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Colibrí®

Package Insert

Colibrí™ System

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PACKAGE INSERT – COLIBRÍ™ SYSTEM

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IVD

"This Package Insert is intended for the US Market only."

[ORIGINAL VERSION]

DOCUMENT HISTORY

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1.1 GENERAL NOTES

Colibrí System documentation (User Manuals and Package Insert) can be also download from https://www.copangroup.com/manuals. A login account is required to access the website. Email request.waspautomations@copangroup.com to request an account setup.

For bioMérieux VITEK MS System documentation visit the bioMérieux Technical Library at www.bioMerieux.com/techlib.

For Bruker MALDI Biotyper documentation contact Bruker Support Team (ms.support.us@bruker.com) they will send the User Manual on request.

1.2 INTENDED USE

The Copan Colibrí™ System is an *in vitro* diagnostic device comprised of the Colibrí Vision System and Colibrí Preparation Station for use with the bioMérieux VITEK® MS or Bruker MALDI Biotyper® CA mass spectrometry systems for qualitative identification of isolated colonies of Gram-negative and Gram-positive bacterial species grown on solid culture media. The Colibrí™ System is a semi-automated pre-analytical processor that picks isolated colonies designated by the operator and uses a pipetting system to prepare MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry) target slides. The Colibrí™ software records the identity of each sample and its position on the target slide and communicates this information electronically to the MALDI-TOF MS analyzers.

The Colibrí™ System is intended for use by trained healthcare professionals in clinical laboratories in conjunction with other clinical and laboratory findings, including Gram staining, to aid in the diagnosis of bacterial infections.

The Colibrí™ System has not been validated for use in identification of yeast species.

1.3 SUMMARY AND PRINCIPLES OF THE PROCEDURE

Timely and accurate identification of microorganisms is the underlying function of any clinical microbiology laboratory. The application of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to cultured microorganism identification represents a paradigm shift in diagnostic microbiology practices (1). However, laboratory errors may occur as a result of colony inoculation in erroneous target locations (transposition errors), testing of impure colonies, smearing between spots, failure to clean target slide or erroneous data entry into laboratory information systems (2).
The Colibrí System standardizes the target slide preparation and automatizes the data transmission reducing the human errors.

1.4 SYSTEM COMPONENTS

The Colibrí System is composed of two modules: the Colibrí Vision System and the Colibrí Preparation Station. The Colibrí Vision System is an Image Acquisition module for digital image acquisition of culture plates showing bacterial species growing on solid culture media that includes a workbench and Image Reading Interface components which allow the user to read the images of the plates, select colonies and assign the appropriate workup. The Colibrí Preparation Station includes a working area, a plates loading/unloading area, a PC (hardware and software), a color LCD (Liquid Crystal Display) monitor with touch screen, a computer keyboard and mouse.

1.5 REQUIRED MATERIALS SUPPLIED

Consumable	Product Description	Product Code (REF)
CO-RE Tips (50 µl) with filters	50 µl Plastic Tips used with Colibrí picking system for the deposition of the designated colony on the target and matrix addition.	235948
Biohazard Disposal Bag for Tips	For the containment and disposal of used consumables	WR090-01-0010

1.6 EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED*

- VITEK® MS-CHCA solution
- MALDI Biotyper® CA System US IVD HCCA solution
- MALDI Biotyper® CA System US IVD BTS solution
- VITEK® MS-DS target slides
- US IVD 48 Spot target
- MBT Biotarget 96 US IVD
- MSP Biotarget Adapter
- Micropipettes (1-10 µl)
- Micropipettes (100-1000 µl)
- Material required by MALDI Biotyper® CA System for US IVD HCCA solution preparation
- Calibrator Strain for VITEK® MS *Escherichia coli* ATCC 8739 cultured on cultured on Columbia Agar + 5% sheep blood or Trypticase Soy Agar + 5% sheep blood.
- Quality Control Strain for VITEK® MS *Klebsiella aerogenes* ATCC® 13048 formerly *Enterobacter aerogenes*) cultured on cultured on Columbia Agar + 5% sheep blood or Trypticase Soy Agar + 5% sheep blood.
- QC Control strain for Colibrí System (*Escherichia coli* ATCC® 25922) cultured on Columbia Agar + 5% sheep blood or Trypticase Soy Agar + 5% sheep blood.
- Lab coat and disposable gloves, powderless

* required equipment and materials may vary according to Colibrí™ System configuration

1.7 WARNINGS AND PRECAUTIONS

- Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- The Copan Colibrí™ System is for *in vitro* diagnostic use only.
- The Copan Colibrí™ should be used only by laboratory professionals trained on the use of the System. Use Copan Colibrí™ System in accordance with the Package Insert and the user manuals.
- Wear protective gloves and other protection commensurate with universal precautions when handling clinical specimens. Observe appropriate CDC Biosafety recommendations. After use, microbial cultures and inoculated products should be considered infectious waste and must be disposed of according to laboratory regulations (3, 4, 5).
- Use of this device outside of the recommended temperature ranges may compromise the stability of reagents leading to invalid results. For best results, the environmental temperature is 20-25°C (+68°F-+77° F).
- Failure to follow maintenance and cleaning procedures illustrated in the user manual HPACUEN - Colibrí Preparation Station Operator Manual (Extract for Id) HPACVEN - Colibrí Vision System Operator Manual could lead to erroneous results.
- Identification should be performed on single isolated colonies. Mixed samples may result in erroneous identifications. Refer to HPACVEN - Colibrí Vision System Operator Manual for correct colony selection procedure.
- The amount of colony spotted on the target impacts a successful identification result: refer to HPACVEN - Colibrí Vision System Operator Manual for the selection of adequate colonies.
- The performance of identification varies between Gram-positive and Gram negative; consider the proportion of Nonreportable results with Gram-positive species to evaluate the suitability of Copan Colibrí System.
- When used in combination with Bruker MALDI Biotyper® CA System, Colibrí™ System can process both MBT Biotarget 96 US IVD (96-position disposable targets) and US IVD 48 Spot target (48-position reusable steel target).
- Culture Requirements: it is the responsibility of the user to select culture media compatible with bioMérieux VITEK® MS and Bruker MALDI Biotyper® CA System. The Colibrí System has been validated to be used with culture media incubated for the time ranges indicated in the following Table:

List of Culture Media and Incubation Time Ranges validated with Colibrí System

Culture Media	Incubation Condition for VITEK MS® *	Incubation Condition for MALDI Biotyper® CA System**
Columbia Agar + 5% sheep blood	18-72 hours	18-48 hours
Trypticase Soy Agar + 5% sheep blood	18-72 hours	18-48 hours
MacConkey Agar	18-72 hours	18-48 hours
Chocolate Agar	18-48 hours	18-48 hours
Columbia CNA Agar	NA	18-48 hours
Bordet-Gengou Agar	NA	5-7 days
Trypticase Soy Agar + 5% sheep blood / MacConkey Agar	18-72 hours	18-48 hours
Columbia CNA Agar / Mac-Conkey Agar	NA	18-48 hours

*fresh culture

**fresh culture or within 12 hours post-incubation at Room Temperature.

- Specific requirements: isolated colonies of *Bordetella pertussis* grown on Bordet Gengou + 15% sheep blood can be processed with Colibrí System in conjunction with Bruker MALDI Biotyper® CA system within 7 days of incubation; no post-incubation time of 12 hours at Room Temperature is recommended.
- It is the responsibility of the user to follow the appropriate labelling for preparation, handling and storage of the matrix, and to monitor its expiration on the deck of the Colibrí Preparation Station.

1.8 INSTRUCTIONS FOR USE

The Colibrí™ System is a semi-automated pre-analytical processor that picks isolated colonies designated by the operator and uses a pipetting system to prepare MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry) target slides. For detailed instructions refer to HPACUEN – Colibrí™ Preparation Station Operator Manual (Extract for Id) HPACVEN - Colibrí Vision System Operator Manual. Here follows a general overview of the workflow:

- The microorganisms intended to be identified through the MALDI-TOF MS technique, should be isolated on the suitable culture media. The complete list of culture media and the recommended incubation times are indicated in paragraph 1.7 Warnings and Precautions.
- After incubation and before loading in the Vision System, culture plates to be processed should be visually inspected by a trained operator to verify the presence of growth.
- Gram stain results must be used to determine the applicability of the Copan Colibrí™ to preparation of colonies for analysis. If adequate bacterial growth is present and colonies should be subjected to MALDI-TOF MS identification, Gram stain evaluation should be performed to determine the applicability of the Copan Colibrí™.
- Plates are loaded on the Vision System to allow automatic image acquisition.
- Digital plate images are observed by the operator on the Vision System Image Reading Interface to select isolated colonies to be processed and assign the automatic ID Workup. One isolated colony shall be selected when the single deposition mode is used; two isolated colonies shall be selected for the duplicate deposition mode.
- Plates are then manually loaded by the operator in the Preparation Station as Input Picking Plates. Through plate barcode reading, the Preparation Station retrieves the selected isolated colonies on the plate and automatically picks and spots them on the target slide. Then, the Preparation Station automatically aspirates the matrix solution and overlays the spots.
- Once the target slide is completed, it is retrieved manually by the operator. Before loading it into the MALDI-TOF MS analyzer for mass spectra acquisition, the operator should check the correct crystallization of the matrix as reported in the instructions for use of the bioMérieux VITEK® MS or Bruker MALDI Biotyper® CA System (6, 7).
- After target unloading from Colibrí™ Preparation Station, data related to the spot are automatically transferred from Colibrí System to MALDI-TOF MS analyzer.
- Before loading the target in the IVD analyzer, the operator should manually prepare the control spots as indicated in the bioMérieux VITEK® MS or Bruker MALDI Biotyper® CA System instruction for use.

- In the event of Nonreportable Results (Low Confidence or No Identification/No Peak result), follow the workflows described in the applicable User Manual for the mass spectrometry instrument and/or use an alternative method for organism identification.

1.9 LIMITATIONS

- The Copan Colibrí™ should be used only for preparation of target slides from isolated colonies of Gram-negative and Gram-positive bacterial species grown on solid culture media. The Colibrí™ System is not intended for and has not been validated for use in identification of yeasts, molds, Nocardia or Mycobacteria. Gram stain results must be used to determine the applicability of the Copan Colibrí™ to preparation of colonies for analysis. If yeast is suspected, follow the instructions in the Package Inserts for the bioMérieux VITEK MS or Bruker MALDI Biotyper CA Systems to prepare samples of the isolate manually and/or use an alternative method of organism identification.
- Colibrí™ System has been validated for the direct spotting of isolated bacterial colony(ies) grown on a solid media and overlaid with matrix solution. Other methods of slide/target preparation such as overlay with formic acid or extraction methods have not been validated and should be conducted manually as per the applicable MALDI-TOF MS analyzer instructions for use.
- Use of this device is permitted only in association with bioMérieux VITEK® MS or Bruker MALDI Biotyper® CA System.
- This product is intended for identification of colonies grown on solid agar media plates. Do not use for identification from liquid cultures.
- Identification results obtained using the Copan Colibrí™ System for sample preparation with bioMérieux VITEK® MS or Bruker MALDI Biotyper® CA System should be used as an adjunct to clinical observations and other information available to the physician.
- Copan Colibrí™ Preparation System does not prepare the control spots as indicated in the bioMérieux VITEK® MS and Bruker MALDI Biotyper® CA System instructions for use: it is responsibility of the user to prepare control spots before loading in the IVD analyzer.
- The ability of the Copan Colibrí™ System to prepare samples for analysis with the VITEK® MS or Bruker MALDI Biotyper® CA System was evaluated using only the species listed in the Inclusivity Study, described in the Performance Characteristics section of this Package Insert. The ability of the Copan Colibrí™ System to prepare samples of other species for mass spectrometry analysis has not been evaluated.
- The performance of Copan Colibrí System in conjunction with the VITEK® MS* and Bruker MALDI Biotyper® CA System was evaluated with Trypticase Soy Agar + 5% sheep blood (BD), Columbia Agar + 5% sheep blood (bioMerieux), MacConkey Agar (BD), Columbia CNA Agar (BD), Bordet Gengou Agar (BD), Trypticase Soy Agar + 5% sheep blood / MacConkey Agar (BD), Columbia CNA Agar / MacConkey Agar (BD). The use of other types of culture media has not been validated.

- Use of the Copan Colibrí System to prepare targets from cultures of Gram-positive organisms resulted in a low proportion of High Confidence Log(scores) with the Bruker MALDI Biotyper CA System. If a low-confidence identification or no identification result is obtained using the Copan Colibrí System, follow the instructions in the Package Insert for the Bruker MALDI Biotyper CA Reference Library to repeat testing of the isolate manually using the Direct Transfer (DT), extended Direct Transfer (eDT) or Extraction (Ext) Sample Preparation Procedure.
- Use of the Copan Colibrí System to prepare targets for the Bruker MALDI Biotyper CA from cultures of Bordetella pertussis on Bordet Gengou Agar + 15% sheep blood and held at ambient temperature for 12 hours after incubation for 7 days at 35 ± 2°C is not recommended. Holding cultures at ambient temperature after incubation for 7 days resulted in a decrease in the proportion of High Confidence Log(scores) obtained on the MALDI Biotyper CA when samples are prepared using the Colibrí System. Isolated colonies of B. pertussis can be processed with Colibrí System in conjunction with Bruker MALDI Biotyper CA system within following 5 to 7 days of incubation. Cultures should be processed immediately following observation of colonies; delay in processing after observation of suspected colonies of B. pertussis may lead to low confidence Log(scores) and failure to obtain reliable identification.
- Use of the Copan Colibrí System to prepare 96-spot disposable targets for the Bruker MALDI Biotyper CA System resulted in a low proportion of High Confidence Log(scores) than the 48-spot reusable target format. If a low-confidence identification or no identification result is obtained using the Copan Colibrí System, follow the instructions in the Package Insert for the Bruker MALDI Biotyper CA Reference Library to repeat testing of the isolate manually using the Direct Transfer (DT), extended Direct Transfer (eDT) or Extraction (Ext) Sample Preparation Procedure.
- Target spots prepared on the Copan Colibrí System are stable up to 48 hours for use on the bioMérieux VITEK MS and up to 24 hours for use on the Bruker MALDI Biotyper CA System. Targets prepared by the Copan Colibrí Preparation Station should be processed within 48 hours for the bioMérieux VITEK MS and within 24 hours for the Bruker MALDI Biotyper; delays in processing beyond the recommended hold times may lead to an unreliable identification.

1.10 QUALITY CONTROL

Quality Control procedures monitor the performance of the Colibrí System. Laboratories must establish the number, type and frequency of testing control material according to guidelines or requirements of local, provincial, state, federal and/or country regulations of accreditation organizations in order to monitor the effectiveness of the entire analytical process. For general Quality Control guidance, the user may wish to refer to Clinical Laboratories Standard Institute EP12 (8).

Quality Control should be run at least daily, until adequate process validation is achieved on the Colibrí System in each laboratory setting. Reduced frequency of control testing should be in accordance with applicable regulations.

- Culture Plates showing isolated colonies are treated as Input Picking Plates for the purpose of Quality Control testing and are processed as described in the HPACUEN - Colibrí Preparation Station Operator Manual (Extract for Id) HPACVEN - Colibrí Vision System Operator Manual.
- For Quality Control it is recommended to prepare 3 culture plates (2 plates will be used only in case of failure root cause analysis for No Identification Results) showing isolated colonies of *Escherichia coli* (ATCC® 25922) grown on Columbia Agar + 5% sheep blood or Trypticase Soy Agar + 5% sheep blood.
- Acquire digital image in the Vision System and select the ID Workup that will result in the picking of 1 colony if the Colibrí System is set in Single Deposition Mode or 2 colonies if set in the Duplicate Deposition Mode. Repeat this operation 3 times.
- Load plates in the Preparation Station as Input Picking Plates and prepare MALDI-TOF MS target.
- Manually spot the appropriate MALDI-TOF MS controls/calibrators that are applicable to the downstream MALDI-TOF MS analyzer.
- Identification Results of spots prepared should be reported with a high Confidence Value/Log (Score).
- In case of “No identification results”, perform a root cause evaluation: unload the processed plates from the Preparation Station and check that the designated colony was picked; if the colony was correctly picked, repeat the quality control using a new Quality Control Plate and visually check that the tip deposits the colony and overlays the spot with the matrix. If the colony was correctly spotted and overlaid, change the matrix with a fresh vial and repeat Quality Control with a new plate. In case of repeated “No identification results” contact Technical Support.

1.11 PERFORMANCE CHARACTERISTICS

The performance of Copan Colibrí™ system for preparation of samples to be used for bacterial identification in conjunction with bioMérieux VITEK® MS or Bruker MALDI Biotyper® CA was determined in the analytical studies summarized below.

1.12 INCLUSIVITY

A variety of bacteria included in the knowledge databases of the bioMérieux VITEK® MS or Bruker MALDI Biotyper® CA System (on-panel strains) were included in this study to demonstrate that spots prepared with the Copan Colibrí™ System provide the expected identification. Strains were selected for inclusion in the study as representatives of different genera and organism groups that exhibited a broad range of colony characteristics (size, morphology and viscoelastic properties). The study was designed to include multiple strains of the most commonly isolated Gram-positive and Gram-negative species in the US, as well as examples of less common/rare pathogens. The identification results obtained by the bioMérieux VITEK® MS and Bruker MALDI Biotyper® CA System using the Copan Colibrí™ System for sample preparation were compared to the expected organism identity. Manual sample preparation was used as reference method to verify Colibrí™ System performance. Fresh cultures of the selected strains were grown on different culture media plates selected among those recommended for use with the MALDI-TOF MS analyzers. The results are summarized in Tables 1-3 stratified per species. The study conducted in conjunction with the Bruker MALDI Biotyper® CA System was performed on both validated targets, the MBT Biotarget 96 US IVD (96-position disposable target) and US IVD 48 Spot target (48 positions reusable steel target).

When Copan Colibrí™ System was used in conjunction with the bioMérieux VITEK® MS, an overall agreement of 97.2% (381/392) in the identification results between organisms spotted automatically and the expected strain identity was found, and no wrong identification results were obtained with the automatic preparation. More specifically, 85.2% of picked colonies (334/392) provided an identification corresponding to the expected strain identity with a Confidence Value ≥ 60%. In addition, the calculation of agreement includes 47/48 colonies of *Enterobacter cloacae* and *Proteus vulgaris* were reported with Low Discrimination as *Enterobacter cloacae/Enterobacter asburiae* and *Proteus penneri/Proteus vulgaris*, in accordance with the labeling for the VITEK MS analyzer.

When Copan Colibrí™ System was used in conjunction with the Bruker MALDI Biotyper® CA System on the US IVD 48 Spot Target, 93.2% of picked colonies (436/468) provided an identification corresponding to the expected strain identity with high confidence (Log(Score) ≥ 2). For Gram-positive species, 22/156 colonies (14.1%) were identified with Low Confidence (Log(Score) 1.70-1.99), compared with 4/312 colonies (1.3%) of Gram-negative species. In addition, 6/156 Gram-positive colonies (3.8%) produced no identification result.

When Copan Colibrí™ System was used in conjunction with Bruker MALDI Biotyper® CA System with the MBT Biotarget 96 US IVD, 85.7% of picked colonies provided an identification corresponding to the expected strain identity with a High confidence ID Log (Score) ≥ 2. For Gram-positive species, 29/156 colonies (18.6%) were identified with Low Confidence (Log(Score) 1.70-1.99), compared with 10/312 colonies (3.2%) of Gram-negative species. In addition, 22/156 Gram-positive colonies (14.1%) produced no identification result compared with 6/312 Gram-negative colonies (1.9%).

As confirmed by the reference manual sample preparation, the identification performance varies according to genera/species (9); none of the colonies in the study was incorrectly identified. Performance of the Copan Colibrí System for preparation of Gram-positive target organisms for the Bruker MALDI Biotyper CA is lower when compared to manual preparation. If a low-confidence identification or no identification result is obtained, the operator is instructed to repeat testing of the isolate manually using the extended Direct Transfer (eDT) or Extraction (Ext) Sample Preparation Procedure.

Table 1: Inclusivity Study, identification results obtained with bioMérieux VITEK® MS

Test strain	Total no. of picked colonies	Correct Single Choice (≥60% Confidence value)		Low discrimination (<60% Confidence value)		No ID		Wrong ID		% agreement* between automatic and expected ID	% agreement** between manual and expected ID
		Auto	Manual	Auto	Manual	Auto	Manual	Auto	Manual		
Gram Positive											
Enterococcus faecalis	12	11	12	0	0	1	0	0	0	91.7%	100.0%
Enterococcus faecium	12	11	11	0	0	1	1	0	0	91.7%	91.7%
Listeria monocytogenes	4	4	3	0	0	0	1	0	0	100.0%	75.0%
Staphylococcus aureus	12	12	12	0	0	0	0	0	0	100.0%	100.0%
Staphylococcus epidermidis	12	12	12	0	0	0	0	0	0	100.0%	100.0%
Staphylococcus saprophyticus	8	6	6	0	0	2	2	0	0	75.0%	75.0%
Streptococcus agalactiae	16	13	15	0	0	3	1	0	0	81.3%	93.8%
Streptococcus pyogenes	8	7	7	0	0	1	1	0	0	87.5%	87.5%
Total Gram-positive	**84**	**76**	**78**	**0**	**0**	**8**	**6**	**0**	**0**	**90.5%**	**92.9%**
Gram Negative											
Acinetobacter baumannii	24	24	20	0	0	0	4	0	0	100.0%	83.3%
Bacteroides fragilis	2	2	2	0	0	0	0	0	0	100.0%	100.0%
Citrobacter koseri	24	24	21	0	0	0	3	0	0	100.0%	87.5%
Eikenella corrodens	2	2	2	0	0	0	0	0	0	100.0%	100.0%
Enterobacter aerogenes/Klebsiella aerogenes	24	23	22	0	0	1	2	0	0	95.8%	91.7%
Enterobacter cloacae	24	0	0	23[a]	20[a]	1	4	0	0	95.8%[a]	83.3%[a]
Escherichia coli	24	24	23	0	0	0	1	0	0	100.0%	95.8%
Haemophilus influenzae	4	4	3	0	0	0	1	0	0	100.0%	75.0%
Klebsiella oxytoca	24	24	20	0	0	0	4	0	0	100.0%	83.3%
Klebsiella pneumoniae	24	23	21	0	0	1	3	0	0	95.8%	87.5%
Moraxella catarrhalis	4	4	4	0	0	0	0	0	0	100.0%	100.0%
Morganella morganii	16	16	15	0	0	0	1	0	0	100.0%	93.8%
Neisseria gonorrhoeae	4	4	4	0	0	0	0	0	0	100.0%	100.0%
Neisseria meningitidis	2	2	1	0	0	0	1	0	0	100.0%	50.0%
Proteus mirabilis	24	24	23	0	0	0	1	0	0	100.0%	95.8%
Proteus vulgaris	24	0	0	24[b]	23[b]	0	1	0	0	100.0%[b]	95.8%[b]

Test strain	Total no. of picked colonies	Correct Single Choice (≥60% Confidence value)		Low discrimination (<60% Confidence value)		No ID		Wrong ID		% agreement* between automatic and expected ID	% agreement** between manual and expected ID
		Auto	Manual	Auto	Manual	Auto	Manual	Auto	Manual		
Pseudomonas aeruginosa	24	24	24	0	0	0	0	0	0	100.0%	100.0%
Salmonella typhimurium	8	8	8	0	0	0	0	0	0	100.0%	100.0%
Serratia marcescens	16	16	16	0	0	0	0	0	0	100.0%	100.0%
Stenotrophomonas maltophilia	8	8	7	0	0	0	1	0	0	100.0%	87.5%
Vibrio parahaemolyticus	2	2	2	0	0	0	0	0	0	100.0%	100.0%
Total Gram-negative	**308**	**258**	**238**	**47**	**43**	**3**	**27**	**0**	**0**	**99.0%** [a, b]	**91.2%** [a, b]
Total	**392**	**334**	**316**	**47** [a, b]	**43** [a, b]	**11**	**33**	**0**	**0**	**97.2%** [a, b]	**91.6%** [a, b]

[a]According to VITEK® MS instrument, *Enterobacter cloacae* identifications are considered as a slashline result, *Enterobacter cloacae/ Enterobacter asburiae* (50%/50%). Therefore, the Low discrimination results for this strain are included in the Agreement calculation

[b] According to VITEK® MS instrument, *Proteus vulgaris* identifications are considered as a slashline result, *Proteus penneri/ Proteus vulgaris* (50%/50%). Therefore, the Low discrimination results for this strain are included in the Agreement calculation.

*Calculated as: $\text{Colibrí™ performance identification}(\%) = \frac{No.\ of\ \text{automatic}\ correct\ results\ with\ Good\ Confidence\ value\ (\geq 60\%)}{Total\ number\ of\ picked\ colonies} x100$

**Calculated as: $\text{Manual performance identification}(\%) = \frac{No.\ of\ manual\ correct\ results\ with\ Good\ Confidence\ value\ (\geq 60\%)}{Total\ number\ of\ picked\ colonies} x100$

Table 2: Inclusivity Study, identification results obtained with Bruker MALDI Biotyper® CA System on US IVD 48 Spot target

Test strain	Total no. of picked colonies	High confidence ID Log (Score) ≥2		Low confidence ID 1.7≤ Log (Score)<2		Combined performance		No ID		Wrong ID		% agreement* between automatic and expected ID	% agreement** between manual and expected ID
		Auto	Manual	Auto	Manual	Auto	Manual	Auto	Manual	Auto	Manual		
Gram Positive													
Enterococcus faecalis	24	19	24	4	0	23	24	1	0	0	0	79.2%	100.0%
Enterococcus faecium	24	21	24	3	0	24	24	0	0	0	0	87.5%	100.0%
Listeria monocytogenes	4	4	4	0	0	4	4	0	0	0	0	100.0%	100.0%
Staphylococcus aureus	24	21	24	3	0	24	24	0	0	0	0	87.5%	100.0%
Staphylococcus epidermidis	24	18	20	6	4	24	24	0	0	0	0	75.0%	83.3%
Staphylococcus saprophyticus	16	12	15	2	1	14	16	2	0	0	0	75.0%	93.8%
Streptococcus agalactiae	24	19	21	2	3	21	24	3	0	0	0	79.2%	87.5%
Streptococcus pyogenes	16	14	16	2	0	16	16	0	0	0	0	87.5%	100.0%
Total Gram-positive	**156**	**128**	**148**	**22**	**8**	**150**	**156**	**6**	**0**	**0**	**0**	**82.1%**	**94.9%**
Gram Negative													
Acinetobacter baumannii	24	23	22	1	2	24	24	0	0	0	0	95.8%	91.7%
Bacteroides fragilis	2	2	2	0	0	2	2	0	0	0	0	100.0%	100.0%
B. pertussis	2	2	2	0	0	2	2	0	0	0	0	100.0%	100.0%
Citrobacter koseri	24	24	24	0	0	24	24	0	0	0	0	100.0%	100.0%
Eikenella corrodens	4	4	4	0	0	4	4	0	0	0	0	100.0%	100.0%
Enterobacter aerogenes	24	24	24	0	0	24	24	0	0	0	0	100.0%	100.0%
Enterobacter cloacae	24	24	24	0	0	24	24	0	0	0	0	100.0%	100.0%
Escherichia coli	24	22	23	2	1	24	24	0	0	0	0	91.7%	95.8%
Haemophilus influenzae	4	4	4	0	0	4	4	0	0	0	0	100.0%	100.0%

Test strain	Total no. of picked colonies	High confidence ID Log (Score) ≥2		Low confidence ID 1.7≤ Log (Score)<2		Combined performance		No ID		Wrong ID		% agreement* between automatic and expected ID	% agreement** between manual and expected ID
		Auto	Manual	Auto	Manual	Auto	Manual	Auto	Manual	Auto	Manual		
Klebsiella oxytoca	24	24	24	0	0	24	24	0	0	0	0	100.0%	100.0%
Klebsiella pneumoniae	24	24	24	0	0	24	24	0	0	0	0	100.0%	100.0%
Moraxella catarrhalis	4	4	4	0	0	4	4	0	0	0	0	100.0%	100.0%
Morganella morganii	16	16	15	0	0	16	15	0	1	0	0	100.0%	93.8%
Neisseria gonorrhoeae	4	4	4	0	0	4	4	0	0	0	0	100.0%	100.0%
Neisseria meningitidis	2	2	2	0	0	2	2	0	0	0	0	100.0%	100.0%
Proteus mirabilis	24	24	24	0	0	24	24	0	0	0	0	100.0%	100.0%
Proteus vulgaris	24	23	23	1	1	24	24	0	0	0	0	95.8%	95.8%
Pseudomonas aeruginosa	24	24	24	0	0	24	24	0	0	0	0	100.0%	100.0%
Salmonella typhimurium and spp	8	8	8	0	0	8	8	0	0	0	0	100.0%	100.0%
Serratia marcescens	16	16	16	0	0	16	16	0	0	0	0	100.0%	100.0%
Stenotrophomonas maltophilia	8	8	8	0	0	8	8	0	0	0	0	100.0%	100.0%
Vibrio parahaemolyticus	2	2	2	0	0	2	2	0	0	0	0	100.0%	100.0%
Total Gram-negative	**312**	**308**	**307**	**4**	**4**	**312**	**311**	**0**	**1**	**0**	**0**	**98.7%**	**98.4%**
Total	**468**	**436**	**455**	**26**	**12**	**462**	**467**	**6**	**1**	**0**	**0**	**93.2%**	**97.2%**

*Calculated as: Colibrí™ performance identification$(\%) = \frac{No.\ of\ \text{automatic}\ correct\ results\ with\ High\ Confidence\ Log\ (Score)\geq 2}{Total\ number\ of\ picked\ colonies} x100$

**Calculated as: Manual performance identification$(\%) = \frac{No.\ of\ manual\ correct\ results\ with\ High\ Confidence\ Log\ (Score)\geq 2}{Total\ number\ of\ picked\ colonies} x100$

Table 3: Inclusivity Study, identification results obtained with Bruker MALDI Biotyper® CA System on MBT Biotarget 96 US IVD

Test strain	Total no. of picked colonies	High confidence ID Log(Score)≥2		Low confidence ID 1.7≤Log(Score)<2		Combined performance		No ID		Wrong ID		% agreement* between automatic and expected ID	% agreement** between manual and expected ID
		Auto	Manual	Auto	Manual	Auto	Manual	Auto	Manual	Auto	Manual		
Gram-positive													
Enterococcus faecalis	24	21	24	0	0	21	24	3	0	0	0	87.5%	100.0%
Enterococcus faecium	24	20	24	2	0	22	24	2	0	0	0	83.3%	100.0%
Listeria monocytogenes	4	4	4	0	0	4	4	0	0	0	0	100.0%	100.0%
Staphylococcus aureus	24	22	23	1	1	23	24	1	0	0	0	91.7%	95.8%
Staphylococcus epidermidis	24	9	10	11	11	20	21	4	3	0	0	37.5%	41.7%
Staphylococcus saprophyticus	16	9	13	2	2	11	15	5	1	0	0	56.3%	81.3%
Streptococcus agalactiae	24	7	9	10	9	17	18	7	6	0	0	29.2%	37.5%
Streptococcus pyogenes	16	13	16	3	0	16	16	0	0	0	0	81.3%	100.0%
Total Gram-positive	**156**	**105**	**123**	**29**	**23**	**134**	**146**	**22**	**10**	**0**	**0**	**67.3%**	**78.8%**
Gram-negative													
Acinetobacter baumannii	24	24	20	0	3	24	23	0	1	0	0	100.0%	83.3%
Bacteroides fragilis	2	2	2	0	0	2	2	0	0	0	0	100.0%	100.0%
B. pertussis	2	2	2	0	0	2	2	0	0	0	0	100.0%	100.0%
Citrobacter koseri	24	23	24	1	0	24	24	0	0	0	0	95.8%	100.0%
Eikenella corrodens	4	4	4	0	0	4	4	0	0	0	0	100.0%	100.0%
Enterobacter aerogenes	24	24	24	0	0	24	24	0	0	0	0	100.0%	100.0%
Enterobacter cloacae	24	21	22	2	2	23	24	1	0	0	0	87.5%	91.7%
Escherichia coli	24	20	18	4	5	24	23	0	1	0	0	83.3%	75.0%
Haemophilus influenzae	4	4	4	0	0	4	4	0	0	0	0	100.0%	100.0%
Klebsiella oxytoca	24	19	21	2	0	21	21	3	3	0	0	79.2%	87.5%
Klebsiella pneumoniae	24	22	20	1	1	23	21	1	3	0	0	91.7%	83.3%
Moraxella catarrhalis	4	4	4	0	0	4	4	0	0	0	0	100.0%	100.0%
Morganella morganii	16	16	16	0	0	16	16	0	0	0	0	100.0%	100.0%
Neisseria gonorrhoeae	4	4	4	0	0	4	4	0	0	0	0	100.0%	100.0%
Neisseria meningitidis	2	2	2	0	0	2	2	0	0	0	0	100.0%	100.0%
Proteus mirabilis	24	23	24	0	0	23	24	1	0	0	0	95.8%	100.0%

Test strain	Total no. of picked colonies	High confidence ID Log(Score)≥2		Low confidence ID 1.7≤Log(Score)<2		Combined performance		No ID		Wrong ID		% agreement* between automatic and expected ID	% agreement** between manual and expected ID
		Auto	Manual	Auto	Manual	Auto	Manual	Auto	Manual	Auto	Manual		
Proteus vulgaris	24	24	24	0	0	24	24	0	0	0	0	100.0%	100.0%
Pseudomonas aeruginosa	24	24	22	0	0	24	22	0	2	0	0	100.0%	91.7%
Salmonella typhimurium and spp	8	8	7	0	0	8	7	0	1	0	0	100.0%	87.5%
Serratia marcescens	16	16	15	0	1	16	16	0	0	0	0	100.0%	93.8%
Stenotrophomonas maltophilia	8	8	8	0	0	8	8	0	0	0	0	100.0%	100.0%
Vibrio parahaemolyticus	2	2	2	0	0	2	2	0	0	0	0	100.0%	100.0%
Total Gram-negative	**312**	**296**	**289**	**10**	**12**	**306**	**301**	**6**	**11**	**0**	**0**	**94.9%**	**92.6%**
Total	**468**	**401**	**412**	**39**	**35**	**440**	**447**	**28**	**21**	**0**	**0**	**85.7%**	**88.0%**

*Calculated as: Colibrí™ performance identification$(\%) = \frac{No.\ of\ \text{automatic}\ correct\ results\ with\ High\ Confidence\ Log\ (Score)\geq 2}{Total\ number\ of\ picked\ colonies} x100$

**Calculated as: Manual performance identification$(\%) = \frac{No.\ of\ manual\ correct\ results\ with\ High\ Confidence\ Log\ (Score)\geq 2}{Total\ number\ of\ picked\ colonies} x100$

1.13 SPECIFICITY

A variety of bacteria not included in the knowledge databases of the bioMérieux VITEK® MS or Bruker MALDI Biotyper® CA System (off-panel strains) were included in this study. Strains were selected to include representative isolates of different genera and organism groups exhibiting a broad range of colony characteristics (size, morphology and viscoelastic properties). The identification results obtained by bioMérieux VITEK® MS and Bruker MALDI Biotyper® CA System using the Copan Colibrí™ System for sample preparation were compared to the expected organism identities. Fresh cultures of selected strains were grown on the appropriate culture media plates selected among those recommended by the MALDI-TOF MS analyzers providing the results indicated in Tables 4-5 stratified per species. The study conducted in conjunction with Bruker MALDI Biotyper® CA System was performed using the US IVD 48 Spot target (48 positions reusable steel target).

With both MALDI-TOF MS analyzers each of the "off-panel" species produced the expected no identification results and in no case was a wrong identification obtained.

Table 4: Specificity Study, identification results obtained with bioMérieux VITEK® MS

Test strain	Total no. of picked colonies	Correct Single Choice (≥60% Confidence value)	Low discrimination (<60% Confidence value)	No ID	Wrong ID	% agreement* between automatic and expected ID
Gram-positive						
Aneuribacillus migulanus	2	0	0	2	0	100.0%
Exiguobacterium aurantiacum	2	0	0	2	0	100.0%
Janibacter melonis	2	0	0	2	0	100.0%
Leuconostoc carnosum	2	0	0	2	0	100.0%
Leuconostoc fallax	2	0	0	2	0	100.0%
Rothia amarae	2	0	0	2	0	100.0%
Total Gram-positive	**12**	**0**	**0**	**12**	**0**	**100.0%**
Gram-negative						
Acidovorax delafieldii	2	0	0	2	0	100.0%
Burkholderia thailendensis	2	0	0	2	0	100.0%
Pectobacterium atrosepticum	2	0	0	2	0	100.0%
Pseudocitrobacter faecalis	2	0	0	2	0	100.0%
Total Gram-negative	**8**	**0**	**0**	**8**	**0**	**100.0%**
Total	**20**	**0**	**0**	**20**	**0**	**100.0%**

*Calculated as: $\frac{No.\ of\ \text{automatic}\ No\ Identification\ results}{Total\ number\ of\ picked\ colonies} x100$

Table 5: Specificity Study, identification results obtained with Bruker MALDI Biotyper® CA System

Test strain	Total no. of picked colonies	High confidence ID Log (Score) ≥2	Low confidence ID 1.7≤ Log (Score) <2	Combined performance	No ID	Wrong ID	% agreement* between automatic and expected ID
Gram-positive							
Paenibacillus humin-icus	2	0	0	0	2	0	100.0%
Bacillus licheniformis	2	0	0	0	2	0	100.0%
Bacillus flexus	2	0	0	0	2	0	100.0%
Bacillus infantis	2	0	0	0	2	0	100.0%
Geobacillus stea-rothermophilus	2	0	0	0	2	0	100.0%
Total Gram-positive	**10**	**0**	**0**	**0**	**10**	**0**	**100.0%**
Gram-negative							
Cardiobacterium hominis	2	0	0	0	2	0	100.0%
Cedecea neteri	2	0	0	0	2	0	100.0%
Brachyspira mur-dochii	2	0	0	0	2	0	100.0%
Gallibacterium anatis	2	0	0	0	2	0	100.0%
Novosphingobium capsulatum	2	0	0	0	2	0	100.0%
Total Gram-nega-tive	**10**	**0**	**0**	**0**	**10**	**0**	**100.0%**
Total	**20**	**0**	**0**	**0**	**20**	**0**	**100.0%**

*Calculated as: $\frac{No.\ of\ \text{automatic}\ No\ Identification\ results}{Total\ number\ of\ picked\ colonies} x100$

1.14 COLONY PICKING STUDY

The Colony Picking study was performed to evaluate the accuracy of the Copan Colibrí™ System in picking the designated colonies of various microbial species from different culture media.

For this study, different media plates (whole and bi-plates) showing polymicrobial growth of bacteria included in the knowledge databases of the bioMérieux VITEK® MS or Bruker MALDI Biotyper® CA System (on-panel strains) were used to challenge the accuracy of the Copan Colibrí™ in picking pre-selected colonies. The results are shown in Tables 6-7, stratified per species in the proper Gram classification. The study was conducted on three Colibrí Systems and for the Bruker MALDI Biotyper® CA System was performed using the US IVD 48 Spot target (48-position reusable steel target). After processing by the Copan Colibrí, culture plates were visually inspected to determine whether the designated colonies had been picked. Results show that in all cases with whole and bi-plates, the correct colony was picked (100% pick accuracy) with both plate configurations and no incorrect identification results were obtained. For the Bruker MALDI Biotyper® CA System, a lower proportion of concordant results for Gram-positive species was observed: nevertheless, the overall performance is considered acceptable because no incorrect identification occurred. If a low-confidence identification or a no identification result is obtained, the operator is instructed to repeat testing of the isolate manually using the extended Direct Transfer (eDT) or Extraction (Ext) Sample Preparation Procedure.

Table 6: Colony Picking Study, identification results obtained with bioMérieux VITEK® MS

Test strain	Total no. of picked colonies	No. of colonies correctly picked	Correct Single Choice (≥60% Confidence value)	Low discrimination (<60% Confidence value)	No ID	Wrong ID	Picking accuracy *	% agreement** between automatic and expected ID
Gram-positive								
Enterococcus faecalis	168	168	152	1	15	0	100%	90.5%
Staphylococcus aureus	292	292	292	0	0	0	100%	100.0%
Streptococcus agalactiae	162	162	156	0	6	0	100%	96.3%
Total Gram-positive	**622**	**622**	**600**	**1**	**21**	**0**	**100%**	**96.5%**
Gram-negative								
Escherichia coli	300	300	300	0	0	0	100%	100.0%
Klebsiella pneumoniae	310	310	310	0	0	0	100%	100.0%
Proteus mirabilis	158	158	158	0	0	0	100%	100.0%
Total Gram-negative	**768**	**768**	**768**	**0**	**0**	**0**	**100%**	**100.0%**
Total	**1390**	**1390**	**1368**	**1**	**21**	**0**	**100%**	**98.4%**

* Calculated as $\frac{No.\ of\ designated\ colonies\ correctly\ picked}{Total\ number\ of\ designated\ colonies} x100$

**Calculated as $\frac{No.\ of\ automatic\ correct\ results\ with\ Good\ Confidence\ value\ (\geq 60\%)}{Total\ number\ of\ picked\ colonies} x100$

Table 7: Colony Picking Study, identification results obtained with Bruker MALDI Biotyper® CA System

Test strain	Total no. of picked colonies	High confidence ID Log (Score) ≥ 2	Low confidence ID 1.7≤ Log (Score) < 2	Combined performance	No ID	Wrong ID	% agreement* between automatic and expected ID
Gram-positive							
Enterococcus faecalis	150	122	22	144	6	0	81.3%
Staphylococcus aureus	364	316	48	364	0	0	86.8%
Staphylococcus epidermidis	148	118	30	148	0	0	79.7%
Streptococcus agalactiae	150	105	26	131	19	0	70.0%
Total Gram-positive	**812**	**661**	**126**	**787**	**25**	**0**	**81.4%**
Gram-negative							
Escherichia coli	380	375	5	380	0	0	98.7%
Klebsiella pneumoniae	330	330	0	330	0	0	100.0%
Proteus mirabilis	168	168	0	168	0	0	100.0%
Total Gram-negative	**878**	**873**	**5**	**878**	**0**	**0**	**99.4%**
Total	**1690**	**1534**	**131**	**1665**	**25**	**0**	**90.8%**

*Calculated as $\frac{No.\ of\ \text{automatic}\ correct\ results\ with\ High\ Confidence\ Log\ (Score)\geq 2}{Total\ number\ of\ picked\ colonies} x100$

1.15 POSITIONAL EFFECT STUDY

The Positional Effect Study was performed to demonstrate that there is no positional effect associated with the location of the target spots of the MALDI-TOF MS target slides when prepared by Colibrí™ System. For this test, media plates showing growth of bacteria included in the knowledge databases of the bioMérieux VITEK® MS and Bruker MALDI Biotyper® CA System (on-panel strains) were used to challenge the accuracy of Copan Colibrí™ in spotting the picked colonies in all the target slide positions. The study conducted in conjunction with Bruker MALDI Biotyper® CA System was performed using both the US IVD 48 Spot target (48-position reusable steel target) and the MBT Biotarget 96 US IVD (96-position disposable target) that have different geometry and spot diameters. The results of the study are provided in Table 8-10. No positional effect was detected, and no wrong identification results were obtained with either mass spectrometry analyzer.

Table 8: Positional Effect Study, identification results obtained with bioMérieux VITEK® MS

Test Strain	No. of spots	Correct Single Choice Confidence value ≥ 60%	Low Discrimination Confidence value < 60%	No ID	Wrong ID	% agreement* between automatic and expected ID *
Escherichia coli	432	432	0	0	0	100%
Staphylococcus aureus	432	431	0	1	0	99.8%

*Calculated as $\frac{No.\ of\ automatic\ correct\ results\ with\ Good\ Confidence\ value\ (\geq 60\%)}{Total\ number\ of\ picked\ colonies} x100$

Table 9: Positional Effect Study, identification results obtained with MALDI Biotyper® CA System with US IVD 48 Spot target

Test Strain	No. of spots	Correct Identification with high confidence (Log score value 2.00-3.00)	Low Confidence Identification (Log score value 1.70- 1.99)	Combined performance	No ID	Wrong ID	% agreement* between automatic and expected ID *
Escherichia coli	432	431	1	432	0	0	99.8%
Staphylococcus aureus	432	418	14	432	0	0	96.8%

*Calculated as $\frac{No.\ of\ automatic\ correct\ results\ with\ High\ Confidence\ Log\ (Score)\geq 2}{Total\ number\ of\ picked\ colonies} x100$

Table 10. Positional Effect Study, identification results obtained with MALDI Biotyper® CA System with MBT Biotarget 96 US IVD

Test Strain	No. of spots	Correct Identification with high confidence (Log score value 2.00-3.00)	Low Confidence Identification (Log score value 1.70- 1.99)	Combined performance	No ID	Wrong ID	% agreement* between automatic and expected ID *
Escherichia coli	846	845	1	846	0	0	99.9%
Staphylococcus aureus	846	810	34	844	2	0	95.7%

*Calculated as $\frac{No.\ of\ automatic\ correct\ results\ with\ High\ Confidence\ Log\ (Score)\geq 2}{Total\ number\ of\ picked\ colonies} x100$

1.16 REPRODUCIBILITY

A Reproducibility Study was performed to demonstrate the repeatability of MALDI-TOF MS identification results obtained with samples processed by the Colibrí™ System. Culture media showing isolated colonies of 10 “on-panel” Gram-positive and Gram-negative strains (Test Strains) were processed on 3 Colibrí™ instruments over 5 days involving 2 operators. Each strain was tested in triplicate using the Duplicate Deposition Mode (2 colonies per isolate, 1 colony per spot). The prepared target slides were then analyzed with the bioMérieux VITEK® MS and Bruker MALDI Biotyper® CA System providing the results indicated in Tables 11-12 stratified per species. The study conducted in conjunction with Bruker MALDI Biotyper® CA System was performed using the US IVD 48 Spot target (48-position reusable steel target).

When the Copan Colibrí System was used in conjunction with the VITEK MS, there was 99.9% agreement (1799/1800) between the reported Good Confidence identification results and the expected identity of each colony in the Reproducibility Study. In the agreement calculation were included also 180 colonies of *Enterobacter cloacae* reported with Low Discrimination as *Enterobacter cloacae/Enterobacter asburiae* but in accordance with the labeling for the VITEK MS analyzer.

When Copan Colibrí System was used in conjunction with the Bruker MALDI Biotyper CA, there was 88.1% agreement (1585/1800) between the reported High Confidence identification results (Log (Score) ≥2.00) and the expected identity of each colony in the Reproducibility Study. For Gram-positive species, 179/900 colonies (19.9%) were identified with Low Confidence (Log (Score) 1.70-1.99), compared with 1/900 colonies (0.1%) of Gram-negative species. In addition, 31/900 Gram-positive colonies (3.4%) produced no identification result compared with 4/900 Gram-negative colonies (0.4%).

With both mass spectrometry systems, similar agreement was observed between Copan Colibrí Systems, across days or operators and none of the colonies in the study was incorrectly identified. The lower proportion of concordant results for Gram-positive bacteria with the Bruker MALDI Biotyper CA was noted. Consistent with the labeling for the MALDI Biotyper CA, it is recommended that Gram-positive species or any samples that produce a Low Confidence Identification or No Identification Result should be manually prepared using the Bruker’s extended Direct Transfer Procedure (eDT), Extraction (Ext) Procedure and/or an alternative method of organism identification.

Table 11: Reproducibility Study, identification results obtained with bioMérieux VITEK® MS

Test strain	Total no. of picked colonies	Correct Single Choice (≥60% Confidence value)	Low discrimination (<60% Confidence value)	No ID	Wrong ID	% agreement* between automatic and expected ID
Gram-positive						
Enterococcus faecalis	180	180	0	0	0	100.0%
Staphylococcus aureus	180	180	0	0	0	100.0%
Staphylococcus epidermidis	180	180	0	0	0	100.0%
Staphylococcus saprophyticus	180	180	0	0	0	100.0%
Streptococcus agalactiae	180	179	0	1	0	99.4%
Total Gram-positive	**900**	**899**	**0**	**1**	**0**	**99.9%**
Gram-negative						
Enterobacter cloacae	180	0	180[a]	0	0	100.0%
Escherichia coli	180	180	0	0	0	100.0%
Klebsiella pneumoniae	180	180	0	0	0	100.0%
Proteus mirabilis	180	180	0	0	0	100.0%
Pseudomonas aeruginosa	180	180	0	0	0	100.0%
Total Gram-negative	**900**	**720**	**180 [a]**	**0**	**0**	**100.0%**
Total	**1800**	**1619**	**180 [a]**	**1**	**0**	**100.0% [a]**

[a]According to VITEK® MS instrument, *Enterobacter cloacae* identifications are considered as a slashline result, *Enterobacter cloacae/ Enterobacter asburiae* (50%/50%). Therefore, the Low discrimination results for this strain are included in the Agreement calculation

*Calculated as $\frac{No.\ of\ \text{automatic}\ correct\ results\ with\ Good\ Confidence\ value\ (\geq 60\%)}{Total\ number\ of\ picked\ colonies} x100$

Table 12: Reproducibility Study, identification results obtained with Bruker MALDI Biotyper® CA System

Test strain	Total no. of picked colonies	High confidence ID Log (Score)≥2	Low confidence ID1.7≤ Log (Score)<2	Combined performance	No ID	Wrong ID	% agreement* between automatic and expected ID
Gram-positive							
Enterococcus faecalis	180	139	40	179	1	0	77.2%
Staphylococcus aureus	180	159	21	180	0	0	88.3%
Staphylococcus epidermidis	180	129	42	171	9	0	71.7%
Staphylococcus saprophyticus	180	143	26	169	11	0	79.4%
Streptococcus agalactiae	180	120	50	170	10	0	66.7%
Total Gram-positive	**900**	**690**	**179**	**869**	**31**	**0**	**76.7%**
Gram-negative							
Enterobacter cloacae	180	180	0	180	0	0	100.0%
Escherichia coli	180	178	1	179	1	0	98.9%
Klebsiella pneumoniae	180	180	0	180	0	0	100.0%
Proteus mirabilis	180	180	0	180	0	0	100.0%
Pseudomonas aeruginosa	180	177	0	177	3	0	98.3%
Total Gram-negative	**900**	**895**	**1**	**896**	**4**	**0**	**99.4%**
Total	**1800**	**1585**	**180**	**1765**	**35**	**0**	**88.1%**

*Calculated as $\frac{No.\ of\ automatic\ correct\ results\ with\ High\ Confidence\ Log(Score)\geq 2}{Total\ number\ of\ picked\ colonies} x100$

1.17 CROSS-CONTAMINATION

The cross-contamination study was performed to demonstrate that the use of the Colibrí™ System does not cause false-positive results due to contamination of adjacent spots on the target slide. Alternating culture plates of "on-" and "off-panel"bacteria were used to prepare target slides on the Colibrí System. The results from analysis of the slides are presented in Tables 13-18 stratified per species. The study conducted in conjunction with the Bruker MALDI Biotyper® CA System was performed on both validated targets, the MBT Biotarget 96 US IVD (96-position disposable targets) and US IVD 48 Spot target (48-position reusable steel target).

There was no evidence of cross-contamination with slides prepared for either mass spectrometry system or no incorrect identification results were obtained. However, with the *Enterococcus faecalis* and *Streptococcus agalactiae* on target slides for the Bruker MALDI Biotyper® CA, fewer High Confidence Log (Score) values were observed than for other bacterial species, leading to lower positive percent agreement.

Table 13. Cross-Contamination: identification results obtained with bioMérieux VITEK® MS (on-panel species)

Test strain	Total no. of spots	Correct Single Choice (≥60% Confidence value)	Low discrimination (<60% Confidence value)	No ID	Wrong ID	% colonies providing expected result*
Gram-positive						
Enterococcus faecalis	46	46	0	0	0	
Staphylococcus aureus	48	48	0	0	0	
Streptococcus agalactiae	48	46	0	2	0	
Total Gram positive	**142**	**140**	**0**	**2**	**0**	**98.6%**
Gram-negative						
Escherichia coli	48	48	0	0	0	
Klebsiella pneumoniae	48	48	0	0	0	
Pseudomonas aeruginosa	48	48	0	0	0	
Total Gram negative	**144**	**144**	**0**	**0**	**0**	**100%**
Total	**286**	**284**	**0**	**2**	**0**	**99.3%**

*Calculated as $\frac{No.\ of\ automatic\ correct\ results\ with\ Goog\ Confidence\ value\ (\geq 60\%)}{Total\ number\ of\ picked\ colonies} x100$

Table 14. Cross-Contamination: identification results obtained with bioMérieux VITEK® MS (off-panel species)

Test strain	Total no. of spots	Correct Single Choice (≥60% Confidence value)	Low discrimination (<60% Confidence value)	No ID	Wrong ID	% colonies providing expected result*
Aneuribacillus migulanus	48	0	0	48	0	
Leuconostoc carnosum	48	0	0	48	0	
Rothia amarae	46	0	0	46	0	
Acidovorax delafieldii	48	0	0	48	0	
Burkholderia thailandensis	48	0	0	48	0	
Pseudocitrobacter faecalis	48	0	0	48	0	
Total	**286**	**0**	**0**	**286**	**0**	**100%**

*Calculated as $\frac{No.\ of No\ Identification\ results}{Total\ number\ of\ picked\ colonies} x100$

Table 15. Cross-Contamination: identification results obtained with Bruker MALDI Biotyper® CA on US IVD 48 Spot target (on-panel species)

Test strain	Total no. of spots	High confidence ID Log (Score)≥2	Low confidence ID 1.7≤ Log (Score)<2	Combined performance	No ID	Wrong ID	% colonies providing expected result*
Gram-positive							
Enterococcus faecalis	24	19	5	24	0	0	
Staphylococcus aureus	48	48	0	48	0	0	
Streptococcus agalactiae	24	20	4	24	0	0	
Total Gram-positive	**96**	**87**	**9**	**96**	**0**	**0**	**90.6%**
Gram-negative							
Acinetobacter baumannii	24	23	1	24	0	0	
Escherichia coli	56	56	0	56	0	0	
Klebsiella pneumoniae	24	24	0	24	0	0	
Total Gram-negative	**104**	**103**	**1**	**104**	**0**	**0**	**99.0%**
Total	**200**	**190**	**10**	**200**	**0**	**0**	**95.0%**

*Calculated as $\frac{No.\ of\ automatic\ correct\ results\ with\ High\ Confidence\ Log(Score)\geq 2}{Total\ number\ of\ picked\ colonies} x100$

Table 16. Cross-Contamination: identification results obtained with Bruker MALDI Biotyper® CA on US IVD 48 Spot target (off-panel species)

Test strain	Total no. of spots	High confidence ID Log (Score)≥2	Low confidence ID 1.7≤ Log (Score)<2	Combined performance	No ID	Wrong ID	% colonies providing expected result*
Bacillus flexus	48	0	0	0	48	0	
Bacillus infantis	24	0	0	0	24	0	
Bacillus licheniformis	24	0	0	0	24	0	
Cedecea neteri	56	0	0	0	56	0	
Gallibacterium anatis	24	0	0	0	24	0	
Novosphingobium capsulatum	24	0	0	0	24	0	
Total	**200**	**0**	**0**	**0**	**200**	**0**	**100%**

*Calculated as $\frac{No.\ of\ No\ Identification\ results}{Total\ number\ of\ picked\ colonies} x100$

Table 17. Cross-Contamination: identification results obtained with MALDI Biotyper® CA on MBT Biotarget 96 US IVD (on-panel species)

Test strain	Total no. of spots	High confidence ID Log (Score)≥2	Low confidence ID 1.7≤ Log (Score)<2	Combined performance	No ID	Wrong ID	% colonies providing expected result*
Gram-positive							
Enterococcus faecalis	24	20	1	21	3	0	
Staphylococcus aureus	24	22	2	24	0	0	
Streptococcus agalactiae	23	10	8	18	5	0	
Total Gram-positive	**71**	**52**	**11**	**63**	**8**	**0**	**73.2%**
Gram-negative							
Acinetobacter baumannii	24	23	0	23	1	0	
Escherichia coli	24	24	0	24	0	0	
Klebsiella pneumoniae	24	23	0	23	1	0	
Total Gram-negative	**72**	**70**	**0**	**70**	**2**	**0**	**97.2%**
Total	**143**	**122**	**11**	**133**	**10**	**0**	**85.3%**

*Calculated as $\frac{No.\ of\ automatic\ correct\ results\ with\ High\ Confidence\ Log(Score)\geq 2}{Total\ number\ of\ picked\ colonies} x100$

Table 18. Cross-Contamination: identification results obtained with Bruker MALDI Biotyper® CA on MBT Biotarget 96 US IVD (off-panel species)

Test strain	Total no. of spots	High confidence ID Log (Score)≥2	Low confidence ID 1.7≤ Log (Score)<2	Combined performance	No ID	Wrong ID	% colonies providing expected result*
Bacillus flexus	24	0	0	0	24	0	
Bacillus infantis	24	0	0	0	24	0	
Bacillus licheniformis	23	0	0	0	23	0	
Cedecea neteri	24	0	0	0	24	0	
Gallibacterium anatis	24	0	0	0	24	0	
Novosphingobium capsulatum	24	0	0	0	24	0	
Total	**143**	**0**	**0**	**0**	**143**	**0**	**100%**

*Calculated as $\frac{No.\ of\ No\ Identification\ results}{Total\ number\ of\ picked\ colonies} x100$

1.18 COLONY STABILITY

Colony Stability study provided information about the ability of the Colibrí System to prepare slides from cultures of different ages within the parameters specified by the MALDI-TOF MS device manufacturers. All the culture media, selected among those suggested by the MALDI-TOF MS device manufacturers with which the Colibrí System is compatible, were included in the study. Target/slide preparation by the Colibrí System was performed with clinically relevant, representative organisms using culture plates incubated over the minimum and at maximum periods indicated by the respective MALDI-TOF MS analyzer (Table 19, 21). The study conducted in conjunction with the Bruker MALDI Biotyper® CA System was also performed using culture plates that were incubated an additional 12 hours at room temperature as indicated by the Bruker MALDI Biotyper® CA System instructions for use. For each culture medium, 48 spots were prepared at each time point and results are shown in Table 20-22.

For the bioMérieux VITEK MS, a total of 576 spots were prepared, and 99.8% of samples produced the expected identification at each time point for all agar media plates under evaluation. No false identification result was provided.

With the Bruker MALDI Biotyper CA System, there was generally good agreement with the expected results for Gram-negative species (i.e., the expected organism identity was reported with a High Confidence Log(Score) value), whereas lower agreement was observed with Gram-positive species, irrespective of the culture medium or duration of incubation. Nevertheless, no incorrect identification results were reported for any of the isolates included in the study and therefore colony age was not shown to affect the accuracy organism identification. For *Bordetella pertussis* on Bordet Gengou Agar, holding

cultures at ambient temperature for 12 h after 7 days incubation at 35 ± 2°C resulted in a decrease in the proportion of High Confidence Log(scores) obtained.

Table 19. List of The Representative Organisms Evaluated on Each Culture Media for Colony Stability Study for VITEK® MS

Columbia Agar + 5% sheep blood (COS)	MacConkey (MAC)	Trypticase Soy Agar + 5% sheep blood (BAP)	Chocolate Agar (CHOCO)
Enterococcus faecalis	*Escherichia coli*	*Enterococcus faecalis*	*Haemophilus influenzae*
Enterococcus faecium	*Klebsiella pneumoniae*	*Enterococcus faecium*	*Neisseria gonorrhoeae*
Listeria monocytogenes	*Proteus mirabilis*	*Listeria monocytogenes*	*Neisseria meningitidis*
Staphylococcus aureus	*Salmonella typhimurium*	*Staphylococcus aureus*	
Staphylococcus epidermidis		*Staphylococcus epidermidis*	
Staphylococcus saprophyticus		*Staphylococcus saprophyticus*	
Streptococcus agalactiae		*Streptococcus agalactiae*	
Streptococcus pyogenes		*Streptococcus pyogenes*	
Escherichia coli		*Escherichia coli*	
Klebsiella pneumoniae		*Klebsiella pneumoniae*	
Proteus mirabilis		*Proteus mirabilis*	
Pseudomonas aeruginosa		*Pseudomonas aeruginosa*	

Table 20. Summary of Colony Stability Study: identification results obtained with VITEK® MS

Culture Medium	N° spot per culture medium	Culture Medium incubation time	ID % Agreement at each Culture Medium incubation time*
Columbia Agar + 5% sheep blood	192	18 h	100%
		24h	100%
		48 h	100%
		72 h	100%
MacConkey	144	18 h	100%
		24 h	100%
		72 h	100%
Trypticase Soy Agar + 5% sheep blood	144	18 h	100%
		24 h	100%
		72 h	97.9%
Chocolate Agar	96	18 h	100%
		48 h	100%

*Calculated as $\frac{No.\ of\ automatic\ correct\ results\ with\ Good\ Confidence\ value\ (\geq 60\%)}{Total\ number\ of\ picked\ colonies} x100$

Table 21. List of The Representative Organisms Evaluated on Each Culture Media for Colony Stability Study for Bruker MALDI Biotyper® CA System

Columbia Agar + 5% sheep blood (COS)	**MacConkey (MAC)**	**Trypticase Soy Agar + 5% sheep blood (BAP)**	**Chocolate Agar (CHOCO)**	**Columbia Agar + 5% sheep blood supplemented of colistin and nalidixic acid (CNA)**	**Bordet Gengou + 15% sheep blood**
Enterococcus faecalis	*Escherichia coli*	*Enterococcus faecalis*	*Haemophilus influenzae*	*Enterococcus faecalis*	*Bordetella pertussis*
Enterococcus faecium	*Klebsiella pneumoniae*	*Enterococcus faecium*	*Neisseria gonorrhoeae*	*Enterococcus faecium*	
Listeria monocytogenes	*Proteus mirabilis*	*Listeria monocytogenes*	*Neisseria meningitidis*	*Staphylococcus aureus*	
Staphylococcus aureus	*Salmonella typhimurium*	*Staphylococcus aureus*		*Staphylococcus saprophyticus*	
Staphylococcus epidermidis		*Staphylococcus epidermidis*		*Staphylococcus epidermidis*	
Staphylococcus saprophyticus		*Staphylococcus saprophyticus*		*Streptococcus pyogenes*	
Streptococcus agalactiae		*Streptococcus agalactiae*			
Streptococcus pyogenes		*Streptococcus pyogenes*			
Escherichia coli		*Escherichia coli*			
Klebsiella pneumoniae		*Klebsiella pneumoniae*			
Proteus mirabilis		*Proteus mirabilis*			
Pseudomonas aeruginosa		*Pseudomonas aeruginosa*			

Table 22. Summary of Colony Stability Study: identification results obtained with Bruker MALDI Biotyper® CA System

Culture Medium	No. spot per culture medium	Culture Medium incubation time	ID % Agreement at different incubation times*	ID % Agreement at Culture Medium different incubation time + 12h post-incubation at RT*
Columbia Agar + 5% sheep blood	288	18 h	93.8%	95.8%
		24 h	91.7%	93.8%
		48 h	87.5%	89.6%
MacConkey	288	18 h	97.9%	100%
		24 h	100%	95.8%
		48 h	100%	100%
Trypticase Soy Agar + 5% sheep blood	288	18 h	79.2%	79.2%
		24 h	83.3%	83.3%
		48 h	87.5%	91.7%
Chocolate Agar	192	18 h	100%	100%
		48 h	100%	93.8%
Columbia Agar + 5% sheep blood supplemented of colistin and nalidixic acid	192	18 h	87.5%	91.7%
		48 h	87.5%	85.4%
Bordet Gengou + 15% sheep blood	192	5 days	100%	100%
		7 days	97.9%	68.7%

*Calculated as $\frac{No.\ of\ automatic\ correct\ results\ with\ High\ Confidence\ Log(Score)\geq 2}{Total\ number\ of\ picked\ colonies} x100$

1.19 SPOT STABILITY PRIOR TO AND AFTER MATRIX DEPOSITION

The spot stability prior to and after matrix application was evaluated to provide evidences of the stability of spots before matrix application and the stability of target/slides before MALDI-TOF MS analysis. Spot stability was evaluated comparing the identification performance between the standard matrix deposition mode (application of matrix immediately after the colony spotting) and the delayed deposition mode (matrix application after 60 minutes after the colony spotting). Target stability was investigated for target prepared with both deposition modes and stored on the lab bench and the Colibrí deck for the maximum incubation time indicated by the respective MALDI-TOF MS analyzer before analysis. For each condition, a complete target was spotted randomly alternating Gram-positive (*Staphylococcus aureus* and *Enterococcus faecium*) and Gram-negative (*Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Escherichia coli*) colonies grown on Trypticase Soy Agar + 5% sheep blood.

For Bruker MALDI Biotyper® CA System the evaluation was performed for both validated targets, MBT Biotarget 96 US IVD (96-position disposable target) and US IVD 48 Spot target (48-position reusable steel target).

The data collected indicate that it is possible to store VITEK® MS targets up to 48h at room temperature when held on the Colibrí™ deck and up to 72 h when held in the original box, since the identification performance is not different to the performance in standard conditions. In case of MALDI Biotyper® CA System, colonies spotted by Colibrí™ system on MBT Biotarget 96 US IVD and US IVD 48 Spot targets are stable up to 60 minutes without matrix and for 24h at room temperature after matrix addition when held both on the Colibrí™ deck and on the Lab bench. Lower agreement with the expected results was observed with Gram-positive species using the 96-spot disposable target format

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