	2
H. cynogastricus	NO	
H. equorum	NO	
H. felis	NO	
H. fennelliae	YES	1
H. ganmani	NO	
H. heilmannii	NO	
H. hepaticus	NO	
H. marmotae	NO	
H. mastomyrinus	NO	
H. mesocricetorum	NO	
H. muridarum	NO	
H. mustelae	YES	1
H. pametensis	NO	
H. pullorum	YES	9
H. pylori	YES	7
H. rodentium	NO	
H. salomonis	NO	
H. suis	NO	

H. trogontum	NO	
H. typhlonius	NO	
H. winghamensis	NO	

References:

- Bessède E, Bénéjat L, Sifré E, Chapelle M, Mogabure P, Mégraud F. MALDI-TOF mass spectrometry applied to *H. pylori* diagnosis and typing. CNR Campylobacters et Hélicobacters, Hôpital Pellegrin et Université de Bourdeaux, France.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Taniguchi T, Sekiya A, Higa M, Saeki Y, Umeki K, Okayama A, Hayashi T, Misawa N. Rapid Identification and Subtyping of Helicobacter cinaedi Strains by Intact-Cell Mass Spectrometry Profiling with the Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. J Clin Microbiol 2014;52(1):95-102. doi: 10.1128/JCM.01798-13.
- Winkler MA, Uher J, Cepa S. Direct Analysis and Identification of Helicobacter and Campylobacter Species by MALDI-TOF Mass Spectrometry. Anal Chem 1999;71(16):3416–3419.

Histophilus somni

There are 2 reference profiles or MSPs in the commercial database.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

I

Ignavigranum ruoffiae

There is no reference profile for this microorganism in the commercial database.

Inquilinus limosus

There are 3 MSPs in the database that belong to *Inquilinus limosus.*

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

By presenting a mucous phenotype it can yield low score values, and require the addition of a drop of formic acid to the sample. If the expected result is not achieved, the extraction recommended by the manufacturer can be attempted.

J

Jeotgalicoccus halotolerans

There is only one MSP or reference profile in the commercial database.

It is recommended to report as *Jeotgalicoccus halotolerans* with **score values >1,7.**

K

Kerstersia

There are 2 reference profiles for *Kerstersia gyiorum,* and none for *Kerstersia similis.*

Both species are phenotypically indistinguishable, therefore, it is recommended to use *gyr*B gene sequencing for their differentiation.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

References:

- Almuzara M, Barberis C, Traglia G, Famiglietti A, Ramirez MS, Vay C. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for species identification of nonfermenting Gram-negative bacilli. J Microbiol Methods 2015;112:24-7. doi: 10.1016/j.mimet.2015.03.004.
- Coenye T, Vancanneyt M, Cnockaert MC, Falsen E, Swings J, Vandamme P. Kerstersia gyiorum gen. nov., sp. nov., a novel Alcaligenes faecalis-like organism isolated from human clinical samples, and reclassification of Alcaligenes denitrificans Rüger and Tan 1983 as Achromobacter denitrificans comb. nov. Int J Syst Evol Microbiol 2003;53(6):1825–1831. doi: 10.1099/ijs.0.02609-0.
- Vandamme P, De Brandt E, Houf K, De Baere T. Kerstersia similis sp. nov., isolated from human clinical samples. Int J Syst Evol Microbiol 2012;62(9):2156-9. doi: 10.1099/ijs.0.037887-0.

Kingella

The following score values have been accepted for a reliable identification, based on data gathered from our experience (not published):

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69= Identification to the genus level

SCORE <1,5= No identification

Table 67. Transcription of species of *Kingella* spp.

Species	Present in BD	No. of MSPs in BD
K. denitrificans	YES	8
K. kingae	YES	10
K. oralis	YES	2
K. potus	YES	1

Klebsiella

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

It is recommended to report as *K. pneumoniae / K. oxytoca* Complex.

MALDI-TOF cannot discriminate between *Klebsiella oxytoca* and *Raoultella ornithinolytica*, due to the close similarity of both spectra. It is recommended to complete the identification with the biochemical profile (see Annex) and/or confirm using *rpo*B gene sequencing.

Genes *pho*E and *scr*A (sucrose regulon) are also used to differentiate *Klebsiella granulomatis* (*pho*E-positive and *scr*A-negative) from the other species of *Klebsiella* (*pho*E and *scr*A-positive).

Table 68. Transcription of *Klebsiella* spp. species.

Species	Present in BD	No. of MSPs in BD
K. granulomatis	NO	
K. michiganensis	NO	
K. oxytoca	YES	8
K. ozaenae	NO	
K. pneumoniae	YES	11
K. quasipneumoniae	NO	
K. rhinoscleromatis	NO	
K. singaporensis	NO	
K. varicola	YES	12

References:

- Brisse S, Passet V, Grimont PA. Description of *Klebsiella quasipneumoniae* sp. nov., isolated from human infections, with two subspecies, *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* subsp. nov. and *Klebsiella quasipneumoniae* subsp. *similipneumoniae* subsp. nov., and demonstration that *Klebsiella singaporensis* is a junior heterotypic synonym of *Klebsiella variicola*. Int J Syst Evol Microbiol 2014;64(9):3146-3152. doi: 10.1099/ijs.0.062737-0.
- Carter JS, Bowden FJ, Bastian I, Myers GM, Sriprakash KS, Kemp DJ. Phylogenetic evidence for reclassification of *Calymmatobacterium granulomatis* as *Klebsiella granulomatis* comb. nov. Int J Syst Bacteriol 1999;49(4):1695-1700. doi: 10.1099/00207713-49-4-1695.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Saha R, Farrance CE, Verghese B, Hong S, Donofrio RS. *Klebsiella michiganensis* sp. nov., a new bacterium isolated from a tooth brush holder. Curr Microbiol 2013;66(1):72-78. doi: 10.1007/s00284-012-0245-x.

Kocuria

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

The equipment may not discriminate between *K. rosea* and *K. polaris,* although the latter has not been isolated from humans. However, the identification to the species level can be completed by *rpo*B gene sequencing.

Refer to the Annex for microbial identification by additional phenotypic testing.

Table 69. Transcription of *Kocuria* spp. species.

Species	Present in BD	No. of MSPs in BD
K. carniphila	YES	1
K. kristinae	YES	9
K. rhizophila	YES	6
K. rosea	YES	5
K. varians	YES	1

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Reddy GS, Prakash JS, Prabahar V, Matsumoto GI, Stackebrandt E, Shivaji S. *Kocuria polaris* sp. nov., an orange-pigmented psychrophilic bacterium isolated from an Antarctic cyanobacterial mat sample. Int J Syst Evol Microbiol 2003;53(1):183-187. doi: 10.1099/ijs.0.02336-0.

Kytococcus

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 70. Transcription of *Kytococcus* spp. species

Species	Present in BD	No. of MSPs in BD
K. aerolatus	NO	
K. schroeteri	YES	1
K. sedentarius	YES	5

References:

- Becker K, Schumann P, Wüllenweber J, Schulte M, Weil HP, Stackebrandt E, Peters G, von Eiff C. *Kytococcus schroeteri* sp. nov., a novel Gram-positive actinobacterium isolated from a human clinical source. Int J Syst Evol Microbiol 2002;52(5):1609-1614. doi: 10.1099/00207713-52-5-1609.
- Stackebrandt E, Koch C, Gvozdiak O, Schumann P. Taxonomic dissection of the genus *Micrococcus*: *Kocuria* gen. nov., *Nesterenkonia* gen. nov., *Kytococcus* gen. nov., *Dermacoccus* gen. nov., and *Micrococcus* Cohn 1872 gen. emend. Int J Syst Bacteriol 1995;45(4):682-692. doi: 10.1099/00207713-45-4-682.

L

Lactobacillus

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Clarification: This recommendation is based on a limited number of isolates.

Lactobacillus rhamnosus/Lactobacillus casei/Lactobacillus paracasei, and others: for their complete differentiation, it is recommended to perform sequencing of genes *phe*S and *rpo*A.

Table 71. Transcription of species of *Lactobacillus* spp.

Species	Present in BD	No. of MSPs in BD
L. acidifarinae	YES	1
L. acidipiscis	YES	2
L. acidophilus	YES	3
L. agilis	YES	3
L. algidus	YES	1
L. alimentarius	YES	2
L. amyloliticus	YES	1
L. amylophilus	YES	1
L. amylotrophicus	YES	1
L. amylovorus	YES	4
L. antri	YES	2
L. aodemi	YES	1
L. aviarius	YES	2

L. backii	YES	2
L. bifermentans	YES	1
L. brevis	YES	6
L. buchneri	YES	1
L. casei	YES	1
L. coleohominis	YES	1
L. collinoides	YES	2
L. concavus	YES	1
L. confusus	NO	
L. coryniformis	YES	4
L. crispatus	YES	3
L. curvatus	YES	5
L. delbrueckii	YES	8
L. diolivorans	YES	1
L. equi	YES	1
L. farciminis	YES	1
L. fermentum	YES	9
L. fornicalis	NO	
L. fructivorans	YES	3
L. frumenti	YES	1
L. fuchuensis	YES	3
L. gallinarum	YES	1
L. gasseri	YES	9
L. gastricus	YES	2
L. graminis	YES	1

L. hammesii	YES	2
L. hamsteri	YES	1
L. harbinensis	YES	2
L. helveticus	YES	1
L. hilgardii	YES	2
L. hominis	NO	
L. homochiochii	YES	2
L. iners	YES	1
L. ingluviei	YES	2
L. intestinalis	YES	1
L. jensenii	YES	3
L. johnsonii	YES	2
L. kalixensis	YES	2
L. kefiri	YES	3
L. lactis	YES	14
L. paracasei	YES	15
L. paraplantarum	YES	2
L. pentosus	YES	3
L. perolens	YES	2
L. piscium	YES	1
L. plantarum	YES	9
L. raffinolactis	YES	2
L. rhamnosus	YES	13
L. salivarius	YES	3
L. uli	NO	

L. ultunensis	YES	2
L. vitulinus	NO	

References:

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- Roos S, Engstrand L, Jonsson H. *Lactobacillus gastricus* sp. nov., *Lactobacillus antri* sp. nov., *Lactobacillus kalixensis* sp. nov. and *Lactobacillus ultunensis* sp. nov., isolated from human stomach mucosa. Int J Syst Evol Microbiol 2005;55(1):77-82. doi: 10.1099/ijs.0.63083-0.

Lactococcus

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Table 72. Transcription of species of *Lactococcus* spp.

Species	Present in BD	No. of MSPs in BD
L. garviae	YES	3
L. lactis	YES	14

References:

- Almuzara M, Barberis C, Rojas Velázquez V, Ramirez MS, Famiglietti A, Vay C. Matrix-assisted Laser Desorption Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) as a Reliable Tool to Identify Species of Catalase-negative Gram-positive Cocci not Belonging to *the Streptococcus* Genus. Open Microbiol J 2016; 10:202–208. doi: 10.2174/1874285801610010202.

Legionella

Based on the NRLs' limited experience with this genus, we can only assert that MALDI-TOF correctly identifies species *Legionella pneumophila* and *Legionella micdadei,* with **score values >2.**

MALDI-TOF has an extensive database of spectra of the species within the genus, which makes it a potential tool for culture identification.

Leuconostoc

This genus is vancomycin-resistant, just like *Pediococcus;* but *Leuconostoc* sp. produces gas and is always ADH-negative (refer to the Annex).

It can be isolated from blood, CSF, peritoneal fluid and wounds, as a causative agent of osteomyelitis, brain abscess, endophthalmitis and bacteremia.

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Table 73. Transcription of species within *Leuconostoc* spp.

Species	Present in BD	No. of MSPs in BD
L. carnosum	YES	1
L. citreum	YES	5
L. gelidum	YES	5
L. holzapfelii	YES	1
L. inhae	YES	1
L. mesenteroides	YES	6
L. palmae	YES	1
L. pseudomesenteroides	YES	2

References:

- Almuzara M, Barberis C, Rojas Velázquez V, Ramirez MS, Famiglietti A, Vay C. Matrix-assisted Laser Desorption Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) as a Reliable Tool to Identify Species of Catalase-negative Gram-positive Cocci not Belonging to *the Streptococcus* Genus. Open Microbiol J 2016; 10:202–208. doi: 10.2174/1874285801610010202.

Listeria

MALDI-TOF achieves an accurate identification to the genus level, but cannot discriminate among species (generally between *L. monocytogenes* and *L. innocua*) even with score values >2,0. The manufacturer recommends ethanolic extraction for a correct identification at the species level; however, it is recommended to perform phenotypic tests for species confirmation (Table 74).

Table 74. Transcription of species within *Listeria* spp.

Species	Present in BD	No. of MSPs in BD
L. fleischmannii	NO	
L. grayi	YES	2
L. innocua	YES	1
L. ivanovii	YES	2
L. monocytogenes	YES	5
L. seeligeri	YES	1
L. whelsimeri	YES	1

Table 75. Phenotypic tests for the differentiation of species within *Listeria* spp.

Assay	*L. grayi*	*L. innocua*	*L. ivanovii ss ivanovii*	*L. ivanovii ss lodoniensis*	*L. marthii*	*L. monocytogenes*	*L. seeligeri*	*L. welshimeri*
β-**Hemolysis**	-	-	++	++	-	+	+	-
CAMP *S. aureus*	-	-	-	-	ND	+	+	-
CAMP *R. equi*	-	-	+	+	ND	V	-	-
Hippurate	-	+	+	+	ND	+	ND	ND
NO_3 reduction	V	-	-	-	-	-	ND	ND
Mannitol acid	+	-	-	-	-	-	-	-
Rhamnose acid	V	V	-	-	-	+	-	V
Xylose acid	-	-	+	+	-	-	+	+
Ribose acid	V	-	+	-	ND	-	-	-

Symbols: V, variable; ND, not determined.

M

Microbacterium

Currently, more than 80 species have been described within this genus, but only a minority are clinically relevant.

In Gram staining, they are seen as short or thin coccobacilli with no branching. Catalase activity and motility are variable, and they can produce fermentation or have an oxidative metabolism.

The most frequent associated pathologies are bacteremia and bone infections.

It is imposible to achieve an identification to the species level by the phenotypic annalysis, which is why molecular methods are required (16S rRNA).

Clinical isolates are usually *M. oxydans, M. paraoxydans* and *M. foliorum.*

Since they are Gram-positive rods that belong to the pigmented group, MALDI-TOF can achieve the identification to the genus level with a **score value> 1.5.**

In the GPB (especially in the pigmented rods group) the failure to achieve identification to the species level has no major impact. The importance of identification lies in the genus ID.

Adding 1ul of formic acid improves the identification (score values).

The following score values have been accepted for a reliable identification, based on the experience with limited isolates:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Table 76. Transcription of species within *Microbacterium* spp.

Species	Present in BD	No. of MSPs in BD
M. foliorum	YES	1
M. resistens	YES	1
M. oxydans	YES	1
M. paraoxydans	YES	1

References:

- Barberis C, Almuzara M, Join-Lambert O, Ramırez MS, Famiglietti A, Vay C. Comparison of the Bruker MALDI-TOF mass spectrometry system and conventional phenotypic methods for identification of Gram-positive rods. PLoS One 2014;9(9):e106303. doi: 10.1371/journal.pone.0106303.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Micrococcus

This genus was redefined, keeping only the species *M. luteus* and *M. lylae*.

The main habitat of *Micrococcus* and *Dermacoccus* is human and animal skin, and they can act as opportunistic pathogens, causing endocarditis, pneumonia and sepsis in immunocompromised patients.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 77. Transcription of species within *Micrococcus* spp.

Species	Present in BD	No. of MSPs in BD
M. flavus	YES	1
M. luteus	YES	12
M. lylae	NO	
M. terreus	YES	1

Refer to the Annex for the discrimination between *Micrococcus* and *Dermacoccus* isolated from clinical samples.

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Moraxella

This genus comprises about 20 species, some of which are part of the microbiome of the upper respiratory tract and others are animal species.

They are seen as cocci or cocobacilli that are arranged in pairs or short chains and tend to resist discoloration. All species are asaccharolytic and strong-positive oxidase. *M. catarrhalis* and *M. canis* are also catalase and DNAse positive, and most isolates reduce nitrates to nitrites.

There are phenotypic tests that allow to discriminate among the clinically relevant species within the genus (refer to the Annex).

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 78. Transcription of species within *Moraxella* spp.

Species	Present in BD	No. of MSPs in BD
M. atlantae	YES	1
M. boevrei	YES	2
M. bovis	YES	3
M. bovoculi	YES	1
M. canis	YES	2
M. caprae	YES	1
M. catarrhalis	YES	10
M.caviae	NO	
M. cuniculi	NO	
M. equi	YES	1
M. lacunata	YES	1
M. lincolnii	YES	2
M. nonliquefaciens	YES	6
M. oblonga	YES	1
M. osloensis	YES	9
M. ovis	YES	1
M. phenylpyruvica	NO	
M. pluranimalium	YES	1

Molecular methods include sequencing of specific genes such as 16S rRNA, 16S – 23S, *rpo*B, *gyr*B, *rec*A.

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

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- Schaller A, Troller R, Molina D, Gallati S, Aebi C, Stutzmann Meier P. Rapid typing of Moraxella catarrhalis subpopulations based on outer membrane proteins using mass spectrometry. Proteomics 2006;6(1):172–180. doi: 10.1002/pmic.200500086.

Morganella

Due to the limited experience with this genus, it is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 79. Transcription of species within *Morganella* sp.

Species	Present in BD	No. of MSPs in BD
Morganella morganii	YES	16

Mycoplasma hominis

There are no reference profiles for this species in the commercial database.

A supplementary database is currently being validated by the STIs Laboratory within the NRL, and will soon be transferred to the platform users in Argentina.

Myroides

This genus includes two species, *M. odoratimimus* and *M. odoratus*, which can be isolated from clinical samples. They are immobile rods, with a fruity smell, similar to that of *Alcaligenes faecalis*. They have a yellow pigment and grow on most of the media that are commonly used, with optimal growth temperatures raging from 18 to 37°C. They are asaccharolytic, urease positive, nitrate-negative and nitrite-positive. *M. odoratus* is susceptible to desferrioxamine, while *M. odoratimimus* is resistant.

Most isolates come from urine, blood and ear infections. Although infections caused by *Myroides* are very rare, *M. odoratimimus* is known to be 5 times more frequent than *M. odoratus*.

Most strains are resistant to penicillins, cephalosporins, aminoglycosides, aztreonam and carbapenems.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 80. Transcription of *Myroides* spp. species

Species	Present in BD	No. of MSPs in BD
M. odoratimimus	YES	7
M. odoratus	YES	7
M. phaeus	YES	2

References:

- Almuzara M, Barberis C, Traglia G, Famiglietti A, Ramirez MS, Vay C. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for species identification of nonfermenting Gram-negative bacilli. J Microbiol Methods 2015;112:24-7. doi: 10.1016/j.mimet.2015.03.004.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

N

Neisseria

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 81. Transcription of *Neisseria* spp. species

Species	Present in BD	No. of MSPs in BD
N. animaloris	YES	7
N. baciliformis	YES	8
N. cinerea	YES	6
N. elongata	YES	9
N. flavescens	YES	3
N. gonorrhoeae	YES	40
N. lactamica	YES	9
N. meningitidis	YES	27
N. mucosa	YES	2
N. polysaccharea	YES	1
N. sicca	YES	3
N. subflava bv. flava	NO	
N. subflava bv. perflava	YES	2
N. subflava bv. subflava	YES	7

N. weaveri	YES	10
N. zoodegmatis	YES	4

MALDI-TOF cannot discriminate between species *Neisseria cinerea* and *Neisseria flavescens/subflava.* Thus, it may identify *Neisseria polysaccharea* as *Neisseria meningitidis.*

There are differential phenotypic tests for some species within the genus (refer to Annex).

After the incorporation of the supplementary database with reference profiles for *Neisseria gonorrhoeae,* which was created by the STIs Laboratory within the NRL, the correct discrimination between species of *Neisseria gonorrhoeae* and *Neisseria meningitidis* can be achieved.

MALDI-TOF correctly identifies species *Neisseria bacilliformis.*

Given the existing genetic similarities among the species of the genus, it is recommended to confirm the identification by Molecular Biology (16S rRNA, 23S rRNA). Specific genetic targets of *Neisseria meningitidis*: *sod*C*, por*A*, por*B*, fet*A.

References:

- Cunningham SA, Mainella JM, Patel R. Misidentification of *Neisseria polysaccharea* as *Neisseria meningitidis* with the Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. J Clin Microbiol 2014;52(6):2270–2271. doi: 10.1128/JCM.00664-14.
- Hong E, Bakhalek Y, Taha MK. The identification of Neisseria meningitidis by MALDI-TOF may not be reliable. Clin Microbiol Infect 2018;pii: S1198-743X(18)30637-2. doi: 10.1016/j.cmi.2018.09.015.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Nocardia

Species of *Nocardia* are usually considered environmental, but some of them can cause disease in humans, specially in immunocompromised patients.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Due to the complex taxonomy of the genus, the reference identification is currently performed by the sequence of several genetic targets, such as 16S rRNA and *sec*A, *hsp*65, *gyr*B and *rpo*B genes (these are often used even concatenated).

Most of the equipment's limitations in the identification take place because of the difficulty presented in generating quality spectra that need to be compared with reference profiles; this is mostly due to the complex chemical composition of their cell walls.

The extraction methods recommended by the manufacturer were evaluated, and it became evident that complex procedures are not necessary if the conditions are optimized when culturing, by previously performing simple extractions on fresh colonies.

Reproducible results are obtained when performing the direct culture method and with disruption into the well with a drop of formic acid. If the result is not as expected, the EFAE Bruker extraction technique can be performed.

Overall, the results have showned that MALDI-TOF can effectively identify Actinomycetal species, but supplementing the equipment with personalized spectra can produce substantial improvements in the performance of the current commercial databases.

A supplementary database with reference profiles of local strains, fully characterized by NRLs is currently under development.

MALDI-TOF can achieve an accurate identification to the species level of ***Nocardia brasiliensis, Nocardia farcinica, Nocardia cyriacigeorgica***; which are usually multidrug-resistant.

We suggest using the following recommendations of the expected identification and susceptibility profile for the different complexes:

- ***N. brasiliensis***

The expected antibiotic susceptibility profile for this species is: resistance to imipenem, ciprofloxacin and clarithromycin. It may also be resistant to ceftriaxone. A significant number of isolates with resistance to Trimethoprim-sulfamethoxazole has been documented.

- ***N. farcinica***

The expected antibiotic susceptibility profile for this species is: resistance to ceftriaxone, tobramycin and clarithromycin. It may also be resistant to imipenem and minocycline.

- ***N. cyriacigeorgica***

The expected antibiotic susceptibility profile for this species is: resistance to Amoxicillin–Clavulanic Acid, ciprofloxacin and clarithromycin. It may also be resistant to minocycline.

- ***N. abscessus* Complex *(N. abscessus, N. arthritidis, N. asiática, N. beijingensis, N. pneumoniae)***

It is not posible to predict an antibiotic susceptibility profile for this complex. MALDI-TOF cannot achieve the identification to the species level due to its limitations; therefore, only the complex should be reported. It is recommended to perform sequencing of *sec*A and *gyr*B genes in order to achieve species level discrimination.

Due to the close homology, the species identified by MALDI-TOF as *N. exalbida*, should be reported as ***N. exalbida/gankensis/ abscessus* complex**.

- ***N. nova* Complex *(N. nova, N. cerradoensis, N. kruczakiae, N. veterana, N. aobensis, N. africana, N. elegans, N. mikamii, N. vermiculata)***

For all of these species, report identification to the complex level by MALDI-TOF. Sequencing of *sec*A and *gyr*B genes is necessary for the discrimination at the species level.

The expected antibiotic susceptibility profile for this species is: resistance to Amoxicillin–Clavulanic Acid, ciprofloxacin and tobramycin. It may also be resistant to minocycline.

- ***N. transvalensis* Complex *(N. blacklockiae, N. transvalensis, N. wallacei)***

The expected antibiotic susceptibility profile for this species is: resistant to amikacin, tobramycin, and clarithromycin. It may also be resistant to Amoxicillin–Clavulanic Acid, imipenem and minocycline. A significant number of isolates with resistance to Trimethoprim-sulfamethoxazole has been documented. These species may also be resistant to the four drugs used in empirical treatment.

- ***N. pseudobrasiliensis***

The expected antibiotic susceptibility profile for this species is: resistance to Amoxicillin–Clavulanic Acid, imipenem and minocycline. It may also be resistant to ceftriaxone and Trimethoprim-sulfamethoxazole. These species may also be resistant to the four drugs used in empirical treatment.

- ***N. brevicatena, N. paucivorans***

MALDI-TOF cannot achieve identification to the species level. This should be confirmed with *sec*A, *gyr*B and 16S rRNA genes.

- ***N. otitidiscaviarum***

The expected antibiotic susceptibility profile for this species is: resistance to Amoxicillin–Clavulanic Acid, ceftriaxone and imipenem. It may also be resistant to minocycline and clarithromycin.

Table 82. Transcription of *Nocardia* spp. species.

Species	Present in BD	No. of MSPs in BD	Observations
N. abscessus	YES	3	Report *N. exalbida/gankensis/ abscessus* complex
N. africana	YES	1	Report *N. nova* complex
N. amikacinitolerans	YES	1	Report genus
N. anaemiae	YES	1	Report genus
N. aobensis	YES	1	Report *N. nova* complex
N. araoensis	YES	2	Report genus
N. arthritidis	YES	1	Report *N. abscessus* complex
N. asiatica	YES	1	Report *N. abscessus* complex
N. asteroides	YES	2	Report *N. asteroides* complex
N. beijingensis	NO		Report *N. abscessus* complex
N. blacklockiae	YES	2	Report *N. transvalensis* complex

N. brasiliensis	YES	1	Report **species**
N. brevicatena	YES	1	Report genus
N. carnea	YES	8	Report genus
N. caviae	NO		Report genus
N. concave	YES	1	Report genus
N. corynebacterioides	NO		Report genus
N. cyriacigeorgica	YES	19	Report **species**
N. dassonvillei	NO		
N. elegans	YES	1	Report *N. nova* complex
N. exalbida	YES	1	Report *N. exalbida/gankensis/ abscessus* complex
N. farcinica	YES	13	Report **species**
N. gankensis	NO		Report *N. exalbida/gankensis/ abscessus* complex
N. harenae	NO		Report genus
N. higoensis	YES	1	Report genus
N. ignorata	YES	1	Report genus
N. inohanensis	YES	1	Report genus
N. kruczakiae	YES	1	Report *N. nova* complex
N. mexicana	YES	1	Report genus
N. neocaledoniensis	YES	1	Report genus
N. mikamii	NO		Report *N. nova* complex
N. niigatensis	YES	2	Report genus

N. ninae	YES	1	Report genus
N. nivae	YES	1	Report genus
N. nova	YES	8	Report *N. nova* complex
N. otitidiscaviarum	YES	8	Report genus
N. paucivorans	YES	3	Report genus
N. pneumoniae	YES	2	Report *N. abscessus* complex
N. pseudobrasiliensis	YES	2	Report genus
N. puris	YES	2	Report genus
N. salmonicida	YES	1	
N. seriolae	YES	1	
N. sienata	YES	1	Report genus
N. shimofusensis	NO		Report genus
Nocardia sp.	YES	4	
N. sungurluensis	YES	1	
N. testacea	YES	1	Report genus
N. takedensis	NO		Report genus
N. terpenica	NO		Report genus
N. thailandica	YES	2	Report genus
N. thraciensis	YES	1	
N. transvaslensis	YES	2	Report *N. transvalensis* complex
N. vermiculata	YES	2	Report *N. nova* complex
N. veterana	YES	5	Report *N. nova* complex

N. vinacea	NO		Report genus
N. wallacei	YES	1	Report *N. transvalensis* complex
N. yamanashiensis	YES	1	Report genus

References:

- CLSI. Susceptibility testing of Mycobacteria, Nocardiae, and Other Actinomycetes; Approved Standard - Second Edition. CLSI document M24-A2, Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
- Lebeaux D, Bergeron E, Berthet J, Djadi-Prat J, Mouniée D, Boiron P, Lortholary O, Rodriguez-Nava V. Antibiotic susceptibility testing and species identification of Nocardia isolates: a retrospective analysis of data from a French expert laboratory, 2010-2015. Clin Microbiol Infect 2019;25(4):489-495. doi: 10.1016/j.cmi.2018.06.013.
- Rocca MF, Barrios R, Zintgraff J, Martínez C, Irazu L, Vay C, Prieto M. Utility of platforms Viteks MS and Microflex LT for the identification of complex clinical isolates that require molecular methods for their taxonomic classification. PLoS ONE 2019;14(7):e0218077. doi: 10.1371/journal.pone.0218077.

O

Ochrobactrum

Ochrobactrum genus comprises 18 species, out of which O. *anthrophi* and *O. intermedium* are the two species most frequently associated to opportunistic infections in humans.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Given the close phylogenetic relationship between *O. anthrophi* and *O. intermedium*, there are many cases in which there is no 10% divergence between the two species when reviewing the Top Ten. Therefore, it is convenient to include the susceptibility to colistin as additional evidence.

Recommended additional tests: colistin, tetracycline, urea, 41C, NO3 (refer to the Annex).

Table 83. Transcription of *Ochrobactrum* spp. species with clinical relevance.

Species	Present in BD	No. of MSPs in BD	Report
O. anthropi	YES	8	*Ochrobactrum anthropi* group
O. ciceri	NO		
O. cytisi	NO		
O. daejeonense	NO		
O. endophyticum	YES	1	
O. gallinifaecis	YES	1	
O. grignonense	YES	1	
O. haematophilum	NO		
O. intermedium	YES	1	*Ochrobactrum Intermedium* group
O. lupini	NO		
O. oryzae	NO		
O. pecoris	NO		
O. pituitosum	NO		
O. pseudointermedium	NO		
O. pseudogrignonense	NO		
O. rhizosphaerae	NO		
Ochrobactrum sp.	YES	2	If score >2: report as *Ochrobactrum* sp.
O. thiophenivorans	NO		
O. tritici	YES	1	*Ochrobactrum anthropi* group

***Ochrobactrum anthrophi* group** gathers the following species: *O. anthropi, O. lupini, O. tritici* and *O. cytisi.* All of this are susceptible to colistin, but *O.cytisi* and *O. lupini* are resistant to 300 U polymyxin B.

***O. intermedium* group** includes species: *O. intermedium, O. pseudointermedium* and *O. pseudogrignonense.* However, they are different regarding their susceptibilty to other antimicrobial agents, as shown in Table 83.

Table 84. Susceptibility profile of species within group *O. intermedium*.

Assay	*O. intermedium*	*O. pseudointermedium*	*O. pseudogrignonense*
Colistin	R	R	R
Tetracycline	S	R	S
Netilmicin	R	S	S
Desferrioxamine	R	R	S

References:

- Almuzara M, Barberis C, Traglia G, Famiglietti A, Ramirez MS, Vay C. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for species identification of Nonfermenting Gram-Negative Bacilli. J Microbiol Methods 2015;112,24-27. doi: 10.1016/j.mimet.2015.03.004.
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Oligella

This genus comprises two species: *O. urethralis* and *O. ureolytica*.

O. urethralis is a gram negative cocobacillus, catalase positive, oxidase positive and immobile, which does not oxidize or ferment carbohydrates, and does not hydrolyze gelatin nor sculin. It is also urease negative.

These tests differentiate it from *O. ureolytica,* which is a mobile species with rapid urease positive.

The species within this genus have been described as causative agents of urinary tract infection, vulvovaginitis, bacteremia and other less common systemic diseases, usually in immunosuppressed patients.

Phenotypic tests are necessary to differentiate *Oligella* from *Brevundimonas diminuta*, which is a closely related species, and from which it can be discriminated for being immobile coccobacilli , colistin susceptible.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

If necessary, the phenotypic tests detailed above can be performed.

Table 85. Transcription of *Oligella* spp. species with clinical relevance.

Species	Present in BD	No. of MSPs in BD
O. ureolytica	YES	9
O. urethralis	YES	8

References:

- Almuzara M, Barberis C, Traglia G, Famiglietti A, Ramirez MS, Vay C. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for species identification of Nonfermenting Gram-Negative Bacilli. J Microbiol Methods 2015;112,24-27. doi: 10.1016/j.mimet.2015.03.004.
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P

Paenalcaligenes

The only species found in human isolates, *Paenalcaligenes hominis,* is not included in the commercial database.

Paenibacillus

There is little scientific evidence to assess the reliability of species level identification of the genus *Paenibacillus*. Given its rare occurence in clinical isolates, and due to the limited experience with our own isolates, it is suggested to only report the identification to the genus level, using the criteria suggested by the manufacturer.

That is, with **score values >1,7** it should be reported as *Paenibacillus* sp.

Table 86. Transcription of *Paenibacillus* spp. species.

Species	Present in BD	No. of MSPs in BD
P. agarexedens	YES	4
P. agaridevorans	YES	3
P. alginolyticus	YES	1
P. alvei	YES	3
P. amylolyticus	YES	3
P. anaericanus	YES	1
P. apiarius	YES	2
P. assamensis	YES	1
P. azoreducens	YES	1
P. barcinonensis	YES	1
P. barengoltzil	YES	1

P. borealis	YES	1
P. bovis	NO	
P. brasilensis	YES	1
P. chibensis	YES	3
P. chinjuensis	YES	1
P. chitinolyticus	YES	1
P. chondroitinus	YES	1
P. cineris	YES	1
P. cooki	YES	1
P. curdanolyticus	YES	2
P. dakarensis	NO	
P. daejeonensis	YES	1
P. dentritiformis	YES	1
P. dururs	YES	2
P. edaphicus	YES	1
P. eimensis	YES	1
P. faecis	NO	
P. favisporus	YES	1
P. gansuensis	YES	1
P. glucanolyticus	YES	2
P. graminis	YES	1
P. illinoisensis	YES	2

P. jamilae	YES	1
P. kobensis	YES	2
P. konkukensis	NO	

P. konsidensis	NO	
P. lactis	YES	2
P. larvae	YES	2
P. lautus	YES	1
P. lentus	YES	1
P. macerans	YES	5
P. macquariensis	YES	2
P. massiliensis	YES	1
P. mendelii	YES	1
P. motobuensis	YES	1
P. naphtalenovorans	YES	1
P. nematophilus	YES	1
P. odorifer	YES	1
P. pabuli	YES	1
P. pasadenensis	YES	1
P. peoriae	YES	1
P. phyllosphaerae	YES	1
P. polymyxa	YES	8
P. provencesis	NO	

P. rhizosphaerae	YES	1
P. sanguinis	NO	
P. sabinae	YES	1

Paenibacillus sp.	YES	8
P. sputi	NO	
P. stelifer	YES	1
P. taiwanensis	YES	1
Pa. terrae	YES	1
P. thiaminolyticus	YES	5
P. timonensis	YES	1
P. urinalis	YES	2
P. validus	YES	5
P. vulneris	NO	
P. wynnii	YES	1
P. xinjiangensis	YES	1
P. xylanilyticus	YES	1
P. zanthoxyli	YES	1

References:

- Celandroni F, Salvetti S, Gueye SA, Mazzantini D, Lupetti A, Senesi S, Ghelardi E. Identification and Pathogenic Potential of Clinical *Bacillus* and *Paenibacillus* Isolates. PLoS One 2016;11(3):e0152831. doi: 10.1371/journal.pone.0152831.

Pandoraea

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 87. Transcription of *Pandoraea* spp. species.

Species	Present in BD	No. of MSPs in BD
P. apista	YES	1
P. norimbergensis	YES	1
P. pnomenusa	YES	1
P. pulmonicola	YES	1
P. sputorum	YES	1
Pandoraea sp.	YES	2

References:

- Almuzara M, Barberis C, Traglia G, Famiglietti A, Ramirez MS, Vay C. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for species identification of nonfermenting Gram-negative bacilli. J Microbiol Methods 2015;112:24-7. doi: 10.1016/j.mimet.2015.03.004.
- Fernández-Olmos A, García-Castillo M, Morosini MI, Lamas A, Máiz L, Cantón R. MALDI-TOF MS improves routine identification of non-fermenting Gram negative isolates from cystic fibrosis patients. J Cyst Fibros 2012; 11(1):59–62. doi: 10.1016/j.jcf.2011.09.001.
- Fernández-Olmos A, Morosini MI, Lamas A, García-Castillo M, García-García L, Cantón R, Máiz L. Clinical and Microbiological Features of a Cystic Fibrosis Patient Chronically Colonized with *Pandoraea sputorum* Identified by Combining 16S rRNA Sequencing and Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. J Clin Microbiol 2012;50(3):1096–1098. doi: 10.1128/JCM.05730-11.
- Martina PF, Martínez M, Frada G, Alvarez F, Leguizamón L, Prieto C, Barrias C, Bettiol M, Lagares A, Bosch A, Ferreras J, Von Specht M. First time identification of *Pandoraea sputorum* from a patient with cystic fibrosis in Argentina: a case report. BMC Pulm Med 2017;17:33. doi: 10.1186/s12890-017-0373-y.

Pannonibacter

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 88. Transcription of *Pannonibacter* spp. species

Species	Present in BD	No. of MSPs in BD
P. carbonis	NO	
P. indicus	NO	
P. phragmitetus	YES	2

References:

- Almuzara M, Barberis C, Traglia G, Famiglietti A, Ramirez MS, Vay C. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for species identification of nonfermenting Gram-negative bacilli. J Microbiol Methods 2015;112:24-7. doi: 10.1016/j.mimet.2015.03.004.

Pantoea

Limited experience with this genus.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 89. Transcription of *Pantoea* spp. species.

Species	Present in BD	No. of MSPs in BD
P. agglomerans	YES	12
P. allii	NO	
P. anantis	YES	3
P. brenneri	NO	
P. calida	YES	1
P. citrea	NO	
P. conspicua	NO	
P. deleyi	NO	
P. dispersa	YES	3
P. eucrina	NO	
P. gaviniae	YES	1
P. intestinalis	NO	
P. punctata	NO	
P. septica	YES	1
P. terrea	NO	

Pasteurella

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69= Identification to the genus level

SCORE <1,5= No identification

Table 90. Transcription of *Pasteurella* spp. species.

Species	Present in BD	No. of MSPs in BD
P. aerogenes	YES	1
P. bettyae	YES	3
P. caballi	NO	
P. canis	YES	3
P. dagmatis	YES	5
P. mairii	YES	1
P. multocida	YES	12
P. oralis	NO	
P. pneumotropica	YES	2
P. stomatis	YES	2

References:

- Bardou M, Honnorat E, Dubourg G, Couderc C, Fournier PE, Seng P, Stein A. Meningitis caused by *Pasteurella multocida* in a dog owner without a dog bite: clonal lineage identification by MALDI-TOF mass spectrometry. BMC Res Notes 2015;8:626. doi: 10.1186/s13104-015-1615-9.
- Zangenah S, Güleryüz G, Boräng S, Ullberg M, Bergman P, Ozenci V. Identification of clinical Pasteurella isolates by MALDI-TOF -- a comparison with VITEK 2 and conventional microbiological methods. Diagn Microbiol Infect Dis 2013;77(2):96-8. doi: 10.1016/j.diagmicrobio.2013.06.024.

Pediococcus

The following score values have been accepted for a reliable identification, based on *Pediococcus acidilactici* and *Pediococcus pentosaceus* isolates (which are the species most frequently found in clinical samples):

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69= Identification to the genus level

SCORE <1,5= No identification

Additional phenotypic tests:

PYR: –

LAP: +

NaCl: +

Vancomycin: R

Glucose Gas: –

ADH: V

Table 91. Transcription of *Pediococcus* spp. species.

Species	Present in BD	No. of MSPs in BD
P. acidilactici	YES	4
P. pentosaceus	YES	9

References:

- Almuzara M, Barberis C, Rojas Velázquez V, Ramirez MS, Famiglietti A, Vay C. Matrix-assisted Laser Desorption Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) as a Reliable Tool to Identify Species of Catalase-negative Gram-positive Cocci not Belonging to *the Streptococcus* Genus. Open Microbiol J 2016; 10:202–208. doi: 10.2174/1874285801610010202.

Peptococcus

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 92. Transcription of *Peptococcus* spp. species.

Species	Present in BD	No. of MSPs in BD
P. niger	YES	1

References:

- Veloo AC, de Vries ED, Jean-Pierre H, Justesen US, Morris T, Urban E, Wybo I, van Winkelhoff AF, ENRIA workgroup. The optimization and validation of the Biotyper MALDI-TOF MS database for the identification of gram positive anaerobic cocci. Clin Microbiol Infect 2016;22(9)793-798. Doi: 10.1016/j.cmi.2016.06.016.

Peptoniphilus

It is recommended to aply the following criteria based on publications:

SCORE ≥ 1,8 = Identification to the species level

SCORE 1,79-1,60 = Identification to the genus level

SCORE < 1,60 = No identification

Table 93. Transcription of *Peptoniphilus* spp. species.

Species	Present in BD	No. of MSPs in BD
P. assacharolyticus	NO	
P. catoniae	NO	
P. coxii	YES	1
P. duerdenii	NO	
P. gorbachii	YES	1
P. grossensis	NO	
P. harei	YES	4
P. indolicus	YES	2
P. ivorii	YES	2
P. koenoeneniae	YES	1
P. lacrimalis	YES	1
P. lacydonensis	YES	1
P. methioninivorax	NO	
P. olsenii	YES	1
P. rhinitidis	NO	
P. senegalensis	NO	
P. timonensis	NO	
P. tyrreliae	YES	1

Regarding these results are based on a limited number of isolates, *P. assacharolyticus* (which is not included in the equipment's database) may ocassionally be confused with *P. harei.*

References:

- Rodríguez-Sánchez B, Alcalá L, Marín M, Ruiz A, Alonso E, Bouza E. Evaluation of MALDI-TOF MS (Matrix-Assisted Laser Desorption-Ionization

Time-of- Flight Mass Spectrometry) for routine identification of anaerobic bacteria. Anaerobe 2016;42:101-107. doi: 10.1016/j.anaerobe.2016.09.009.
- Veloo AC, de Vries ED, Jean-Pierre H, Justesen US, Morris T, Urban E, Wybo I, van Winkelhoff AJ, ENRIA workgroup. The optimization and validation of the Biotyper MALDI-TOF MS database for the identification of gram positive anaerobic cocci. Clin Microbiol Infect 2016;22(9):793-798. doi: 10.1016/j.cmi.2016.06.016.

Peptostreptococcus

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 94. Transcription of *Peptostreptococcus* spp. species.

Species	Present in BD	No. of MSPs in BD
P. anaerobius	YES	4
P. assacharolyticus	NO	
P. canis	YES	1
P. russellii	NO	
P. stomatis	NO	

References:

- Rodríguez-Sánchez B, Alcalá L, Marín M, Ruiz A, Alonso E, Bouza E. Evaluation of MALDI-TOF MS (Matrix-Assisted Laser Desorption-Ionization Time-of- Flight Mass Spectrometry) for routine identification of anaerobic bacteria. Anaerobe 2016;42:101-107. doi: 10.1016/j.anaerobe.2016.09.009.
- Veloo AC, de Vries ED, Jean-Pierre H, Justesen US, Morris T, Urban E, Wybo I, van Winkelhoff AJ, ENRIA workgroup. The optimization and validation of the Biotyper MALDI-TOF MS database for the identification of gram positive anaerobic cocci. Clin Microbiol Infect 2016;22(9):793-798. doi: 10.1016/j.cmi.2016.06.016.

Porphyromonas

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

There are limitations on its identification. In our experience, the obtained score values are usually low, and it is not very common to achieve an identification. Therefore, if there is an obligate anaerobic Gram negative rod, the antibiotic disc pattern should be used:

- Vancomycin (5ug) S
- Colistin (10 ug) R
- Kanamycin (1000 ug) R
- 20% Bilis (oxgall) S

If MALDI-TOF yields the identification of a species of *Porphyromonas* with a **score value > 1,7**, it should be reported to the genus level (*Porphyromonas* sp.).

Species *P. assacharolytica* and *P. uenonis* cannot be discriminated by MALDI-TOF nor by 16S rRNA, and they have a limited differentiation with other genes, such as *hsp*60; therefore, it should be reported as *P. assacharolytica/uenonis*.

Table 95. Transcription of *Porphyromonas* spp. species.

Species	Present in BD	No. of MSPs in BD
P. assacharolytica	YES	1
P. bennonis	NO	
P. cangingivalis	NO	
P. canoris	NO	
P. catoniae	NO	
P. circumdentaria	NO	
P. crevioricans	NO	
P. endodontalis	NO	
P. gingivalis	YES	2
P. gulae	YES	1
P. levii	YES	1
P. macacae	YES	1
P. pasteri	NO	
P. somerae	NO	
P. uenonis	YES	2

References:

- Rodríguez-Sánchez B, Alcalá L, Marín M, Ruiz A, Alonso E, Bouza E. Evaluation of MALDI-TOF MS (Matrix-Assisted Laser Desorption-Ionization Time-of- Flight Mass Spectrometry) for routine identification of anaerobic bacteria. Anaerobe 2016;42:101-107. doi: 10.1016/j.anaerobe.2016.09.009.
- Veloo ACM, Jean-Pierre H, Justesen US, Morris T, Urban E, Wybo I, Kostrzewa M, Friedrich AW. Validation of MALDI-TOF MS Biotyper database optimized for anaerobic bacteria: The ENRIA project. Anaerobe 2018;54:224-230. https://doi.org/10.1016/j.anaerobe.2018.03.007.
- Zamora-Cintas M, Marín M, Quiroga L, Martínez A, Fernández-Chico MA, Bouza E, Rodríguez-Sánchez B, Alcalá L. Identification of Porphyromonas isolates from clinical origin using MALDI-TOF Mass Spectrometry. Anaerobe 2018;54:197-200. doi: 10.1016/j.anaerobe.2018.06.017.

Prevotella

It is recommended to apply the following criteria based on publications:

SCORE ≥ 1,8 = Identification to the species level

SCORE 1,79-1,60 = Identification to the genus level

SCORE < 1,60 = No identification

Other authors (Barba *et al.*) suggest that the correct identification to the species level between *P. nigrescens* and *P. intermedia* is with a score value ≥1,7. However, this may present limitations on the identification.

Table 96. Transcription of *Prevotella* spp. species.

Species	Present in BD	No. of MSPs in BD
P. albensis	NO	
P. amnii	YES	1
P. aurantiaca	NO	
P. baroniae	YES	2
P. bergensis	YES	1
P. bivia	YES	8
P. buccae	YES	6
P. buccalis	YES	2
P. colorans	NO	
P. conceptionensis	NO	
P.corporis	YES	1
P. denticola	YES	5
P. disiens	YES	3
P. enoeca	NO	
P. falsenii	NO	
P. fusca	NO	

P. histicola	YES	1
P. intermedia	YES	1
P. loescheii	YES	4
P. maculosa	YES	1
P. marshii	NO	
P. melaninogenica	YES	4
P. micans	NO	
P. multiformis	NO	
P. multisaccharivorax	YES	1
P. nanceiensis	YES	2
P. nigrescens	YES	2
P. oralis	YES	1
P. oris	YES	2
P. oulorum	YES	1
P. pallens	YES	4
P. pleuritidis	NO	
P. salivae	YES	1
P. saccharolytica	NO	
P. scopos	NO	
P. shahii	YES	1
P. stercorea	YES	1
P. timonensis	YES	6
P. veroralis	NO	
P. zoogleoformans	NO	

References:

- Gürsoy M, Harju I, Matomäki J, Bryk A, Könönen E. Performance of MALDI-TOF MS for identification of oral Prevotella species. Anaerobe 2017;47:89-93. doi: 10.1016/j.anaerobe.2017.04.008.
- La Scola B, Fournier PE, Raoult D. Burden of emerging anaerobes in the MALDI-TOF and 16S rRNA gene sequencing era. Anaerobe 2011;17(3):106-12. doi: 10.1016/j.anaerobe.2011.05.010.
- Rodríguez-Sánchez B, Alcalá L, Marín M, Ruiz A, Alonso E, Bouza E. Evaluation of MALDI-TOF MS (Matrix-Assisted Laser Desorption-Ionization Time-of- Flight Mass Spectrometry) for routine identification of anaerobic bacteria. Anaerobe 2016;42:101-107. doi: 10.1016/j.anaerobe.2016.09.009.

*Propionibacterium (*currently, *Cutibacterium)*

The following score values have been accepted for a reliable identification, based on results of species *P. avidum* and *P. acnes*:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Table 97. Transcription of *Propionibacterium* spp. species (*Cutibacterium* spp.*)

Species	Present in BD	No. of MSPs in BD
P. acidifaciens	YES	1
P. acidipropionici	YES	3
P. acnes	YES	15
P. australiense	YES	1
P. avidum	YES	4
P. cyclohexanicum	YES	1
P. freundenreichii	YES	3
P. granulosum	YES	3
P. jensenii	YES	4
P. microaerophilum	YES	1
P. propionicum	YES	1
Propionibacterium sp.	YES	7
P. thoenii	YES	2

***** *Propionibacterium* has been reclassified as *Cutibacterium.*

Identification should be completed with the phenotypic tests shown below in order to discriminate *P. acnes* (Indole positive) from other species of *Propionibacterium* isolated from clinical samples (Indole negative) and/or *Propioniferax*.

Table 98. Differentiation among species of *Propionibacterium* spp. and *Propioniferax* spp.

Species	Aerotolerance	Catalase	Indole	NO_3	Esc
Propionibacterium acidifaciens	-	-	-	-	-
Propionibacterium acnes	+	+	+	+	-
Propionibacterium avidum	+	+	-	-	+
Propionibacterium granulosum	+	+	-	-	-
Propionibacterium propionicum	-	-	-	+	-
Propioniferax innocua	+	+	-	V	-

Symbols: V, variable.

References:

- Barberis C, Almuzara M, Join-Lambert O, Ramırez MS, Famiglietti A, Vay C. Comparison of the Bruker MALDI-TOF mass spectrometry system and conventional phenotypic methods for identification of Gram-positive rods. PLoS One 2014;9(9):e106303. doi: 10.1371/journal.pone.0106303.
- Rodríguez-Sánchez B, Alcalá L, Marín M, Ruiz A, Alonso E, Bouza E. Evaluation of MALDI-TOF MS (Matrix-Assisted Laser Desorption-Ionization Time-of- Flight Mass Spectrometry) for routine identification of anaerobic bacteria. Anaerobe 2016;42:101-107. doi: 10.1016/j.anaerobe.2016.09.009.

Proteus

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

MALDI-TOF correctly identifies *Proteus mirabilis*. However, it cannot discriminate among species *Proteus vulgaris/penneri/hauseri*; therefore, it should only be reported to the group/complex level, or it should be completed with phenotypic tests for these species (See table below) .

Table 99. Phenotypic tests for the differentiation among species of *Proteus* spp.

Bacteria	TSI	Urease	LDC	IMVIC	ODC	Esc	Salicine	Trehalose
P. vulgaris	Acid Black background	+	Deami-nation	++-V	-	+	+	-
P. penneri	Acid/acid with or without gas Black background	+	Deami-nation	-+- -	-	-	-	V
P. hauseri	Acid/acid without gas Black background	+	Deami-nation	++- -	-	-	-	-

Table 100. Transcription of *Proteus* spp. species.

Species	Present in BD	No. of MSPs in BD	Current nomenclature
P. hauseri	YES	3	
P. mirabilis	YES	9	
P. myxofaciens	YES	2	*Cosenzaea myxofaciens*
P. penneri	YES	3	
P. vulgaris	YES	9	

Pseudomonas

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Given the complexity of this genus, due to the numerous species comprised in it, there are special considerations that need to be taken into account:

- Species *P. oryzihabitans, P. aeruginosa, P. stutzeri, P. chlororaphi, P. indica* are accurately identified to the species level.
- *P. salomonii* is not included in MALDI-TOF's database and it is identified as *P. antarctica/extremorientalis.*
- *Pseudomonas cichorii/syringae*: should be sequenced.
- *Pseudomonas azotoformans* cannot be identified. Sequencing is suggested.
- *P. alcaliphila/oleovorans/pseudoalcaligenes:* MALDI-TOF cannot discriminate among these species, it is recommended to do so with *gyr*B.

The following species identification should be reported to the group/complex level:

- *P. plecoglossicida, P. monteilii, P. mossellii, P. putida, P. fulva:* **are reported as *Pseudomonas putida* group.**
- *P. lundensis, P. vietnamiensis, P. fluorescens, P. libanensis*, *P. koorensis*: **are reported as *Pseudomonas fluorescens* group.**

In these species, the differentiation cannot be achieved by phenotypic tests nor by sequencing of 16S rRNA gene. *gyr*B and *rpo*D are recommended to confirm the identification.

Table 101. Transcription of *Pseudomonas* spp. species.

Species	Present in BD	No. of MSPs in BD	Current Nomenclature
P. aeruginosa	YES	9	
P. aestusnigri	NO		
P. agarici	YES	1	
P. alcaligenes	YES	2	
P. alcaliphila	YES	1	
P. amygdali	NO		
P. anguilliseptica	YES	1	
P. antarctica	YES	1	
P. argentinensis	NO		
P. asplenii	YES	1	
P. asturiensis	NO		

P. asuensis	NO		
P. avellanae	YES	1	
P. azotifigens	YES	1	
P. azotoformans	YES	1	
P. baetica	NO		
P. balearica	YES	2	
P. bauzanensis	NO		
P. benzenivorans	NO		
P. beteli	NO		
P. borbori	NO		
P. boreopolis	YES	1	
P. brassicasearum	NO		
P. brenneri	YES	1	
P. caeni	NO		
P. canadensis	NO		
P. cannabina	NO		
P. carboxydohydrogena	NO		
P. caricapapayae	YES	1	
P. caspiana	NO		
P. cedrina	YES	1	
P. cerasi	NO		
P. chengduensis	NO		
P. chlororaphis	YES	2	
P. cichori	YES	1	
P. cissicola	NO		

P. citronellosis	YES	2	
P. composti	NO		
P. congelans	YES	1	
P. corrugata	YES	1	
P. costantini	NO		
P. cremoricolorata	NO		
P. cuatrocienegasensis	NO		
P. delhiensis	NO		
P. donhuensis	NO		
P. duriflava	NO		
P. endophytica	NO		
P. entomophila	NO		
P. extremaustralis	NO		
P. extremorientalis	YES	1	
P. flavescens	YES	1	
P. flexibilis	NO		
P. floridensis	NO		
P. fluorescens	YES	6	
P. fluvialis	NO		
P. formosensis	NO		
P. fragi	YES	1	
P. frederiksbergensis	YES	1	

P. fulva	YES	2	
P. furukawai	NO		
P. fuscovaginae	YES	1	

P. gelidicola	NO		
P. geniculata	NO		
P. gessardii	YES	1	
P. glareae	NO		
P. graminis	YES	1	
P. granadensis	NO		
P. grimontii	YES	1	
P. guineae	NO		
P. halophila	NO		
P. helleri	NO		
P. helmanticenis	NO		
P. hibiscicola	NO		
P. hussaini	NO		
P. indica	YES	1	
P. japonica	NO		
P. jessenii	YES	1	
P. jinjuensis	YES	1	
P. kilonensis	YES	1	
P. koreensis	YES	3	
P. kunmingensis	NO		
P. lactis	NO		
P. libanensis	YES	1	
P. lini	NO		
P. linyingensis	NO		
P. litoralis	NO		

P. lundensis	YES	1	
P. lutea	YES	1	
P. luteola	YES	3	*Chryseomonas luteola*
P. mandelii	YES	1	
P. marginalis	YES	1	
P. maricola	NO		
P. mediterranea	NO		
P. meliae	NO		
P. mendocina	YES	1	
P. meridiana	NO		
P. miguale	YES	1	
P. monteillii	YES	7	
P. moorei	NO		
P. moraviensis	NO		
P. mosselii	YES	1	
P. mucidolens	YES	1	
P. nitritireducens	NO		
P. nitroreducens	YES	2	
P. oleovorans	YES	3	
P. orientalis	YES	1	
P. oryzihabitans	YES	7	*Flavimonas oryzihabitans*
P. otitidis	YES	1	
P. pachastrellae	NO		
P. palleroniana	NO		

P. panacis	NO		
P. panipatensis	YES	1	
P. parafulva	NO		
P. paralactis	NO		
P. pelagia	NO		
P. peli	NO		
P. pertucinogena	YES	1	
P. pictorum	YES	1	*Stenotrophomonas pictorum*
P. plecoglosicida	YES	6	
P. poae	YES	1	
P. phoangensis	YES	1	
P. populi	NO		
P. profundi	NO		
P. prosekii	NO		
P. proteolytica	YES	1	
P. pseudoalcaligenes	YES	1	*Pseudomonas oleovorans*
P. psycrophila	SI	1	
P. psycrotolerans	NO		
P. punonensis	NO		
P. putida	YES	26	
P. reinekei	NO		
P. resinovorans	NO		
P. rhodesiae	YES	1	
P. rhizosphaerae	YES	1	

P. sabulinigri	NO		
P. sagittaria	NO		
P. salina	NO		
P. salomonii	NO		
P. saponiphila	NO		
P. savastanoi	YES	2	
P. segetis	YES	1	
P. sesami	NO		
P. simiae	NO		
P. soli	NO		
Pseudomonas sp.	YES	8	
P. straminea	YES	1	
P. stutzeri	YES	10	
P. synxantha	YES	1	
P. syringae	YES	2	
P. taeaenensis	NO		
P. taetrolens	YES	1	
P. taiwanensis	NO		
P. tarimensis	YES	1	
P. testosteronii	NO		
P. thermotolerans	YES	1	
P. thivervalensis	YES	1	
P. tolaasii	YES	1	
P. toyotomiensis	NO		
P. tremae	NO		

P. trivialis	YES	1	
P. tuomuorensis	NO		
P. umsongensis	YES	1	
P. vancouverensis	YES	1	
P. veronii	YES	4	
P. versuta	NO		
P. viridiflava	YES	1	
P. vranovensis	NO		
P. wadenswilerensis	NO		
P. xanthomarina	YES	1	
P. xiamenensis	NO		
P. zeshuii	NO		
P. zahodongensis	NO		

References:

- Almuzara M, Barberis C, Traglia G, Famiglietti A, Ramirez MS, Vay C. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for species identification of nonfermenting Gram-negative bacilli. J Microbiol Methods 2015;112:24-7. doi: 10.1016/j.mimet.2015.03.004.

Psychrobacter

There might be difficulties in the identification since these are mucous and pigmented strains. It usually yields low score values.

There is only one species represented in the database: *Psychrobacter lutiphocae*. It is correctly identified, but with a low score value.

P. pulmonis/faecalis are not included in the database.

R

Ralstonia

It is very similar to the *Cupriavidus* genus.

Within this genus, *Ralstonia pickettii* is the best-known species regarding human disease, it causes bacteremias, meningitis, endocarditis and osteomyelitis.

Ralstonia mannitolilytica has been recently described in a nosocomial outbreak and in a case of recurrent meningitis; this species along with *R. insidiosa* mostly affect fibrocystic patients.

Based on our experience, *Ralstonia pickettii* is correctly identified. However, *R. mannitolilytica* can be confused with *R. pickettii;* therefore, it is suggested to report all species within this genus as *Ralstonia* sp.

It is recommended to report the identification to the genus level with a **score value > 1,7.**

Table 102. Transcription of *Ralstonia* spp. species.

Species	Present in BD	No. of MSPs in BD
R. insidiosa	YES	3
R. mannitolilytica	YES	1
R. pickettii	YES	9
R. pseudosolanacearum	NO	
R. solanacearum	NO	
Ralstonia sp.	YES	1
R. syzygii	YES	1

Refer to the Annex for microbial identification using phenotypic tests.

References:

- Almuzara M, Barberis C, Traglia G, Famiglietti A, Ramirez MS, Vay C. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass

spectrometry for species identification of nonfermenting Gram-negative bacilli. J Microbiol Methods 2015;112:24-7. doi: 10.1016/j.mimet.2015.03.004.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Raoultella

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

However:

- ***Raoultella ornitinolytica:*** only report as such if ODC positive and Indole positive. If not, it should be reported as *Klebsiella oxytoca.*
- ***Raoultella planticola*:** report as *Klebsiella pneumoniae* if indole negative, and report as *Klebsiella oxytoca* if indole positive.
- ***Raoultella terrigena*:** identification to the species level is not reliable.

Refer to the Annex for the differentiation with *Klebsiella* spp. by phenotypic tests.

Table 103. Transcription of *Raoultella* spp. species.

Species	Present in BD	No. of MSPs in BD
R. electrica	NO	
R. ornithinolytica	YES	13
R. planticola	YES	6
R. terrigena	YES	5

References:

- Sekowska A, Mikucka A, Gospodarek-Komkowska E. Identification of *Raoultella* spp.: Comparison of three methods. Indian J Med Microbiol 2018;36(2):197-200. doi: 10.4103/ijmm.IJMM_17_99.

Rhizobium

Rhizobium genus comprises several species, however, there are very few spectra included in the database, except for *R. radiobacter*.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 104. Transcription of *Rhizobium* spp. species.

Species	Present in BD	No. of MSPs in BD
R. radiobacter	YES	14
R. rubi	YES	1
R. tropici	YES	1

References:

- Almuzara M, Barberis C, Traglia G, Famiglietti A, Ramirez MS, Vay C. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for species identification of nonfermenting Gram-negative bacilli. J Microbiol Methods 2015;112:24-7. doi: 10.1016/j.mimet.2015.03.004.
- Jia RZ, Zhang RJ, Wei Q, Chen WF, Cho IK, Chen WX, Li QX. Identification and Classification of Rhizobia by Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry. J Proteomics Bioinform 2015;8,98-107. doi: 10.4172/jpb.1000357.
- Uhlik O, Strejcek M, Junkova P, Sanda M, Hroudova M, Vlcek C, Mackova M, Macek T. Matrix-assisted laser desorption ionization (MALDI)-time of flight mass spectrometry- and MALDIbiotyper-based identification of cultured biphenyl-metabolizing bacteria from contaminated horseradish rhizosphere soil. Appl Environ Microbiol 2011;77(19):6858-66. doi: 10.1128/AEM.05465-11.

Rhodococcus

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Based on our experience and on published data, *R. equi* can be correctly identified to the species level with a **score value >1,7.**

Rhodococcus hoagii and *Rhodococcus equi* are considered the same species.

Rhodococcus hoagii and *Rhodococcus soli* are closely related, complete sequencing of the 16S rRNA gene (1500 bp) is required for the resolution of many of these species.

Table 105. Transcription of *Rhodococcus* spp. species.

Species	**Present in BD**	**No. of MSPs in BD**
R. aetherivorans	YES	1
R. aerolatus	NO	
R. antrifimi	NO	
R. artemisiae	NO	
R. baikonurensis	YES	1
R. biphenylivorans	NO	
R. canchipurensis	NO	
R. cerastii	NO	
R. cercidiphylli	NO	
R. coprophilus	YES	2
R. corynebacterioides	YES	1
R. defluvii	NO	
R. degrandans	NO	
R. enclenis	NO	
R. equi	YES	8
R. erythropolis	YES	16
R. fascians	YES	8

R. gannanensis	NO	
R. globerulus	YES	4
R. gordoniae	YES	1
R. hoagii	NO	
R. humicola	NO	
R. imtechensis	YES	1
R. jialingiae	NO	
R. jostii	YES	1
R. koorensis	YES	1
R. kroppenstedtii	YES	1
R. kummingensis	YES	1
R. kyotonensis	NO	
R. lactis	NO	
R. maanshanensis	YES	1
R. marinonascens	YES	1
R. nanhaiencis	NO	
R. olei	NO	
R. opacus	YES	5
R. percolatus	YES	1
R. phenolicus	YES	1
R. pyridinovorans	YES	2
R. rhodnii	YES	5
R. qingshengii	NO	
R. rhodochrous	YES	12
R. ruber	YES	15

R. rubropertinctus	NO	
R. soli	NO	
R. sovatensis	NO	
R. triatomae	YES	2
R. trifolii	NO	
R. tukisamuensis	NO	
R. wratislaviensis	YES	1
R. yunnanensis	NO	
R. zopfii	NO	

References:

- Barberis C, Almuzara M, Join-Lambert O, Ramırez MS, Famiglietti A, Vay C. Comparison of the Bruker MALDI-TOF mass spectrometry system and conventional phenotypic methods for identification of Gram-positive rods. PLoS One 2014;9(9):e106303. doi: 10.1371/journal.pone.0106303.
- de Alegría Puig CR, Pilares L, Marco F, Vila J, Martínez-Martínez L, Navas J. Comparison of the Vitek MS and Bruker Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry Systems for Identification of *Rhodococcus equi* and *Dietzia* spp. J Clin Microbiol 2017;55(7):2255-2260. doi: 10.1128/JCM.00377-17.

Roseomonas

These species are rarely isolated from clinical samples (blood, wound, abscess).

There might be limitations in the identification by MALDI-TOF since they are mucous and pigmented strains (pink-coral), and this does not improve with the extraction methods.

Species of *Roseomonas* may not be identified, and they might also require sequencing of the 16S rRNA gene for confirmation: *R. aestuarii /oryzae /rhizosphareae/aerophila.*

It is recommended to report the identification to the genus level with a **score value > 1,7.**

All *Roseomonas* species strongly hydrolyse urea, but not esculin. If necessary, it is suggested to perform the following phenotypic tests:

Table 106. Differential phenotypic tests for *Roseomonas* spp. species.

Species	**Oxidase**	**PYR**	**Arabinose acid**	**Mannitol**	**Fructose**	**Glucose**	**NO_3**	**Deferroxyamine**
R. cervicalis	+	-	+	-	V	-	-	-
R. gilardi	+	+	+	+	V	-	-	-
R. mucosa	-	+	+	+	+	+	-	-
R. genomospecie 4	+	-	-	-	+	+	+	+
R. genomospecie 5	+	-	-	-	+	-	-	-

Symbols: V, variable.

Table 107. Transcription of *Roseomonas* spp. species

Species	**Present in BD**	**No. of MSPs in BD**
R. aeriglobus	NO	
R. aerilata	NO	
R. aerofrigidensis	NO	
R. aerophila	NO	
R. aestuarii	NO	
R. alkaliterrae	NO	
R. acquatica	NO	
R. arctica	NO	
R. arcticisoli	NO	
R. cervicalis	NO	

R. deserti	NO	
R. eburnea	NO	
R. elaeocarpi	NO	
R. fluminis	NO	
R. frigidaquae	NO	
R. gilardi	NO	
R. hibiscisoli	NO	
R. lacus	NO	
R. mucosa	YES	4
R. musae	NO	
R. nepalensis	NO	
R. oryzae	NO	
R. oryzicola	NO	
R. pecuniae	NO	
R. rhizosphaerae	NO	
R. riguiloci	NO	
R. rosea	NO	
R. rubra	NO	
R. soli	NO	
R. stagni	NO	
R. suffusca	NO	
R. terrae	NO	
R. terricola	NO	
R. vinacea	NO	
R. wooponensis	NO	

References:

- Diesendorf N, Köhler S, Geißdörfer W, Grobecker-Karl T, Karl M, Burkovski A. Characterisation of Roseomonas mucosa isolated from the root canal of an infected tooth. BMC Res Notes 2017;10(1):212. doi:10.1186/s13104-017-2538-4.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Rothia

This genus is presented as gram positive cocci, although they can also appear as coryneform cocobacilli.

The clinically relevant species within this genus are *R. mucilaginosa* (originally, *Stomatococcus mucilaginosus*), *R. dentocariosa* and *R. aeria.*

They are part of the normal flora of the oral cavity and the oropharynx in healthy people, although periodontal lesions could make it possible for bacteremia or other systemic diseases to take place.

R. aeria has been associated with endocarditis and sepsis; while *Rothia mucilaginosa* has caused meningitis and septicemia, especially in children with hematological diseases.

R. dentocariosa colonies tend to be whitish (or rarely grayish-black and adherent), smooth or rough, or “spoke-wheel shaped”, and they grow better in a CO_2 atmosphere. This species is catalase variable, immobile, it reduces nitrates, hydrolyzes esculin, is urease negative, and ferments glucose, maltose and sucrose; but it is lactose, xylose and mannitol negative.

API Coryne correctly identifies the representative species of the genus: *Rothia dentocariosa* (PAL and Bgur positive).

Conventional phenotypic tests cannot discriminate between *R. aeria* and *R. dentocariosa*, but MALDI-TOF **can** achieve identification to the species level.

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Table 108. Transcription of *Rothia* spp. species.

Species	Present in BD	No. of MSPs in BD
R. aeria	YES	6
R. aerolata	NO	
R. amarae	YES	2
R. dentocariosa	YES	6
R. endophytica	YES	1
R. mucilaginosa	YES	9
R. nasimurium	YES	4
R. terrae	YES	4

References:

- Barberis C, Almuzara M, Join-Lambert O, Ramırez MS, Famiglietti A, Vay C. Comparison of the Bruker MALDI-TOF mass spectrometry system and conventional phenotypic methods for identification of Gram-positive rods. PLoS One 2014;9(9):e106303. doi: 10.1371/journal.pone.0106303.
- Crowe A, Ding NS, Yong E, Sheorey H, Waters MJ, Daffy J. Rothia aeria mitral valve endocarditis complicated by multiple mycotic aneurysms: laboratory identification expedited using MALDI-TOF MS. Infection 2014;42(2):419-23. doi: 10.1007/s15010-013-0532-x.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Nicodemo AC, Gonçalves LG, Odongo FC, Martino MD, Sampaio JL. Rothia aeria endocarditis in a patient with a bicuspid aortic valve: case report. Braz J Infect Dis 2014;18(5):561-4. doi: 10.1016/j.bjid.2014.05.001.
- WEBSITE: *www.antimicrobe.org*

S

Salmonella

The NRLs are not experienced enough with this genus.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 109. Transcription of *Salmonella* spp. species.

Species	**Present in BD**	**No. of MSPs in BD**
Salmonella sp. *(bongori)*	YES	2
Salmonella sp. *(choleraesuis)*	YES	1
Salmonella sp. *(enterica st Anatum)*	YES	1
Salmonella sp. *(enterica st Arizonae)*	YES	3
Salmonella sp. *(enterica st Diarizonae)*	YES	2
Salmonella sp. *(enterica st Dublin)*	YES	1
Salmonella sp. *(enterica st Enterica)*	YES	1
Salmonella sp. *(enterica st Gallinarum)*	YES	1
Salmonella sp. *(enterica st Hadar)*	YES	1
Salmonella sp. *(enterica st Houtenae)*	YES	1
Salmonella sp. *(enterica st Indica)*	YES	2
Salmonella sp. *(enterica st Salamae)*	YES	1
Salmonella sp. *(enterica st Stanley)*	YES	1
Salmonella sp. *(enteritidis)*	YES	1
Salmonella sp. *(typhimurium)*	YES	1

Serratia

The NRLs are not experienced enough with this genus.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 110. Transcription of *Serratia* spp. species.

Species	Present in BD	No. of MSPs in BD
S. entomophila	YES	1
S. ficaria	YES	1
S. fonticola	YES	10
S. grimesii	YES	1
S. liquefaciens	YES	9
S. marcescens	YES	6
Serratia spp. *marcescens*	YES	1
Serratia spp. *sakuensis*	YES	1
S. odorifera	YES	9
S. plymuthica	YES	6
S. proteamaculans	YES	1
S. proteamaculans	YES	1
S. quinivorans	YES	1
S. rubidaea	YES	7
S. ureilytica	YES	1

Shewanella

It is recommended to report the identification to the genus level with a **score value> 1,7.**

Shewanella spp. is the only genus of the gram negative nonfermenters that produces H_2S in TSI.

Shewanella algae can be wrongly identified as *Shewanella putrefaciens*.

Clarification: *S. algae*, represents the majority of human isolates, and *S. putrefaciens* represents the majority of non-human isolates.

Table 111. Biochemical tests for the differentiation of species of *Shewanella* spp.

Species	Pigment	Growth on 6.5% NaCl	OF Fructose	OF Sucrose	OF Maltose	Growth on SS
S. algae	Tan	+	-	-	-	+
S. putrefaciens	Tan	-	V	+	+	-

Symbols: V, variable.

The complete differentiation of the species within the genus is carried out by sequencing of specific genetic targets such as: 16S rRNA, 16S-23S, 23S rRNA, *gyr*B, *rpo*B, *rec*A.

Table 112. Transcription of *Shewanella* spp. species.

Species	Present in BD	No. of MSPs in BD
S. algae	YES	1
S. baltica	YES	1
S. fetalis	YES	1
S. fidelis	YES	1
S. frigidimarina	YES	1
S. profunda	YES	1
S. putrefaciens	YES	4

References:

- Manual de “Identificación de Bacilos Gram Negativos No Fermentadores de importancia clínica”. Servicio Bacteriología Especial, Departamento Bacteriología, Instituto Nacional de Enfermedades Infecciosas Anlis - “Dr. Carlos G. Malbrán”, Buenos Aires, Argentina.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Shigella

There are no reference protein profiles in the commercial database due to its close similarity to *Escherichia coli* (refer to the Annex).

There is an *in house* database customized by the NRLs that is currently under development and validation.

Sphingobacterium

Currently under evaluation.

For the time being, it is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Refer to the Annex for microbial identification by phenotypic methods.

Table 113. Transcription of *Sphingobacterium* spp. species

Species	Present in BD	No. of MSPs in BD
S. daejeonense	YES	1
S. faecium	YES	1
S. mizutaii	YES	1
S. multivorum	YES	4
S. spiritivorum	YES	8
S. thalpophilum	YES	1

References:

- Curso teórico-práctico "Identificación de Bacilos Gram Negativos No Fermentadores de importancia clínica". Servicio Bacteriología Especial, Departamento Bacteriología, Instituto Nacional de Enfermedades Infecciosas Anlis - "Dr. Carlos G. Malbrán", Buenos Aires, Argentina.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Sphingomonas

Currently under evaluation.

For the time being, it is suggested to report to the genus level with a **score value > 1,7.**

S. paucimobilis is a polymorphic gram negative rod, obligate aerobic, oxidase weak-positive and catalase positive. Colonies grow on blood agar but not on MacConkey agar, and they produce a yellow pigment. Although it has a single polar flagellum, a low percentage of cells are actively mobile, and motility may be difficult to demonstrate in the laboratory (hence the name *paucimobilis*). *Sphingomonas* can be mistakenly identified by conventional identification systems, but is usually correctly identified by MALDI-TOF.

MALDI-TOF cannot achieve the identification of *Sphingomonas oligophenolica.*

Refer to the Annex for microbial identification by phenotypic methods.

Table 114. Transcription of *Sphingomonas* spp. species.

Species	Present in BD	No. of MSPs in BD
S. abaci	YES	1
S. adhaesiva	YES	1
S. aerolata	YES	1
S. aquatilis	YES	2
S. asaccharolytica	YES	1
S. aurantiaca	YES	3
S. azotifigens	YES	1
S. desiccabilis	YES	1
S. dokdonensis	YES	1
S. echinoides	YES	4
S. faeni	YES	1
S. haloaromaticamans	YES	1
S. koreensis	YES	2
S. leidyi	YES	3
S. mali	YES	1
S. melonis	YES	1
S. molluscorum	YES	1
S. mucosissima	YES	2
S. panni	YES	1
S. parapaucimobilis	YES	1
S. paucimobilis	YES	10
S. phyllosphaerae	YES	1
S. pituitasa	YES	1
S. pruni	YES	1

S. pseudosanguinis	YES	2
S. sanguinis	YES	2
S. soli	YES	1
Sphingomonas sp.	YES	7
S. trueperi	YES	2
S. wittichii	YES	2
S. yabuuchiae	YES	2
S. yunnanensis	YES	1

References:

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Staphylococcus

It is suggested to use a direct extraction method (with 1 µl of 70% formic acid) and to consider the following cut-off values:

SCORE ≥1,7 = Correct identification to the species level

SCORE 1,5-1,7 = Correct identification to the genus level

SCORE <1,5 = Unreliable identification

Table 115. *Staphylococcus* spp. species included in the Biotyper Software 3.1 Database (Bruker Daltonics).

ID using MALDI TOF	Present in BD	No. of MSPs in BD	REPORT	Observations	References
S. chromogenes	YES	1	YES	Isolated from animals with split or broken hooves (artiodactyls): sheep, goats, deer, camels, cattle and pigs). It produces bovine mastitis.	
S. arlettae	YES	3	YES (unreliable ID is usually obtained, regardless of the score used)	CoNS species Novobiocin Resistant isolated from animals	Han *et al.* 2015; Ayeni *et al.* 2017
S. aureus	YES	14	YES		
S. auricularis	YES	6	YES	Only identification to the genus level	Han *et al.* 2015
S. capitis ssp *capitis*	YES	6	YES	Subspecies level discrimination	Zhu *et al.* 2015
S. capitis ssp *ureolyticus*	YES	1	YES	Subspecies level discrimination	Zhu *et al.* 2015
S. caprae	YES	8	YES	Species isolated from goat milk. Emerging pathogen in human infections (osteoarticular, endocarditis, etc.)	Seng *et al.* 2014; Kwok *et al.* 2016
S. carnosus ssp *carnosus*	YES	1	YES (at least to the species level)	Different works do not specify whether it can achieve subspecies level discrimination.	

S. carnosus ssp *utilis*	YES	2	YES (at least to the species level)	Different works do not specify whether it can achieve subspecies level discrimination.	
S. cohnii ssp *cohnii*	YES	4	YES	Subspecies level discrimination. CoNS species Novobiocin Resistant	Zhu *et al.* 2015
S. cohnii ssp *urealyticus*	YES	2	YES	Subspecies level discrimination. CoNS species Novobiocin Resistant	Zhu *et al.* 2015
S. condimenti	YES	2	YES	Isolated from food samples (soy sauce). Also isolated from human infections (catheter-related bacteremia)	Misawa *at al.* 2014
S. delphini	YES	19	YES	Coagulase positive species, isolated from dolphins and horses. It is part of the *S. intermedius* group (SIG) along with *S. intermedius* and *S. pseudointermedius*	Han *et al.* 2015; Silva *et al.* 2015; Decristophoris *et al.* 2011
S. epidermidis	YES	10	YES		
S. equorum	YES	4	YES	CoNS species Novobiocin Resistant isolated from animals	
S. felis	YES	8	YES	Species isolated from clinical samples of cats.	
S. fleurettii	YES	1	Yields low score values.	Using >2 (for species) can only discriminate at genus level.	Han *et al.* 2015

				Using >1,7 (for species) wrongly identifies as *S. sciuri*. Novobiocin Resistant species.	
S. gallinarum	YES	5	ND	CoNS species Novobiocin Resistant isolated from animals.	
S. haemolyticus	YES	12	YES		
S. hominis ssp *hominis*	YES	6	YES	Subspecies level discrimination.	Zhu *et al.* 2015
S. hominis ssp *novobiosepticus*	YES	1	YES	Subspecies level discrimination. Novobiocin Resistant	Zhu 2015
S. hyicus	YES	2	YES	Coagulase variable species isolated from pigs.	
S. intermedius	YES	12	YES	Correct ID at the species level, regardless of the score used. Coagulase positive species, isolated from pigeons. It is part of the *S. intermedius* group (SIG) along with *S. delphini* and *S. pseudointermedius*	Han *et al.* 2015; Silva *et al.* 2015; Decristophoris *et al.* 2011
S. kloosii	YES	4	YES	CoNS species Novobiocin Resistant isolated from animals	
S. lentus	YES	9	YES	Ex *Staphylococcus sciuri* ssp *lentus*. CoNS species Novobiocin Resistant	
S. lugdunensis	YES	7	YES		
S. lutrae	YES	6	YES	Coagulase positive species, isolated from otters	

S. microti	YES	4	ND	Species isolated from *Microtus arvalis* (a species of rodent from the *Cricetidae* family widely distributed throughout Europe and some areas of Asia). Species Novobiocin Resistant	
S. muscae	YES	1	YES	Species isolated from flies	
S. nepalensis	YES	5	YES	Species isolated from Himalayan goats. Novobiocin Resistant	
S. pasteuri	YES	8	YES	Species isolated from human, animal and food samples. Named in honour of the french microbiologist Louis Pasteur for his contribution in 1878 to the recognition of staphylococci as pathogens, and in honour of the research Pasteur Institute, in Paris, France, where the new species was characterized.	
S. pettenkoferi	YES	6	YES	CoNS species Isolated from human samples	Trulzsch *et al.* 2002
S. piscifermentans	YES	2	YES	CoNS species isolated from fermented fish in Thailand	Tanasupawat *et al.* 1992
S. pseudointermedius	YES	5	**NO** (Only report as *Staphylococcus*	**Wrongly identified as *S. intermedius*** regardless of the score used.	Han *et al.* 2015; Silva *et al.*

			Intermedius group): SIG	Coagulase positive species, isolated from domestic dogs and cats. It is part of the *S. intermedius* group (SIG) along with *S. intermedius* and *S. delphini*	2015; Murugaiyan *et al.* 2014; Devriese *et al.* 2005
S. saccharolyticus	YES	5	YES	Anaerobic *Staphylococcus* species. Formerly classified as *Peptococcus saccharolyticus*	Young *et al.* 2017
S. saprophyticus ssp *bovis*	YES	1	YES (at least to the species level)	Different works do not specify whether it can achieve subspecies level discrimination. Isolated from animals (bovine nostrils). Novobiocin Resistant	
S. saprophyticus ssp *saprophyticus*	YES	9	YES (at least to the species level)	Different works do not specify whether it can achieve subspecies level discrimination . Novobiocin Resistant	
S. schleiferi ssp *coagulans*	YES	3	YES	Coagulose positive species	
S. schleiferi ssp *schleiferi*	YES	4	YES		
S. sciuri ssp *carnaticus*	YES	2	YES (at least to the species level)	Different works do not specify whether it can achieve subspecies level discrimination . Novobiocin Resistant	
S. sciuri ssp *rodentium*	YES	2	YES (at least to the species level)	Different works do not specify whether it can achieve	

				subspecies level discrimination. Novobiocin Resistant	
S. sciuri ssp *sciuri*	YES	4	YES	Novobiocin Resistant	
S. simiae	YES	4	YES	Species isolated from squirrel monkeys in South America	
S. simulans	YES	9	YES	Named as such because of its similarity to certain species of coagulase positive staphylococci (including *S. aureus*)	
S. succinus ssp *casei*	YES	1	YES (at least to the species level)	Different works do not specify whether it can achieve subspecies level discrimination. Species isolated from surface of matured cheese. Novobiocin Resistant	
S. succinus ssp *succinus*	YES	1	YES (at least to the species level)	Different works do not specify whether it can achieve subspecies level discrimination. Novobiocin Resistant species, isolated from Dominican amber.	
S. vitulinus	YES	6	YES	Current name of *S. pulvereri*. Novobiocin Resistant	Švec *et al.* 2004
S. warneri	YES	7	YES	Named after Arthur Warner, who originally isolated this microorganism.	
S. xylosus	YES	7	YES	Novobiocin Resistant	

Staphylococcus spp. species **NOT** included in Bruker's database:

1) From animal or environmental isolates:

Staphylococcus agnetis (Taponen *et al.* 2012, sp. nov.). Coagulase variable species isolated from bovine milk.

Staphylococcus argensis (Heβ and Gallert 2015, sp. nov.)

***Staphylococcus caseolyticus* (ex Evans 1916)** Schleifer *et al*. 1982, nom. rev., comb. nov. (Now: *Macrococcus caseolyticus* (Schleifer *et al.* 1982) Kloos *et al.* 1998, comb. nov.)

Staphylococcus edaphicus (Pantůček *et al.* 2018, sp. nov.)

Staphylococcus rostri (Riesen and Perreten 2010, sp. nov.). Isolated from healthy pigs nasal passages (Riesen *et al.,* 2010).

Staphylococcus schweitzeri (Tong *et al.* 2015, sp. nov.). Belongs to the *Staphylococcus aureus* complex but has been isolated from non-human primates (Tong *et al.,*2015)

Staphylococcus stepanovicii (Hauschild *et al.* 2012, sp. nov.). Novobiocin Resistant and Oxidase positive species isolated from certain small wild mammals (Hauschild *et al.,*2010).

2) From human isolates:

Staphylococcus argenteus (Tong *et al.* 2015, sp. nov.). Species of the *Staphylococcus aureus* complex, an emerging pathogen in osteoarticular infections, identified by MALDI-TOF with score values between 1,39 and 1,87 as *S. aureus*, *S. simiae* or *S. epidermidis* (Rigaill *et al*., 2017)

Staphylococcus jettensis (De Bel *et al.* 2013, sp. nov.).

Staphylococcus massiliensis (Al Masalma *et al.* 2010, sp. nov.) Isolated from a brain abscess (Al Masalma *et al*., 2010).

Staphylococcus petrasii (Pantůček *et al.* 2013, sp. nov.). Isolated from ear infections (Pantucek *et al.,* 2013).

References:

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Maldi Tof y estafilococos

216 aislados de Estafilococos
ID Fenotipia convencional/ 16 s cuando hubo discrepancias
177 Score > 2 (84,7 %)
32 Score > 1,7 > 2 (13,3%)
Total con score > 1,7 : 96,8 %
La distribución de scores más bajos es dependiente de la especie:

~ 5% *S epidermidis y Saureus*

100% *S cohnii*
75% *S sciuri*
60% *S caprae*


Journal of Microbiological Methods

Evaluation of the Biotyper MALDI-TOF MS system for identification of *Staphylococcus* species☆

Wenming Zhu, Krzysztof Sieradzki, Valerie Albrecht, Sigrid McAllister, Wen Lin, Olga Stuchlik, Brandi Limbago, Jan Pohl, J. Kamile Rasheed

IMP Discrimina correctamente *S lugdunensis*!!

Malditof en Estafilococos coagulasa positivos:

S aureus, Complejo *S intermedius*, *S hyicus*, *S chromogenes* y *S schleiferii* ss *coagulans*.

Secuenciación del gen *pta*
5 aislados de S pseudointermedius
(3 inf asociadas Protesis-implantes osteoarticulares, 1 NAC , IPPB por mordedura de perro)

Fenotipo: No diferenció
S intermedius –pseudointermedius , si de S aureus
2 fueron coagulasa negativos!!!
Vitek: en 2 cepas: 1 S pseudintermedius, 1 S intermedius
Maldi-tof (bruker) No discrimina S intermedius-S pseudintermedius (score > 1,7-<1,9)

***S. pseudintermedius* puede ser identificado erróneamente como *S. intermedius*.**
Usando como método patrón la sec de 4 genes


Article published online: 8 May 2014
Clin Microbiol Infect 2014; 20: 1007–1015
10.1111/1469-0691.12662

Species differentiation within the Staphylococcus intermedius group using a refined MALDI-TOF MS database

Recomendación: Informar grupo INTERMEDIUS

Stenotrophomonas

Although it is not typically pathogenic for healthy persons, S*. maltophila* is a well-known opportunistic human pathogen. It is among the most common causes of wound infection due to trauma involving agricultural machinery. It is also an important nosocomial pathogen, associated with high morbidity and mortality, particularly in weakened or immunocompromised patients, and in patients that require artificial ventilation in the ICU. The incidence of human infections has increased in recent years, and a variety of clinical syndromes has been described, including bacteremia, pneumonia, urinary tract infection, ocular infection, endocarditis, meningitis, wound and soft tissue infection, mastoiditis, epididymitis, cholangitis, osteochondritis, bursitis and peritonitis. Septicemia may be accompanied by ecthyma gangrenosa, a skin lesion more commonly associated with *P. aeruginosa* and *Vibrio* spp. The incidence of *S. maltophila* respiratory tract infection in people with cystic fibrosis also seems to be increasing.

It is not posible to discriminate among the species of the genus, which usually yield low score values.

Report identification to the genus level with a **score value > 1,7.**

On the other hand, MALDI-TOF may wrongly identify *Stenotrophomonas maltophilia* as *Pseudomonas* species, so when suspected, it is suggested to perform the following additional phenotypic tests, specific of the genus:

Oxidation of glucose and maltose (intense), DNase, Lysine Decarboxylase, motility:

all yield <u>positive</u> results.

Table 116. Transcription of *Stenotrophomonas* spp. species.

Species	Present in BD	No. of MSPs in BD
S. acidaminiphila	YES	1
S. maltophilia (Pseudomonas beteli)	YES	1
S. maltophilia (Pseudomonas geniculata)	YES	1
S. maltophilia (Pseudomonas hibiscicola)	YES	1
S. maltophilia	YES	7
S. nitritireducens	YES	1
S. rhizophila	YES	1
Strenotrophomonas sp.	YES	1

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Streptococcus

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

MALDI-TOF may wrongly identify *Streptococcus mitis* as *Streptococcus pneumoniae* and vice versa. The bile solubility test and optochin (in O_2 and in 5% CO_2) must be performed.

The equipment cannot discriminate between *S. pneumoniae* and *S. pseudopneumoniae*: perform optochin test in O_2 and CO_2.

It cannot distinguish among species within *mitis* Group (less than 10% divergence): report as *mitis* Group.

Streptococcus mitis / suis: specific PCR.

MALDI-TOF correctly identifies species of *Streptococcus pyogenes*.

Additional tests:

- Beta-Hemolytic *Streptococci:* Bacitracin, PYR, CAMP, serology, VP, Sorbitol, Trehalose.

Refer to the Annex for microbial identification by phenotypic tests.

Viridans Group Streptococci. The following species should be reported to the GROUP level:

***Streptococcus mitis* Group**	*S. mitis*
	S. sanguinis
	S. parasanguinis
	S. gordonii
	S. cristatus
	S. oralis
	S. infantis
	S. peroris
	S. australis
	S.oligofermentans
	S. massiliensis
	S. sinensis
	S. orisratti
	S. pseudopneumoniae
	S. pneumoniae
	S. tigurinus
	S. lactarius

***Streptococcus anginosus* Group**	*S. anginosus*
	S. constellatus
	S. intermedius

***Streptococcus salivarius* Group**	*S. salivarius*
	S. vestibularis
	S. thermophilus

***Streptococcus mutans* Group**	*S. mutans*
	S. sobrinus
	S. cricetti
	S. ratti
	S. downei

The following can be reported to the SPECIES level:

***Streptococcus bovis* Group**	*S. lutetiensis*	Report to the species level
	S.equinus	Confirm with *rpo*B and *sod*A genes
	S. gallolyticus ss gallolyticus	Report to the species level
	S. gallolyticus ss pasteurianus	Report to the species level
	S. infantarius	Report to the group level

Table 117. Transcription of *Streptococcus* spp. species.

Species	Present in BD	No. of MSPs in BD
S. acidominimus	YES	1
S. agalactiae	YES	9
S. alactoyticus	YES	6
S. anginosus	YES	9
S. australis	YES	1
S. caballi	YES	1
S. canis	YES	11
S. constellatus	YES	6
S. costoreus	YES	1
S. criceti	YES	4
S. cristatus	YES	2
S. dentirousetti	YES	1
S. devriersei	YES	2
S. didelphis	YES	1
S. downey	YES	1
S. dysgalactiae	YES	8
S. entericus	YES	1
Streptococcus equi spp ruminatorum	YES	1
Streptococcus equi spp zooepidemicus	YES	7
S. equinus	YES	2
S. ferus	YES	1
S. gallalyticus	YES	2
Streptococcus gallalyticus ssp gallalyticus	YES	2

Streptococcus gallalyticus ssp macedoneces	YES	2
Streptococcus gallalyticus ssp pasteurianus	YES	6
S. gallinaceus	YES	1
S. gordonii	YES	3
S. halichoeri	YES	1
S. henryi	YES	1
S. hyointestinalis	YES	2
S. hyovaginalis	YES	2
S. inaiae	YES	1
S. infantarius	YES	1
S. infantis	YES	7
S. intemedius	YES	6
S. lutetiensis	YES	6
S. macacae	YES	1
S. marimammalium	YES	1
S. massiliensis	YES	1
S. merionis	YES	1
S. minor	YES	3
S. mitis	YES	39
S. mutans	YES	2
S. oralis	YES	38
S. orisratti	YES	1
S. orisurs	YES	1
S. ovis	YES	1
S. parasanguinis	YES	10

S. parauheris	YES	2
S. peroris	YES	2
S. phocae	YES	2
S. pleomorphus	YES	7
S. pluronimalium	YES	1
S. pneumoniae	YES	1
S. porcinus	YES	6
S. pseudopneumoniae	YES	6
S. pyogenes	YES	8
S. ratti	YES	2
S. salivarius	YES	5
Streptococcus salivarius spp salivarius	YES	1
Streptococcus salivarius spp thermophilus	YES	8
S. sanguinis	YES	11
S. sinensis	YES	1
S. sobrinus	YES	7
Streptococcus sp.	YES	2
Streptococcus ssp equi	YES	11
S. suis	YES	9
S. thoraltensis	YES	2
S. uberis	YES	6
S. urinalis	YES	2
S. vestibularis	YES	9

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Manual de procedimientos para la identificación de Cocos Gram Positivos Catalasa Negativa. Servicio Bacteriología Especial, Departamento Bacteriología, Instituto Nacional de Enfermedades Infecciosas ANLIS - "Dr. Carlos G. Malbrán", Buenos Aires, Argentina. Edición 2015.

Streptomyces

The taxonomy of this genus, which comprises more than 525 species and subspecies, continues to be a problem. Many of these species have been patented because the products they synthesize are used for commercial purposes.

So far, sequencing of 16S rRNA and *sec*A genes makes it possible to distinguish between the most frequent species of *Nocardia* and *Gordonia* / *Streptomyces* / *Tsukamurella*.

It may cause disease in immunocompromised patients, but rarely in healthy individuals, mycetoma being the most common, whose etiologic agent is usually *Streptomyces somaliensis*.

There are a few reports that imply that other species within this genus are opportunistic pathogens. Due to the large number of *Streptomyces* species described, and to the lack of information about the clinical significance of many of them, identification to the genus level is probably sufficient in most cases.

In a study on the susceptibility of 92 *Streptomyces* species of clinical specimens, 100% were susceptible to amikacin and linezolid, 77% were susceptible to minocycline, 67% to imipenem, and 51% to clarithromycin and amoxicillin-clavulanic acid.

Mycobacteria, *Nocardia* and Aerobic Actinomycetes represent a diagnostic challenge due to their complex cell walls, which is why they may require special processing prior to MALDI-TOF analysis in order to obtain more accurate results.

Based on the experience of the Reference Laboratories, there is a need to increase the representative profiles for this group of microorganisms in the commercial database. On the other hand, due to the characteristics of the cell wall, MALDI-TOF usually fails to achieve an identification or yields low score values, which is why it is recommended to follow the steps listed bellow:

a) inoculate sample using the direct method,

b) inoculate sample with the subsequent addition of 1ul of formic acid,

c) cover sample with 2ul of HCCA matrix,

d) perform the direct inoculation method, but from older plates, inoculated several days before,

e) if results are not as expected, the extraction process must be carried out with ethanol and formic acid. The extraction process with pearls can also be tested for Actinomycetals, which is recommended by the manufacturer.

It is recommended to report the identification to the genus level with a **score value ≥ 1,7.**

Table 118. Transcription of clinically relevant *Streptomyces* spp. species.

Species	**Present in BD**	**No. of MSPs in BD**
S. albus	YES	1
S. bikiniensis	NO	
S. cinereoruber	NO	
S. griseus	YES	1
S. somaliensis	NO	

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

T

Terribacillus

Species within this genus are *T. aidingensis, T goriensis, T. halophilus, T. saccharophilus*. These are usually environmental and are not clinically relevant.

In our experience, *Terribacillus goriensis / saccharophilus* (identified using Molecular Biology), might be identified by MALDI-TOF as *Brevibacillus brevis* with a score value > 2.

There are no reference protein profiles for *Terribacillus* spp. in today's commercial database.

References:

- An SY, Asahara M, Goto K, Kasai H, Yokota A. Terribacillus saccharophilus gen. nov., sp. nov. and Terribacillus halophilus sp. nov., spore-forming bacteria isolated from field soil in Japan. Int J Syst Evol Microbiol 2007;57(1):51-55. doi: 10.1099/ijs.0.64340-0.
- Krishnamurthi S, Chakrabarti T. Proposal for transfer of Pelagibacillus goriensis Kim et al. 2007 to the genus Terribacillus as Terribacillus goriensis comb. nov. Int J Syst Evol Microbiol 2008;58(10):2287-2291. doi: 10.1099/ijs.0.65579-0.
- Liu W, Jiang L, Guo C, Yang SS. Terribacillus aidingensis sp. nov., a moderately halophilic bacterium. Int J Syst Evol Microbiol 2010;60(12):2940-2945. doi: 10.1099/ijs.0.017228-0.

Tetragenococcus solitarius

Its role in causing infection in humans is unknown.

There is only a single MSP in Maldi Biotyper 3.1. commercial database.

Trueperella

This genus comprises five species, out of which *Trueperella pyogenes* and *Trueperella bernardiae (Arcanobacterium bernardiae* until year 2011) may be isolated from clinical specimens, more frequently from skin infections and abscesses.

Arcanobacterium species are CAMP / reverse CAMP positive, whereas *Trueperella* yields a negative result.

T. pyogenes is a veterinary pathogen and rarely causes infection in humans. It is the only species of *Arcanobacteria* / *Trueperella* of clinical relevance, with positive Beta Glucuronidase and Xylose Fermentation activity.

T. bernardiae reduces Maltose and Glucose.

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Table 119. Transcription of clinically relevant *Trueperella* spp. species.

Especie	Present in BD	No. of MSPs in BD
T. abortisius	YES	4
T. bernardiae	YES	10
T. pyogenes	YES	9

References:

- Barberis C, Almuzara M, Join-Lambert O, Ramırez MS, Famiglietti A, Vay C. Comparison of the Bruker MALDI-TOF mass spectrometry system and conventional phenotypic methods for identification of Gram-positive rods. PLoS One 2014;9(9):e106303. doi: 10.1371/journal.pone.0106303.
- Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry: a Fundamental Shift in the Routine Practice of Clinical Microbiology. Clin Microbiol Rev 2013;26(3):547–603. doi: 10.1128/CMR.00072-12.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

Tsukamurella

The characteristic chemical composition of its cell wall separates it from the rest of the Actinomycetals. The type species of the genus is *Tsukamurella paurometabola* (originally *Corynebacterium paurometabolum*), followed by twelve species.

Infections are usually associated with foreign bodies, such as intravenous catheters.

T. tyrosinosolvens has been implicated in cases of keratitis, bacteremia and catheter-related infection.

Liu *et al.* reported drug susceptibility data from *T. tyrosinosolvens, T. spumae* and *T. pulmonis* using a short incubation period (CLSI standards continue to indicate reading after 48 hours), and the three species resulted susceptible to Amoxicillin- Clavulanic acid, Ciprofloxacin and Linezolid.

T. tyrosinosolvens and *T. spumae* were suscpetible to sulfamethoxazole, whereas *T. pulmonis* was reported as resistant.

Table 120. Transcription of clinically relevant *Tsukamurella* spp. species.

Species	Present in BD	No. of MSPs in BD
T. hongkongensis	NO	
T. inchonensis	YES	1
T. paurometabola	YES	5
T. pulmonis	NO	
T. sinensis	NO	
T. strandjordii	NO	
T. spumae	NO	
T. tyrosinosolvens	NO	
Tsukamurella sp.	YES	1

Based on the experience of the Reference Laboratories, there is a need to increase the representative profiles for this group of microorganisms in the commercial database. On the other hand, due to the characteristics of their cell wall, MALDI-TOF usually fails to achieve an identification or yields low score values, so it is recommended to follow the steps listed bellow:

a) inoculate sample using the direct method

b) inoculate sample with the subsequent addition of 1ul of formic acid

c) cover sample with 2ul of HCCA matrix

d) perform the direct inoculation method but starting from older plates (inoculated several days before)

e) if results are not as expected, the extraction process must be carried out with ethanol and formic acid. The extraction process with pearls can also be tested for Actinomycetals, which is suggested by the manufacturer.

It is recommended to report the identification to the genus level with a **score value ≥ 1,7.**

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Teng JL, Tang Y, Wong SS, Ngan AH, Huang Y, Tsang CC, Choi GK, Lau SK, Woo PC. *Tsukamurella hongkongensis* sp. nov. and *Tsukamurella sinensis* sp. nov., isolated from patients with keratitis, catheter-related bacteraemia and conjunctivitis. Int J Syst Evol Microbiol 2016;66(1):391-397. doi: 10.1099/ijsem.0.000733.
- WEBSITE: *www.bacterio.net/-sz.html*

Turicella

Turicella genus is phylogenetically related to the *Corynebacterium* genus, but the only representative species of this genus is *Turicella otitidis*.

T. otitidis is catalase positive, immobile, oxidative, and is usually located in the external auditory region; which is why clinical isolates are almost exclusively found around the ear, although it is not a causative agent of otitis media in children.

Turicella otitidis isolates are CAMP positive and have a specific code in the API Coryne system: 2100004. Refer to the annex to see its morphological appearance in Gram staining.

There are 8 reference protein profiles in the commercial database.

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69 = Identification to the genus level

SCORE <1,5 = No identification

Table 121. Phenotypic differentiation of *Turicella* spp. from related coryneform species.

Species	Methabolism	CAMP	DNase	LAP
Turicella otitidis	Oxidative	+	+	+
Corynebacterium auris	Oxidative	+	-	+
Corynebacterium coyleae	Oxidative	+	-	+
Corynebacterium afermentans ss afermentans	Oxidative	+	-	-

References:

- Barberis C, Almuzara M, Join-Lambert O, Ramırez MS, Famiglietti A, Vay C. Comparison of the Bruker MALDI-TOF mass spectrometry system and conventional phenotypic methods for identification of Gram-positive rods. PLoS One 2014;9(9):e106303. doi: 10.1371/journal.pone.0106303.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

U

Ureaplasma

There is no reference profile in the commercial database.

V

Vagococcus

Until now, the number of *Vagococcus* isolated from clinical specimens (blood culture, wound, peritoneal fluid) has been very low. The difficulties that arise in the identification are due to its low frequency.

It is recommended to report the identification to the genus level with **score values >1,5.**

A reliable identification to the species level is not achieved; the same happens with phenotypic methods (refer to Annex) and with sequencing of the 16S rRNA and the specific gene *sod*A. The number of reference profiles in the database for this group of microorganisms needs to be increased.

Table 122. Transcription of clinically relevant *Vagococcus* spp. species.

Species	Present in BD	No. of MSPs in BD	Observations
V. carniphilus			In our experinece, it has not been identified by MALDI-TOF
V. fluvialis	YES	4	
V. lutrae	YES	1	It may not be identified due to its poor representation in the commercial database.
V. fessus	NO		

References:

- Almuzara M, Barberis C, Velázquez VR, Ramirez MS, Famiglietti A, Vay C. Matrix-assisted Laser Desorption Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) as a Reliable Tool to Identify Species of Catalase-negative Gram-positive Cocci not Belonging to the *Streptococcus* Genus. Open Microbiol J 2016;10,202–208. doi: 10.2174/1874285801610010202.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

- Wang L, Cui YS, Kwon CS, Lee ST, Lee JS, Im WT. Vagococcus acidifermentans sp. nov., isolated from an acidogenic fermentation bioreactor. Int J Syst Evol Microbiol 2011;61(5):1123–1126. doi: 10.1099/ijs.0.022087-0.

Veillonella

The *Veillonella* genus comprises 13 species, out of which *V. alcalescens, V. atypica, V. denticariosi, V. dispar, V. parvula, V. rogosae* and *V. tobetsuensis* have been isolated from the human oral cavity.

The risk factors for a *Veillonella* infection include periodontitis, immunodeficiency, intravenous drug use and premature birth.

They are etiological agents of severe infections such as meningitis, osteomyelitis, prosthetic joint infection, bacteremia and endocarditis; although the virulence mechanisms of these microorganisms are unclear.

It is resistant to tetracycline, erythromycin, gentamicin and kanamycin, and is susceptible to penicillin G, cephalothin and clindamycin.

Due to the Reference Laboratories' limited experencie with this genus, and based on the scientific literature listed below, where the limitation of mass spectrometry regarding identification to the species level is evident, it is recommended to only report the identification of *Veillonella spp.* to the genus level.

SCORE > 1,7 = Identification to the genus level

Table 123. Transcription of *Veillonella* spp. species isolated from clinical samples.

Species	Present in BD	No. of MSPs in BD
V. alcalescens	NO	
V. atypica	YES	4
V. caviae	YES	1
V. criceti	YES	1
V. denticariosi	YES	2
V. dispar	YES	2
V. magna	YES	1
V. montpellierensis	YES	2
V. parvula	YES	7
V. ratti	YES	1
V. rogosae	YES	1
V. rodentium	NO	
V. seminalis	NO	

References:

- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Justesen U, Holm A, Knudsen E, Andersen L, Gorm Jensen T, Kemp M, Skov M, Gahrn-Hansen B, Møller J. Species Identification of Clinical Isolates of Anaerobic Bacteria: a Comparison of Two Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Systems. J Clin Microbiol 2011; 49(12):4314–4318. doi: 10.1128/JCM.05788-11.

Vibrio

Members of the *Vibrionaceae* family can cause a wide variety of intestinal and extraintestinal human illnesses; these include diarrhea, cellulitis, necrotizing fasciitis, septicemia, and eye and ear infections.

Species that are clinically relevant are listed below:

Species	**Clinical manifestation**
V. cholerae	It is divided into more than 200 serogroups, of which only serogroups O1 and O139 are responsible for epidemic and pandemic cholera.
V. mimicus	Diarrhea.
V. parahaemolyticus	Intestinal infection; associated with the consumption of raw fish.
V. vulnificus	Septicemia, wound and ear infection.
V. fluvialis	Gastroenteritis, bacteremia.
V. furnissii	Diarrhea.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Vibrio cholerae is not represented in the commercial database because it is considered a bioterrorism agent.

In the case of agents that require Biosafety Level 3, the method of choice for the preparation of the samples that will be processed in MALDI-TOF is very important, since it must ensure both the inactivation of the microorganism and the optimal quality of the generated spectrum. Based on the literature, the extraction method with ethanol / formic acid / acetonitrile is recommended.

Table 124. Transcription of *Vibrio* spp. species isolated from clinical samples.

Species	Present in BD	No. of MSPs in BD
V. cholerae	NO	
V. fluvialis	YES	3
V. furnissii	YES	2
V. mimicus	YES	1
V. parahaemolyticus	YES	8
V. vulnificus	YES	9

References:

- Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry: a Fundamental Shift in the Routine Practice of Clinical Microbiology. Clin Microbiol Rev 2013;26(3):547–603. doi: 10.1128/CMR.00072-12.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.

W

Weeksella

The natural habitat of gram negative nonfermenters, oxidase and indole positive, is usually the soil, plants, and water, including those in a hospital environment. The most clinically relevant species is *Weeksella virosa*, frequently isolated from the urogenital tract.

It is recommended to use the following criteria suggested by the manufacturer:

SCORE ≥ 2,00 = Identification to the species level

SCORE 1,7-1,99 = Identification to the genus level

SCORE < 1,70 = No identification

Table 125. Transcription of *Weeksella* spp. species isolated from clinical samples.

Species	Present in BD	No. of MSPs in BD	Observations
W. massiliensis	NO		In today's nomenclature, it is equivalent to *Vaginella massiliensis*
W. virosa	YES	9	
W. zoohelcum	NO		In today's nomenclature, it is equivalent to *Bergeyella zoohelcum.* It differs by being PYR -, rapid urease + and Col / Pol R

References:

- Bergeyella zoohelcum (Holmes et al. 1987) Vandamme et al. 1994, comb. nov.
- Diop K, Bretelle F, Michelle C, Richez M, Rathored J, Raoult D, Fournier PE, Fenollar F. Taxonogenomics and description of *Vaginella massiliensis* gen. nov., sp. nov., strain Marseille P2517^T, a new bacterial genus isolated from the human vagina. New Microbes New Infect 2017;15: 94–103. doi: 10.1016/j.nmni.2016.11.006.

- Holmes B, Steigerwalt AG, Weaver RE, Brenner Don J. *Weeksella virosa* gen. nov., sp. nov. (Formerly Group IIf), found in human clinical specimens. Syst Appl Microbiol 1986;8(3):185-190. doi: 10.1016/S0723-2020(86)80075-3.
- Holmes B, Steigerwalt AG, Weaver RE, Brenner Don J. *Weeksella zoohelcum* sp. nov. (Formerly group IIj), from human clinical specimens. Syst Appl Microbiol 1986;8(3):191-196. doi: 10.1016/S0723-2020(86)80076-5.
- Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology, 11th edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.
- Sankar SA, Lo CI, Fall B, Sambe-Ba B, Mediannikov O, Diallo I, Labas N, Faye N, Wade B, Raoult D, Fournier PE, Fenollar F. Noncontiguous finished genome sequence and description of *Weeksella massiliensis* sp. nov. New Microbes New Infect 2015;8, 89-98. doi: 10.1016/j.nmni.2015.09.013.

Weissella

It is a member of the group of Gram positive, catalase negative, PYR negative, vancomycin resistant cocci, along with *Leuconostoc* and *Pediococcus*.

The most frequent species within this genus is *Weissella confusa*, causative agent of bacteremia and endocarditis.

The following score values have been accepted for a reliable identification:

SCORE >1,7 = Identification to the species level

SCORE 1,5-1,69= Identification to the genus level

SCORE <1,5= No identification

Identification failures (No Identification) may occur in the case of *Weissella paramesenteroides* and *Weissella confusa.*

It is recommended to perform sequencing of the 16S rRNA or *sod*A gene in order to confirm the identification to the species level.

Table 126. Phenotypic features of *Weissella* spp. species isolated from clinical samples.

Assay	*W. confusa*	*W. cibaria*	*W. viridescens*	*W. paramesenteroides*
Arabinose Acid	-	+	-	d
Galactose Acid	+	-	-	+
Ribose Acid	+	-	-	d
Sucrose Acid	+	+	d	+
ADH	+	+	-	-
Esculin Hydrolysis	+	+	-	v

Table 127. Transcription of *Weissella* spp. species.

Species	**Present in BD**	**No. of MSPs in BD**
W. confusa	YES	2
W. cibaria	NO	
W. halotolerans	YES	2
W. hellenica	YES	1
W. minor	YES	2
W. paramesenteroides	NO	
W. viridescens	YES	4

References:

- Almuzara M, Barberis C, Velázquez VR, Ramirez MS, Famiglietti A, Vay C. Matrix-assisted Laser Desorption Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) as a Reliable Tool to Identify Species of Catalase-negative

Gram-positive Cocci not Belonging to the *Streptococcus* Genus. Open Microbiol J 2016;10,202–208. doi: 10.2174/1874285801610010202.

- Björkroth KJ, Schillinger U, Geisen R, Weiss N, Hoste B, Holzapfel WH, Korkeala HJ, Vandamme P. Taxonomic study of Weissella confusa and description of Weissella cibaria sp. nov., detected in food and clinical samples. Int J Syst Evol Microbiol 2002;52(1):141-148. doi: 10.1099/00207713-52-1-141.
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Y

Yersinia

The NRL has no experience with this genus.

Yersinia represents a group of clinically relevant microorganisms that are uncommon in clinical isolates. The species of the genus that cause disease in humans are *Yersinia pseudotuberculosis, Yersinia enterocolitica* and *Yersinia pestis*. Infections caused by *Y. pseudotuberculosis* and *Y. enterocolitica* occur after the ingestion of contaminated food or water, and they are primarily manifested as gastroenteritis; while *Yersinia pestis* (ethiological agent of the plague), is transmitted to humans through the bite of an infected flea and it results in a serious disease with a high degree of mortality.

Within this genus, there are other 14 environmental species that are non-pathogenic to humans.

Yersinia pestis is considered a bioterrorism agent, which needs to be manipulated in Class II Biosafety Cabinets (BSL-2, BSL-3), so it is not included in the commercial database.

The use of MALDI-TOF for this type of microorganisms has been evaluated in multiple ways; in 2010 Lasch *et al.* developed a reference database through which characteristic peaks for the genus and species level could be detected. This database could also detect biomarker peaks between *Y. pestis / Y. pseudotuberculosis,* which are organisms with genetical similarities.

On the other hand, Ayyadurai *et al.* were able to achieve the differentiation of environmental and pathogenic species of *Yersinia*, and the detection of biotypes of *Y. pestis* by creating a database that represented 12 species and 3 biotypes of *Yersinia pestis.*

Regarding the scientific literature on this matter, MALDI-TOF could be considered a reliable and robust method for the identification of *Yersinia*, which can also provide epidemiological information when detecting *Yersinia pestis* biotypes. However, the most important aspect to consider is the inactivation protocol of the microorganisms to be tested; since, in addition to biosafety, it must have minimal influence on the generated spectrum. Nowadays, the most commonly used methodology is the extraction with TFA.

Table 128. Transcription of *Yersinia* spp. species.

Species	Present in BD	No. of MSPs in BD	Observations
Y. enterocolitica	YES	7	
Y. pestis	NO		Bioterrorism agent
Y. pseudotuberculosis	YES	12	

References:

- Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry: a Fundamental Shift in the Routine Practice of Clinical Microbiology. Clin Microbiol Rev 2013;26(3):547–603. doi: 10.1128/CMR.00072-12.
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