# 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
# DECISION SUMMARY
INSTRUMENT ONLY

## I Background Information:

### A 510(k) Number

K220546

### B Applicant

Copan WASP S.r.l.

### C Proprietary and Established Names

Colibrí System

### D Regulatory Information

| Product Code(s) | Classification | Regulation Section | Panel |
|---|---|---|---|
| LON | Class II | 21 CFR 866.1645 - Fully Automated Short-Term Incubation Cycle Antimicrobial Susceptibility System | MI - Microbiology |
| QQV | Class II | 21 CFR 866.3378 - Clinical mass spectrometry microorganism identification and differentiation system | MI - Microbiology |
| QBN | Class II | 21 CFR 866.3378 - Clinical mass spectrometry microorganism identification and differentiation system | MI - Microbiology |

## II Submission/Device Overview:

### A Purpose for Submission:

To obtain a substantial equivalence determination for the Copan Colibrí System for use with the VITEK 2 Antimicrobial Susceptibility Testing System.

**B Type of Test:**

Qualitative *in vitro* diagnostic device for identification of bacteria cultured from human specimens by automation of target preparation for mass spectrometry analysis and antimicrobial susceptibility test (AST) assessment of bacteria cultured from human specimens by automation of culture suspensions for AST analysis.

## III Intended Use/Indications for Use:

**A Intended Use(s):**
See Indications for Use below.

**B Indication(s) for Use:**
The 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 and with the bioMérieux VITEK 2 Antimicrobial Susceptibility Testing (AST) system for qualitative testing 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 for bacterial identification and microbial suspension at known concentration for antimicrobial susceptibility testing and purity assessment.

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. Bacterial suspensions for AST and purity plates are identified by barcode label.

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 the identification or processing of yeast species, molds, Nocardia, or mycobacteria.

**C Special Conditions for Use Statement(s):**

- Rx - For Prescription Use Only
- IVD - For In Vitro Diagnostic Use Only
- Special Instruments for Use:
  Bruker MALDI Biotyper for Clinical Applications (MBT-CA)
  bioMérieux VITEK MS
  bioMérieux VITEK 2

[Refer to the K193138 Decision Summary for Special Conditions for Use Statements that are only applicable to the sample preparation for bacterial identification and MALDI-TOF MS workflow.]

- Use of this device is permitted only in association with bioMérieux VITEK MS or Bruker MALDI Biotyper CA System for microbial identification and in association with bioMérieux VITEK 2 for AST.
- This product is intended for target slides preparation for identification and suspensions preparation for susceptibility testing from colonies grown on solid agar media plates. Do not use for identification from liquid cultures.
- For AST application, make sure that the selected colonies are morphologically similar.
- Results obtained using the Copan Colibrí System for sample preparation with the compatible analyzers should be used as an adjunct to clinical observations and other information available to the physician.
- The ability of the Copan Colibrí System to prepare samples for analysis with the compatible analyzers was evaluated using only the species listed in the Inclusivity and AST Challenge studies, described in the Performance Characteristics section of this Package Insert [Section VII "Performance Characteristics" section of this submission]. The ability of the Copan Colibrí System to prepare samples of other species for mass spectrometry analysis and AST has not been evaluated.
- The performance of Copan Colibrí System in conjunction with the VITEK 2 was evaluated with Trypticase Soy Agar + 5% sheep blood (BD), Columbia Agar + 5% sheep blood (bioMerieux), MacConkey Agar (bioMerieux), Trypticase Soy Agar + 5% sheep blood / MacConkey Agar (BD). The use of other types of culture media has not been validated.
- The Copan Colibrí System should be used only for preparation of target slides and microbial suspension from isolated colonies of Gram-negative and Gram-positive bacterial species grown on solid culture media. Colibrí System is not intended for processing of yeasts, moulds, Nocardia or Mycobacteria.

## IV Device/System Characteristics:

### A Device Description:

The Colibrí System is designed to be used as an accessory for the downstream matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) and antimicrobial susceptibility testing (AST) analyzers. It automates various manual sample preparation steps in the workflow for the identification and AST of isolated colonies of gram-negative and gram-positive bacterial species grown on solid culture media.

The Colibrí System automates the preparation of MALDI target slides for the bioMérieux VITEK MS or the Bruker MALDI Biotyper CA systems that are used in clinical laboratories for identification (ID) of organisms grown on plated media by MALDI-TOF MS. [Refer to the K193138 Decision Summary for the device description and performance characteristics of the sample preparation for bacterial identification and MALDI-TOF MS workflow.] The Colibrí System automates the preparation of microbial suspensions at a known concentration for the bioMérieux VITEK 2 System that is used in clinical laboratories for AST analyses. Moreover, the Colibrí System is used for purity plate preparation for purity assessments.

The Colibrí system comprises the Colibrí Vision System and Colibrí Preparation Station hardware modules and pipette tips, Primary Tubes, Spreader and nephelometer Verification Kit

as consumables. After appropriate plate incubation, the operator loads the plates into the Colibrí Vision System and uses the Graphical User Interface (Image Reading Interface) to choose the plates exhibiting adequate growth. The operator selects the isolated colonies on a digital plate to be processed by assigning the automatic ID or AST tasks. After selecting colonies, the operator manually loads the plates in the Colibrí Preparation Station where colonies are automatically picked, spotted on the target slide and overlayed with the matrix for ID or suspended into the dedicated solution for the preparation of the microbial suspension for AST testing and purity assessment.

When used in conjunction with the bioMérieux VITEK 2, the Colibrí System can prepare microbial suspensions by the direct colony suspension method. The onboard nephelometer and pipetting system allow for the preparation of Secondary Tubes (AST suspensions) at the appropriate concentration for AST analysis and the Colibrí Spreader is used for purity plate preparation. The traceability of prepared secondary tubes and purity plates is maintained by application of dedicated labels.

## B Instrument Description Information:

1. Instrument Name:

   Colibrí System comprised of the Colibrí Vision System and Colibrí Preparation Station

2. Specimen Sampling and Handling:

   Culture plates used for processing with the Colibrí System are prepared and sorted manually to identify those with isolated colonies from gram-positive or gram-negative bacteria and then labeled by applying a linear barcode to the base of the plate. After loading the plates into the Colibrí Vision System, the operator must select the appropriate culture medium for image acquisition to ensure that the appropriate optical parameters are applied. Following image acquisition, the operator designates well-isolated colonies for picking and then manually transfers the plates from the Colibrí Vision System to the Colibrí Preparation Station, where the colonies are picked automatically and used to prepare a culture suspension at specific turbidity for antimicrobial susceptibility testing (AST) using the bioMérieux VITEK 2 system and bioMérieux VITEK 2 AST cards.

3. Microbial Suspension Preparation and AST Analysis:

   Culture plates for processing are identified by the Colibrí Vision System by scanning the manually applied linear barcode on the side of each plate. The barcode is used to orientate the plate and, together with the plate's geometric center, also used to define the Cartesian coordinates of each of the colonies that are designated for picking. This information is retrieved by the Colibrí Preparation Station, which picks the designated colonies from the agar surface and deposits them into the Primary Tube containing a saline solution. The Primary Tube is mixed with the on-board vortex and the turbidity is measured with the on-board nephelometer. If needed, the suspension is adjusted with additional colonies, vortexed, and measured again with the nephelometer. A specific aliquot is automatically transferred from the Primary Tube into the labeled Secondary Tube to yield a microbial suspension with appropriate turbidity for AST analysis with the bioMérieux VITEK 2 system and bioMérieux VITEK 2 AST cards. There are no changes to the methods of data analysis or result

algorithms for the VITEK 2 system compared to those used in the 510(k)-cleared devices. After preparation of the microbial suspension in the Secondary Tube, a labeled Purity Plate may optionally be automatically prepared from the Secondary Tube.

4. Calibration:

   Set-up calibration, auto-calibration, and run-time calibration checks are performed for the Colibrí System, as described in the K193138 Decision Summary.

   For the AST function, the Colibrí System requires a daily nephelometer verification to check the proper reading of suspensions at different turbidity values. In addition, a nephelometer set-up calibration is performed by the technical engineer as part of the system setup during the initial device installation.

5. Quality Control:

   Quality Control for AST testing was performed in accordance with the manufacturer's instructions for the bioMérieux VITEK 2 system. CLSI-recommended reference strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212 were grown on trypticase soy agar + 5% sheep blood. Microbial suspensions were prepared using three separate Colibrí Systems and loaded in the appropriate VITEK 2 AST card. MIC values for each drug/organism combination were compared to the expected MIC range. Purity of all the suspensions was confirmed by purity plates prepared by the Colibrí System, with 100% of purity plates showing monomicrobial growth. Quality control testing was performed daily during analytical performance testing using the quality control bacterial strains in rotation, and all results obtained were in-range. Results are summarized in **Table 1**. The quality control testing is acceptable.

**Table 1. Quality Control Organisms and Antimicrobial Agents Tested**

| QC Organism | Antimicrobial Agent | QC Range | No. MIC within QC Range |
|---|---|---|---|
| *Escherichia coli* ATCC 25922 | Ampicillin/Sulbactam | 2/1-8/4 | 47/47 (100%) |
| | Piperacillin/Tazobactam | 1/4-4/4 | 47/47 (100%) |
| | Cefazolin | 1-4 | 47/47 (100%) |
| | Cefoxitin | 2-8 | 47/47 (100%) |
| | Ceftazidime | 0.06-0.5 | 47/47 (100%) |
| | Ceftriaxone | 0.03-0.12 | 47/47 (100%) |
| | Cefepime | 0.016-0.12 | 47/47 (100%) |
| | Aztreonam | 0.06-0.25 | 47/47 (100%) |
| | Ertapenem | 0.004-0.016 | 47/47 (100%) |
| | Meropenem | 0.008-0.006 | 47/47 (100%) |
| | Imipenem | 0.06-0.25 | 47/47 (100%) |
| | Amikacin | 0.5-4 | 47/47 (100%) |
| | Gentamicin | 0.25-1 | 47/47 (100%) |

| QC Organism | Antimicrobial Agent | QC Range | No. MIC within QC Range |
|---|---|---|---|
| | Tobramycin | 0.25-1 | 47/47 (100%) |
| | Levofloxacin | 0.008-0.06 | 47/47 (100%) |
| | Tetracycline | 0.5-2 | 47/47 (100%) |
| | Tigecycline | 0.03-0.25 | 47/47 (100%) |
| | Nitrofurantoin | 4-16 | 47/47 (100%) |
| | Trimethoprim/Sulfamethoxazole | ≤0.5/9.5 | 47/47 (100%) |
| *Pseudomonas aeruginosa* ATCC 27853 | Piperacillin/Tazobactam | 1/4-8/4 | 38/38 (100%) |
| | Ceftazidime | 1-4 | 38/38 (100%) |
| | Ceftriaxone | 8-64 | 38/38 (100%) |
| | Cefepime | 0.5-4 | 38/38 (100%) |
| | Aztreonam | 2-8 | 38/38 (100%) |
| | Ertapenem | 2-8 | 38/38 (100%) |
| | Meropenem | 0.12-1 | 38/38 (100%) |
| | Imipenem | 1/4 | 38/38 (100%) |
| | Amikacin | 1-4 | 38/38 (100%) |
| | Gentamicin | 0.5-2 | 38/38 (100%) |
| | Tobramycin | 0.25-1 | 38/38 (100%) |
| | Levofloxacin | 0.5-4 | 38/38 (100%) |
| | Trimethoprim/Sulfamethoxazole | 8/152-32/608 | 38/38 (100%) |
| | Ciprofloxacin | 0.12-1 | 38/38 (100%) |
| *Staphylococcus aureus* ATCC 29213 | Gentamicin | 0.12-1 | 40/40 (100%) |
| | Levofloxacin | 0.06-0.5 | 40/40 (100%) |
| | Tetracycline | 0.12-1 | 40/40 (100%) |
| | Tigecycline | 0.03-0.25 | 40/40 (100%) |
| | Nitrofurantoin | 8-32 | 40/40 (100%) |
| | Trimethoprim/Sulfamethoxazole | ≤0.5/9.5 | 40/40 (100%) |
| | Ciprofloxacin | 0.12-0.5 | 40/40 (100%) |
| | Benzylpenicillin (Penicillin) | 0.25-2 | 40/40 (100%) |
| | Ampicillin | 0.5-2 | 40/40 (100%) |
| | Oxacillin | 0.12-0.5 | 40/40 (100%) |
| | Moxifloxacin | 0.016-0.12 | 40/40 (100%) |
| | Erythromycin | 0.25-1 | 40/40 (100%) |
| | Clindamycin | 0.06-.025 | 40/40 (100%) |
| | Quinupristin/Dalfopristin | 0.25-1 | 40/40 (100%) |
| | Linezolid | 1-4 | 40/40 (100%) |
| | Vancomycin | 0.5-2 | 40/40 (100%) |

| QC Organism | Antimicrobial Agent | QC Range | No. MIC within QC Range |
|---|---|---|---|
| | Rifampicin (Rifampin) | 0.004-0.016 | 40/40 (100%) |
| *Enterococcus faecalis* ATCC 29212 | Levofloxacin | 0.25-2 | 39/39 (100%) |
| | Tetracycline | 8-32 | 39/39 (100%) |
| | Tigecycline | 0.03-0.12 | 39/39 (100%) |
| | Nitrofurantoin | 4-16 | 39/39 (100%) |
| | Ciprofloxacin | 0.25-2 | 39/39 (100%) |
| | Benzylpenicillin (Penicillin) | 1-4 | 39/39 (100%) |
| | Ampicillin | 0.5-2 | 39/39 (100%) |
| | Moxifloxacin | 0.06-0.5 | 39/39 (100%) |
| | Erythromycin | 1-4 | 39/39 (100%) |
| | Clindamycin | 4-16 | 39/39 (100%) |
| | Quinupristin/Dalfopristin | 2-8 | 39/39 (100%) |
| | Linezolid | 1-4 | 39/39 (100%) |
| | Vancomycin | 1-4 | 39/39 (100%) |

## V Substantial Equivalence Information:

### A Predicate Device Name(s):
Vitek 2 Systems (pc) 5.02 Software

### B Predicate 510(k) Number(s):
K103752

### C Comparison with Predicate(s):

| Device & Predicate Device(s): | Device K220546 | Predicate K103752 |
|---|---|---|
| Device Trade Name | Colibrí System | VITEK 2 Systems (PC) 5.02 Software |
| **General Device Characteristic Similarities** | | |
| Intended Use/Indications For Use | The 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 and with the bioMérieux VITEK 2 Antimicrobial Susceptibility Testing (AST) | The VITEK 2 and VITEK 2 Compact Systems using VITEK 2 Systems 5.02 Software are a laboratory aid in the determination of in vitro susceptibility of microorganisms to antimicrobial agents. The VITEK 2 Antimicrobial Susceptibility Test (AST) is intended to be used with the VITEK 2 System for the automated quantitative or qualitative susceptibility |

| Device & Predicate Device(s): | Device<br>K220546 | Predicate<br>K103752 |
|---|---|---|
| | system for qualitative testing 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 for bacterial identification and microbial suspension at known concentration for antimicrobial susceptibility testing and purity assessment.<br><br>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. Bacterial suspensions for AST and purity plates are identified by barcode label. 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.<br><br>The Colibrí System has not been validated for use in the identification or processing of yeast species, molds, Nocardia, or mycobacteria. | testing of isolated colonies for the most clinically significant aerobic Gram-Negative bacilli, *Staphylococcus spp*., *Enterococcus spp., Streptococcus agalactiae, S. pneumoniae* and clinically significant yeast. The VITEK 2 Systems (PC) 5.02 Software is intended for use with VITEK 2 and VITEK 2 Compact Systems (list of claimed organisms omitted for brevity; refer to K103752). |
| Sample Type | Isolated bacterial colonies | Same |
| Solution for Suspension Preparation | 0.45% NaCl Saline Solution (pH 4.5 to 7.0) | Same |
| Inoculum Density Check | Accuracy of the inoculum preparation is verified by an on-board nephelometer | Accuracy of the inoculum preparation is verified by a nephelometer. |
| Quality Control | Suspension of reference strains to be used as quality | Same |

| Device & Predicate Device(s): | Device<br>K220546 | Predicate<br>K103752 |
|---|---|---|
| | control should be prepared manually according to the instruction for use of the used VITEK 2 AST card. | |
| AST Results Interpretation | MIC and categorization of results are provided by VITEK 2. | Same |
| **General Device Characteristic Differences** | | |
| Colony Selection | The colony to be picked is selected by an operator on a digital plate using the Graphical User Interface of a Vision System. | The colony to be picked is manually selected by an operator on the plate through the visual inspection. |
| Method of Colony Picking | Colibrí System has been validated for automatic picking of colonies using a sterile pipette tip. | The colonies to be picked are manually transferred using a sterile stick or swab. |
| Sample Traceability | On each Secondary Tube prepared by the Colibrí System, a barcode label is applied including following data: the sample identification, the hour of the preparation and the Gram classification associated to the processed isolate. Label data are used for sample traceability for further processing on the VITEK 2. | The sample identification is recorded directly in the Cassette Docking Station software manually or scanning the barcode of the culture media plate from which the colonies were collected during the preparation of the microbial suspension. |
| Method of AST Suspension Preparation | Using a pipetting system, a predefined number of morphologically similar colonies are transferred into Primary Tube containing saline solution (0.45% NaCl Saline Solution pH 4.5 to 7.0). A homogenous heavy suspension of organisms is prepared and checked by using the on-board Colibrí nephelometer. In the Secondary Tube containing 3.0mL of the same saline solution, a variable aliquot of the heavy suspension is automatically transferred to obtain the final microbial concentration according to IVD package insert indications. The suspensions prepared by Colibrí System must be tested in MANUAL MODE on the VITEK 2. | Using a sterile stick or swab, a sufficient number of morphologically similar colonies are transferred to a saline tube (0.45% NaCl, Saline Solution pH 4.5 to 7.0). A homogenous suspension with a density equivalent to the 0.5 McFarland is prepared and checked with the nephelometer. In a second tube containing 3.0mL of saline, a predetermined aliquot of 0.5 McFarland is transferred according to IVD package insert indications (MANUAL MODE). Alternatively, the 0.5 McFarland suspension is loaded on the VITEK 2 that automatically prepares the Secondary Tube at proper concentration (AUTO |

| Device & Predicate Device(s): | Device K220546 | Predicate K103752 |
|---|---|---|
| | | DILUTION MODE). |

## VI Standards/Guidance Documents Referenced:

*The following FDA-recognized Consensus Standards were referenced and pertain to device and study design for the Colibrí System's preparation of samples for AST:*

- IEC 62304. Medical Device Software – Software Life Cycle Processes (Edition 1.1; 2015-06).
- CLSI M07. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically (11th Edition)
- CLSI M100. Performance Standards for Antimicrobial Susceptibility Testing. (31st Edition)
- Guidance for Industry and Food and Drug Administration Staff – Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems (August 28, 2009)

## VII Performance Characteristics:

The performance of the Copan Colibrí System was evaluated in conjunction with the bioMérieux VITEK 2 using the criteria for MIC interpretation as described in the VITEK 2 labeling. [Refer to the K193138 Decision Summary for the performance characteristics of the sample preparation for bacterial identification and MALDI-TOF MS workflow.]

### A Analytical Performance:

1. Precision/Reproducibility:

   The reproducibility of MIC results obtained using microbial suspensions automatically prepared by the Colibrí System was evaluated with the bioMérieux VITEK 2. For this study, three Colibrí instruments (each comprised of a Colibrí Vison System and Colibrí Preparation Station) were used to prepare microbial suspensions from overnight (18 to 24 hour) cultures of 15 representative, clinically relevant bacterial isolates (seven gram-negative species and eight gram-positive species including two *Streptococcus spp.,* summarized in **Table 2**) grown on trypticase soy agar with 5% sheep blood. Each isolate was tested with ≥4 drugs representing different drug classes. Samples of each isolate were tested in triplicate on three separate days. On each day that testing was performed, three different operators each designated at least four (for gram-negative) or six (for gram-positive) colonies for automated picking from digital images of each of the three culture plates corresponding to each bacterial species. A total of 81 replicates per antimicrobial/organism combination were analyzed (3 Colibrí instruments x 3 operators x 3 replicates x 3 days).

**Table 2. List of Species for Reproducibility Testing**

| | |
|---|---|
| **Gram negative** | *Acinetobacter baumannii, Citrobacter koseri, Klebsiella (Enterobacter) aerogenes, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa* |
| **Gram positive** | *Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Streptococcus pyogenes* |

Samples were prepared and assessed for purity consistent with the Colibrí System's instructions for use. The final suspension in the Secondary Tube was manually loaded into the appropriate VITEK 2 AST card. The secondary tubes and corresponding AST cards were processed by the VITEK 2 instrument. The reported MIC values were used to calculate within-run, between-run, within-user, and between-user reproducibility.

Reproducibility was calculated as the total number of results that were within one doubling dilution of the mode result divided by total number of results. Off-scale results that were one doubling dilution from the mode were considered reproducible for best-case reproducibility calculations but considered not reproducible for worst-case calculations.

In total, 9,639 MIC results were generated, with 9,597 of those results being on-scale. The results for all samples are summarized and stratified by the tested antimicrobial in **Table 3** for gram-negative species and **Table 4** for gram-positive species. Reproducibility between operators was also evaluated and is summarized in **Table 5**. For all antimicrobials evaluated, best-case and worst-case reproducibility was ≥95%. Additionally, purity plates were analyzed for monomicrobial growth. All purity plates displayed monomicrobial growth. The reproducibility results are acceptable.

**Table 3**. **Reproducibility Results – Gram-negative Organisms Stratified by Antimicrobial**

| Antimicrobial | Colibrí System | Best case (%) | Worst case (%) |
|---|---|---|---|
| **Ampicillin-Sulbactam** | Instrument 1 | 27/27 (100%) | 27/27 (100%) |
| | Instrument 2 | 27/27 (100%) | 27/27 (100%) |
| | Instrument 3 | 27/27 (100%) | 27/27 (100%) |
| | *Combined* | *81/81 (100%)* | *81/81 (100%)* |
| **Piperacillin/ Tazobactam** | Instrument 1 | 134/135 (99.3%) | 133/135 (98.5%) |
| | Instrument 2 | 133/135 (98.5%) | 132/135 (97.8%) |
| | Instrument 3 | 135/135 (100%) | 133/135 (98.5%) |
| | *Combined* | *402/405 (99.3%)* | *398/405 (98.3%)* |
| **Cefoxitin** | Instrument 1 | 81/81 (100%) | 79/81 (97.5%) |
| | Instrument 2 | 81/81 (100%) | 80/81 (98.8%) |
| | Instrument 3 | 80/81 (98.8%) | 79/81 (97.5%) |
| | *Combined* | *242/243 (99.6%)* | *238/243 (97.9%)* |
| **Ceftazidime** | Instrument 1 | 187/189 (98.9%) | 186/189 (98.4%) |
| | Instrument 2 | 189/189 (100%) | 189/189 (100%) |
| | Instrument 3 | 188/189 (99.5%) | 188/189 (99.5%) |
| | *Combined* | *242/243 (99.6%)* | *238/243 (97.9%)* |
| **Ceftriaxone** | Instrument 1 | 27/27 (100%) | 27/27 (100%) |

| Antimicrobial | Colibrí System | Best case (%) | Worst case (%) |
|---|---|---|---|
| | Instrument 2 | 27/27 (100%) | 27/27 (100%) |
| | Instrument 3 | 27/27 (100%) | 27/27 (100%) |
| | *Combined* | *81/81 (100%)* | *81/81 (100%)* |
| **Cefepime** | Instrument 1 | 162/162 (100%) | 162/162 (100%) |
| | Instrument 2 | 162/162 (100%) | 162/162 (100%) |
| | Instrument 3 | 162/162 (100%) | 162/162 (100%) |
| | *Combined* | *486/486 (100%)* | *486/486 (100%)* |
| **Aztreonam** | Instrument 1 | 81/81 (100%) | 81/81 (100%) |
| | Instrument 2 | 81/81 (100%) | 81/81 (100%) |
| | Instrument 3 | 81/81 (100%) | 81/81 (100%) |
| | *Combined* | *243/243 (100%)* | *243/243 (100%)* |
| **Meropenem** | Instrument 1 | 108/108 (100%) | 108/108 (100%) |
| | Instrument 2 | 108/108 (100%) | 108/108 (100%) |
| | Instrument 3 | 108/108 (100%) | 108/108 (100%) |
| | *Combined* | *324/324 (100%)* | *324/324 (100%)* |
| **Amikacin** | Instrument 1 | 189/189 (100%) | 189/189 (100%) |
| | Instrument 2 | 189/189 (100%) | 189/189 (100%) |
| | Instrument 3 | 188/189 (99.5%) | 188/189 (99.5%) |
| | *Combined* | *566/567 (99.8%)* | *566/567 (99.8%)* |
| **Gentamicin** | Instrument 1 | 81/81 (100%) | 81/81 (100%) |
| | Instrument 2 | 81/81 (100%) | 81/81 (100%) |
| | Instrument 3 | 81/81 (100%) | 81/81 (100%) |
| | *Combined* | *243/243 (100%)* | *243/243 (100%)* |
| **Tobramycin** | Instrument 1 | 27/27 (100%) | 27/27 (100%) |
| | Instrument 2 | 27/27 (100%) | 27/27 (100%) |
| | Instrument 3 | 27/27 (100%) | 24/27 (96.3%) |
| | *Combined* | *81/81 (100%)* | *79/81 (97.5%)* |
| **Levofloxacin** | Instrument 1 | 81/81 (100%) | 81/81 (100%) |
| | Instrument 2 | 80/81 (98.8%) | 80/81 (98.8%) |
| | Instrument 3 | 80/81 (98.8%) | 80/81 (98.8%) |
| | *Combined* | *241/243 (99.2%)* | *241/243 (99.2%)* |
| **Tetracycline** | Instrument 1 | 54/54 (100%) | 54/54 (100%) |
| | Instrument 2 | 54/54 (100%) | 54/54 (100%) |
| | Instrument 3 | 54/54 (100%) | 54/54 (100%) |
| | *Combined* | *162/162 (100%)* | *162/162 (100%)* |
| **Tigecycline** | Instrument 1 | 81/81 (100%) | 80/81 (98.8%) |
| | Instrument 2 | 81/81 (100%) | 80/81 (98.8%) |
| | Instrument 3 | 81/81 (100%) | 80/81 (98.8%) |
| | *Combined* | *243/243 (100%)* | *240/243 (98.8%)* |
| **Nitrofurantoin** | Instrument 1 | 135/135 (100%) | 135/135 (100%) |
| | Instrument 2 | 135/135 (100%) | 134/135 (99.3%) |
| | Instrument 3 | 134/135 (99.3%) | 134/135 (99.3%) |
| | *Combined* | *404/405 (99.8%)* | *403/405 (99.5%)* |
| **Imipenem** | Instrument 1 | 27/27 (100%) | 27/27 (100%) |
| | Instrument 2 | 27/27 (100%) | 27/27 (100%) |
| | Instrument 3 | 27/27 (100%) | 27/27 (100%) |
| | *Combined* | *81/81 (100%)* | *81/81 (100%)* |
| **Ciprofloxacin** | Instrument 1 | 27/27 (100%) | 27/27 (100%) |
| | Instrument 2 | 27/27 (100%) | 26/27 (96.3%) |
| | Instrument 3 | 27/27 (100%) | 27/27 (100%) |

| Antimicrobial | Colibrí System | Best case (%) | Worst case (%) |
|---|---|---|---|
| | *Combined* | *81/81 (100%)* | *80/81 (98.8%)* |

**Table 4. Reproducibility Results – Gram-positive Organisms Stratified by Antimicrobial**

| Antimicrobial | Colibrí System | Best case (%) | Worst case (%) |
|---|---|---|---|
| **Levofloxacin** | Instrument 1 | 243/243 (100%) | 243/243 (100%) |
| | Instrument 2 | 241/243 (99.2%) | 241/243 (99.2%) |
| | Instrument 3 | 241/243 (99.2%) | 241/243 (99.2%) |
| | *Combined* | *725/729 (99.5%)* | *725/729 (99.5%)* |
| **Tetracycline** | Instrument 1 | 27/27 (100%) | 27/27 (100%) |
| | Instrument 2 | 27/27 (100%) | 26/27 (96.3%) |
| | Instrument 3 | 27/27 (100%) | 27/27 (100%) |
| | *Combined* | *81/81 (100%)* | *80/81 (98.8%)* |
| **Tigecycline** | Instrument 1 | 27/27 (100%) | 27/27 (100%) |
| | Instrument 2 | 27/27 (100%) | 27/27 (100%) |
| | Instrument 3 | 27/27 (100%) | 27/27 (100%) |
| | *Combined* | *81/81 (100%)* | *81/81 (100%)* |
| **Nitrofurantoin** | Instrument 1 | 54/54 (100%) | 54/54 (100%) |
| | Instrument 2 | 54/54 (100%) | 54/54 (100%) |
| | Instrument 3 | 54/54 (100%) | 54/54 (100%) |
| | *Combined* | *162/162 (100%)* | *162/162 (100%)* |
| **Ciprofloxacin** | Instrument 1 | 81/81 (100%) | 81/81 (100%) |
| | Instrument 2 | 81/81 (100%) | 81/81 (100%) |
| | Instrument 3 | 81/81 (100%) | 81/81 (100%) |
| | *Combined* | *243/243 (100%)* | *243/243 (100%)* |
| **Penicillin (Benzylpenicillin)** | Instrument 1 | 162/162 (100%) | 160/162 (98.8%) |
| | Instrument 2 | 162/162 (100%) | 159/162 (98.1%) |
| | Instrument 3 | 161/162 (99.4%) | 159/162 (98.1%) |
| | *Combined* | *485/486 (99.8%)* | *478/486 (98.4%)* |
| **Ampicillin** | Instrument 1 | 27/27 (100%) | 27/27 (100%) |
| | Instrument 2 | 27/27 (100%) | 27/27 (100%) |
| | Instrument 3 | 27/27 (100%) | 27/27 (100%) |
| | *Combined* | *81/81 (100%)* | *81/81 (100%)* |
| **Oxacillin** | Instrument 1 | 27/27 (100%) | 27/27 (100%) |
| | Instrument 2 | 27/27 (100%) | 26/27 (96.3%) |
| | Instrument 3 | 27/27 (100%) | 26/27 (96.3%) |
| | *Combined* | *81/81 (100%)* | *79/81 (97.5%)* |
| **Erythromycin** | Instrument 1 | 108/108 (100%) | 107/108 (99.1%) |
| | Instrument 2 | 108/108 (100%) | 108/108 (100%) |
| | Instrument 3 | 108/108 (100%) | 107/108 (99.1%) |
| | *Combined* | *324/324 (100%)* | *322/324 (99.4%)* |
| **Quinupristin/ Dalfopristin** | Instrument 1 | 54/54 (100%) | 52/54 (96.3%) |
| | Instrument 2 | 54/54 (100%) | 53/54 (98.1%) |
| | Instrument 3 | 54/54 (100%) | 54/54 (100%) |
| | *Combined* | *162/162 (100%)* | *159/162 (98.1%)* |
| **Linezolid** | Instrument 1 | 108/108 (100%) | 108/108 (100%) |
| | Instrument 2 | 108/108 (100%) | 108/108 (100%) |
| | Instrument 3 | 108/108 (100%) | 108/108 (100%) |
| | *Combined* | *324/324 (100%)* | *324/324 (100%)* |
| **Vancomycin** | Instrument 1 | 189/189 (100%) | 188/189 (99.5%) |

| Antimicrobial | Colibrí System | Best case (%) | Worst case (%) |
|---|---|---|---|
| | Instrument 2 | 189/189 (100%) | 188/189 (99.5%) |
| | Instrument 3 | 189/189 (100%) | 188/189 (99.5%) |
| | *Combined* | *567/567 (99.2%)* | *564/567 (99.5%)* |

**Table 5. Summary of Reproducibility Results – Stratified by Operator**

| Instrument | | Operator 1 | Operator 2 | Operator 3 | Total |
|---|---|---|---|---|---|
| **Colibrí #1** | Best-case | 990/990 (100%) | 980/981 (99.9%) | 979/981 (99.8%) | 2949/2952 (99.9%) |
| | Worst-case | 986/990 (99.6%) | 974/981 (99.3%) | 975/981 (99.4%) | 2935/2952 (99.4%) |
| **Colibrí #2** | Best-case | 980/981 (99.9%) | 988/990 (99.8%) | 980/981 (99.9%) | 2948/2952 (99.9%) |
| | Worst-case | 972/981 (99.1%) | 985/990 (99.5%) | 976/981 (99.5%) | 2933/2952 (99.4%) |
| **Colibrí #3** | Best-case | 977/981 (99.6%) | 981/981 (100%) | 978/981 (99.7%) | 2936/2943 (99.8%) |
| | Worst-case | 975/981 (99.4%) | 975/981 (99.4%) | 974/981 (99.3%) | 2924/2943 (99.4%) |
| **Total** | Best-case | 2947/2952 (99.8%) | 2949/2952 (99.9%) | 2937/2943 (99.8%) | 8833/8847 (99.8%) |
| | Worst-case | 2933/2952 (99.4%) | 2934/2952 (99.4%) | 2925/2943 (99.4%) | 8792/8847 (99.4%) |

2. Linearity:

   Not applicable.

3. Analytical Specificity/Interference:

   Refer to **Section VII (A)(4).**

4. Accuracy (Instrument):

   *Colony Picking Accuracy*

   A total of 1,500 colonies were picked by three Colibrí System instruments to prepare microbial suspensions consistent with those used for AST. Six clinically relevant gram-negative (*E. coli, K. pneumoniae*, and *P. aeruginosa*) and gram-positive (*E. faecalis*, *S. aureus*, and *S. pyogenes*) species were used to prepare polymicrobial whole and bi-plates as test plates for colony picking. The gram-negative species were mixed in a 1:1:1 ratio and plated on tryptic soy agar with 5% sheep blood (whole and bi-plate), MacConkey agar (whole and bi-plate), and chocolate agar (whole plate). The same process was followed for the gram-positive species and suspensions were plated on tryptic soy agar with 5% sheep blood (whole and bi-plate) and chocolate agar (whole plate).

   Test plates were automatically processed by the Colibrí System – digital plate images were acquired by the Vision System and the operator selected at least four isolated colonies for gram-negative species and at least six isolated colonies for gram-positive species. For the bi-plates, both sides were used for colony selection. Plates were then manually loaded into the

Preparation Station as input picking plates. Using the plate barcode, the Preparation Station automatically picked the user-designated colonies and deposited them into the primary tube. Primary tube suspensions were used to automatically prepare purity plates.

At the end of the automatic preparation, processed plates were visually inspected by the operator and compared to the digital image captured by the Vision System to determine the accuracy of the Colibrí System in picking the correct colony designated by the operator. Outcomes were categorized as follows: correct colony picked, no colony picked, or incorrect colony picked. Accuracy was calculated as the percentage of correctly picked colonies.

At least 139 pick points for each microorganism were made between all replicates. All colonies (100%) were accurately and successfully picked. Data stratified by microorganism, culture medium, and instrument are summarized in **Table 6**.

**Table 6. Percentage of Colonies Picked Correctly**

| Organism | Culture medium | Incubation time | No. picking points | | | Overall |
|---|---|---|---|---|---|---|
| | | | Colibrí #1 | Colibrí #2 | Colibrí #3 | |
| ***E. coli*** | Tryptic Soy Agar +5% sheep blood (whole and bi-plates) | 14 h | 16/16 | 16/16 | 16/16 | 48/48 (100%) |
| | | 18 h | 16/16 | 16/16 | 16/16 | 48/48 (100%) |
| | MacConkey (whole and bi-plates) | 14 h | 24/24 | 22/22 | 22/22 | 68/68 (100%) |
| | | 18 h | 22/22 | 20/20 | 22/22 | 64/64 (100%) |
| | Chocolate | 14 h | 8/8 | 12/12 | 10/10 | 30/30 (100%) |
| | | 18 h | 10/10 | 8/8 | 10/10 | 28/28 (100%) |
| **Total *E. coli*** | | | **96/96** | **94/94** | **96/96** | **286/286 (100%)** |
| ***K. pneumoniae*** | Tryptic Soy Agar +5% sheep blood (whole and bi-plates) | 14 h | 18/18 | 22/22 | 20/20 | 60/60 (100%) |
| | | 18 h | 16/16 | 16/16 | 16/16 | 48/48 (100%) |
| | MacConkey (whole and bi-plates) | 14 h | 18/18 | 20/20 | 22/22 | 60/60 (100%) |
| | | 18 h | 20/20 | 22/22 | 22/22 | 64/64 (100%) |
| | Chocolate | 14 h | 12/12 | 12/12 | 12/12 | 36/36 (100%) |
| | | 18 h | 10/10 | 10/10 | 10/10 | 30/30 (100%) |
| **Total *K. pneumoniae*** | | | **94/94** | **102/102** | **102/102** | **298/298 (100%)** |
| ***P. aeruginosa*** | Tryptic Soy Agar +5% sheep blood (whole and bi-plates) | 14 h | 24/24 | 24/24 | 24/24 | 72/72 (100%) |
| | | 18 h | 24/24 | 20/20 | 24/24 | 68/68 (100%) |
| | MacConkey (whole and bi-plates) | 14 h | 24/24 | 24/24 | 24/24 | 72/72 (100%) |
| | | 18 h | 24/24 | 20/20 | 24/24 | 68/68 (100%) |

| Organism | Culture medium | Incubation time | No. picking points | | | Overall |
|---|---|---|---|---|---|---|
| | | | Colibrí #1 | Colibrí #2 | Colibrí #3 | |
| | Chocolate | 14 h | 12/12 | 12/12 | 12/12 | 36/36 (100%) |
| | | 18 h | 12/12 | 10/10 | 12/12 | 34/34 (100%) |
| **Total *P. aeruginosa*** | | | **120/120** | **110/110** | **120/120** | **350/350 (100%)** |
| ***S. aureus*** | Tryptic Soy Agar +5% sheep blood (whole and bi-plates) | 18 h | 36/36 | 36/36 | 36/36 | 108/108 (100%) |
| | Chocolate | 18 h | 18/18 | 18/18 | 18/18 | 54/54 (100%) |
| **Total *S. aureus*** | | | **54/54** | **54/54** | **54/54** | **162/162 (100%)** |
| ***E. faecalis*** | Tryptic Soy Agar +5% sheep blood (whole and bi-plates) | 18 h | 42/42 | 42/42 | 40/40 | 124/124 (100%) |
| | Chocolate | 18 h | 22/22 | 22/22 | 20/20 | 64/64 (100%) |
| **Total *E. faecalis*** | | | **64/64** | **64/64** | **60/60** | **188/188 (100%)** |
| ***S. pyrogenes*** | Tryptic Soy Agar +5% sheep blood (whole and bi-plates) | 18 h | 48/48 | 48/48 | 48/48 | 144/144 (100%) |
| | Chocolate | 18 h | 24/24 | 24/24 | 24/24 | 72/72 (100%) |
| **Total *S. pyrogenes*** | | | **72/72** | **72/72** | **72/72** | **216/216 (100%)** |
| **TOTAL: 1500/1500 (100%)** | | | | | | |

The designated colonies were picked accurately, with no evidence of microbial contamination based on visual inspection of the purity plates. The accuracy of colony picking by the Colibrí System for preparation of microbial suspensions for AST was therefore determined to be acceptable.

*Microbial Suspension Accuracy*

Isolated colonies identified as part of the colony picking study were used by the Colibrí Preparation Station to automatically prepare microbial suspensions in the primary tube. The turbidity of each suspension was measured by the onboard nephelometer. Microbial suspensions were then manually diluted, plated in triplicate, and colonies were counted to determine if the automatically prepared suspensions contained the appropriate and expected concentration of bacteria. Colony counts were deemed to be acceptable if they were within the expected ranges calculated using the reference value of *E. coli* suspensions (a suspension with turbidity equivalent to 0.5 McFarland contains approximately 1-2x$10^8$ CFU/mL for *E. coli* ATCC25955 according to CLSI M07), with the upper limit and lower limit representative of 0.5 x $10^8$ CFU/mL (half a doubling dilution) more or fewer bacteria, respectively. Accuracy was calculated as the percentage of primary tubes with microbial content in the expected range. In total, 60 microbial suspensions were prepared for each

gram-negative species and 27 microbial suspensions were prepared for each gram-positive species. The summary of agreement between the measured concentration and the concentration calculated by colony count is shown in **Table 7**, stratified by Colibrí instrument, culture medium and microorganism.

**Table 7. Percentage Suspensions with Acceptable Microbial Concentration**

| Microorganism | Culture medium | Avg. Calculated Concentration (CFU/mL) | Avg. Expected Concentration (CFU/mL) | Colibrí #1 | Colibrí #2 | Colibrí #3 | Overall |
|---|---|---|---|---|---|---|---|
| *E. coli* | Tryptic Soy Agar +5% sheep blood | $1.44 \times 10^8$ | $1.39 \times 10^8$ | 8/8 | 8/8 | 8/8 | 60/60 (100%) |
| | MacConkey | $1.16 \times 10^8$ | $1.20 \times 10^8$ | 8/8 | 8/8 | 8/8 | |
| | Chocolate | $1.39 \times 10^8$ | $1.35 \times 10^8$ | 4/4 | 4/4 | 4/4 | |
| *K. pneumoniae* | Tryptic Soy Agar +5% sheep blood | $1.34 \times 10^8$ | $1.19 \times 10^8$ | 8/8 | 8/8 | 8/8 | 60/60 (100%) |
| | MacConkey | $1.33 \times 10^8$ | $1.27 \times 10^8$ | 8/8 | 8/8 | 8/8 | |
| | Chocolate | $1.41 \times 10^8$ | $1.30 \times 10^8$ | 4/4 | 4/4 | 4/4 | |
| *P. aeruginosa* | Tryptic Soy Agar +5% sheep blood | $1.17 \times 10^8$ | $1.28 \times 10^8$ | 8/8 | 7/8 | 8/8 | 59/60 (98.3%) |
| | MacConkey | $1.11 \times 10^8$ | $1.22 \times 10^8$ | 8/8 | 8/8 | 8/8 | |
| | Chocolate | $1.05 \times 10^8$ | $1.22 \times 10^8$ | 4/4 | 4/4 | 4/4 | |
| *S. aureus* | Tryptic Soy Agar +5% sheep blood | $5.29 \times 10^8$ | $4.26 \times 10^8$ | 6/6 | 6/6 | 6/6 | 26/27 (96.3%) |
| | Chocolate | $4.93 \times 10^8$ | $3.97 \times 10^8$ | 3/3 | 3/3 | 2/3 | |
| *S. pyogenes* | Tryptic Soy Agar +5% sheep blood | $7.84 \times 10^8$ | $9.10 \times 10^8$ | 6/6 | 6/6 | 6/6 | 27/27 (100%) |
| | Chocolate | $8.64 \times 10^8$ | $9.19 \times 10^8$ | 3/3 | 3/3 | 3/3 | |
| *E. faecalis* | Tryptic Soy Agar +5% sheep blood | $1.44 \times 10^8$ | $1.45 \times 10^8$ | 6/6 | 6/6 | 6/6 | 27/27 (100%) |
| | Chocolate | $1.73 \times 10^8$ | $1.71 \times 10^8$ | 3/3 | 3/3 | 3/3 | |
| *Total* | | *$1.76 \times 10^8$* | *$1.64 \times 10^8$* | *87/87 (100%)* | *86/87 (98.8%)* | *86/87 (98.8%)* | *259/261 (99.2%)* |

Over 98% of microbial suspensions prepared using picked colonies were within the acceptable limits. Therefore, the accuracy of microbial suspension preparation was determined to be acceptable. These results further support the accuracy of colony picking by the Colibrí System for preparation of microbial suspensions.

*Accuracy of AST Results*

The accuracy of MICs obtained by bioMérieux VITEK 2 with microbial suspensions prepared by the Colibrí System was evaluated by testing representative strains of clinically relevant gram-positive and gram-negative bacteria. Three different Colibrí instruments operated by three different operators were used to prepare suspensions from isolated colonies of representative isolates of different species of *Enterobacterales* (n=62), *Staphylococcus* spp. (n=16), *Streptococcus* spp. (n=30), *Enterococcus* spp. (n=16) and non-fermenting gram-negative bacilli (n=32) grown on trypticase soy agar + 5% sheep blood, MacConkey agar,

and Columbia agar + 5% sheep blood. **Table 8** shows the test strains used for the AST challenge study.

**Table 8. Species Tested in AST Challenge Study**

| Group | Microorganisms Included (n = strains tested) |
|---|---|
| *Enterobacterales* | *Escherichia coli (n=12), Klebsiella pneumoniae (n=11), Citrobacter freundii (n=6), Citrobacter koseri (n=4), Enterobacter cloacae (n=9), Klebsiella aerogenes (n=3), Klebsiella oxytoca (n=3), Morganella morganii (n=2), Proteus mirabilis (n=4), Proteus vulgaris (n=2), Raoultella planticola (n=2), Salmonella ser. typhimurium (n=1), Serratia marcescens (n=3)* |
| Non-fermenters | *Acinetobacter baumannii (n=8), Pseudomonas aeruginosa (n=27)* |
| *Staphylococci* | *Staphylococcus aureus (n=11), Staphylococcus epidermidis (n=3), Staphylococcus saprophyticus (n=2)* |
| *Enterococci* | *Enterococcus faecalis (n=7), Enterococcus faecium (n=6), Enterococcus casseliflavus (n=1), Enterococcus durans (n=1), Enterococcus hirae (n=1)* |
| *Streptococci* | *Streptococcus agalactiae (n=16), Streptococcus pyogenes (n=14)* |

Both susceptible and resistant strains exhibiting a range of on-scale MIC values were tested against at least four antimicrobials representative of major drug classes. One VITEK 2 AST system was used with various AST card types containing a broad range of concentrations of specific drugs (VITEK cards used: GN74, *Enterobacterales* species; GN82, Non-fermenters species; G67, *Staphylococcus* species; GP67, *Enterococcus* species; ST03, *Streptococcus* species). Thirty-one different antimicrobial agents were analyzed, resulting in a total of 1,883 evaluable MIC results.

The MIC results obtained using the Colibrí System and VITEK 2 system were compared to the results from manually prepared samples and the VITEK 2 system, consistent with the VITEK 2 system instructions for use.

Essential Agreement (EA) was defined as MIC results from Colibrí System prepared samples that were within one doubling dilution of the MIC results from the manually prepared samples. Category Agreement (CA) was defined as MIC interpretations (S/I/R) that were the same between the Colibrí System-prepared and manually prepared samples. Very major errors were defined as false susceptible results from the Colibrí System prepared samples, major errors were defined as false resistance results from the Colibrí System prepared samples, and minor errors were defined as results with minor discrepancies (i.e., an intermediate result reported as either resistant or susceptible, or vice versa). Since this is a method-to-method comparison, results were considered acceptable if the EA and CA were ≥95% with no major or very major errors. Additionally, no significant differences should be observed between the Colibrí Systems, operators, culture medium and incubation time.

For all species and antimicrobial agents, 1882/1883 (99.9%) of MIC results were within one doubling dilution of the comparator result and 5947/5991 (99.26%) of SIR categorizations were in agreement. Therefore, the EA of the evaluable MIC results was >99.9% and the CA was 99.3%. For each microorganism, antimicrobial agent, instrument, operator, culture medium and incubation time, both essential agreement and categorical agreement were >95%. No very major errors occurred. One major error (false resistance) occurred for the

preparation of *P. aeruginosa* with Cefepime. All purity plates (100%) exhibited monomicrobial growth.

**Table 9** summarizes the results stratified by antimicrobial/organism group combinations. Due to the high degree of agreement between MICs determined from manual and Colibrí preparations, the AST accuracy was determined to be acceptable**.**

**Table 9. Summary of Contingency Results, Stratified by Antimicrobial Agent**

| Agent | Organism group | Total tested | # EA | % EA | Total Evalu-able | # EA of Evalu-able | % EA of Evalu-able | Total cat. | # CA | % CA | # S | # R | # vmj | # maj | # min |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amikacin | *Enterobacterales* | 186 | 186 | 100% | 81 | 81 | 100% | 186 | 182 | 97.8% | 141 | 39 | 0 | 0 | 4 |
| | *Non-fermenters* | 81 | 81 | 100% | 39 | 39 | 100% | 81 | 79 | 97.5% | 66 | 6 | 0 | 0 | 2 |
| Ampicillin | *Enterococcus* | 48 | 48 | 100% | 4 | 4 | 100% | 48 | 48 | 100% | 36 | 12 | 0 | 0 | 0 |
| | *Streptococcus* | 90 | 90 | 100% | 0 | 0 | N/A | 90 | 90 | 100% | 90 | 0 | 0 | 0 | 0 |
| Ampicillin / Sulbactam | *Enterobacterales* | 111 | 111 | 100% | 9 | 9 | 100% | 111 | 111 | 100% | 21 | 90 | 0 | 0 | 0 |
| | *Non-fermenters* | 24 | 24 | 100% | 5 | 5 | 100% | 24 | 23 | 95.8% | 15 | 6 | 0 | 0 | 1 |
| Aztreonam | *Enterobacterales* | 186 | 185 | 99.5% | 54 | 54 | 100% | 186 | 185 | 99.5% | 57 | 123 | 0 | 0 | 1 |
| Cefepime | *Enterobacterales* | 186 | 186 | 100% | 58 | 58 | 100% | 186 | 185 | 99.5% | 93 | 54 | 0 | 0 | 1 |
| | *Non-fermenters* | 81 | 81 | 100% | 50 | 50 | 100% | 81 | 80 | 98.8% | 48 | 33 | 0 | 1 | 0 |
| Cefotaxime | *Streptococcus* | 90 | 90 | 100% | 0 | 0 | N/A | 90 | 90 | 100% | 90 | 0 | 0 | 0 | 0 |
| Cefoxitin | *Enterobacterales* | 186 | 186 | 100% | 42 | 42 | 100% | 186 | 184 | 98.9% | 36 | 132 | 0 | 0 | 2 |
| Ceftazidime | *Enterobacterales* | 180 | 180 | 100% | 43 | 43 | 100% | 180 | 179 | 99.4% | 33 | 132 | 0 | 0 | 1 |
| | *Non-fermenters* | 105 | 105 | 100% | 68 | 68 | 100% | 105 | 105 | 100% | 57 | 36 | 0 | 0 | 0 |
| Ceftriaxone | *Enterobacterales* | 186 | 185 | 99.5% | 31 | 30 | 96.8% | 186 | 185 | 99.5% | 36 | 144 | 0 | 0 | 1 |
| | *Streptococcus* | 90 | 90 | 100% | 0 | 0 | N/A | 90 | 90 | 100% | 90 | 0 | 0 | 0 | 0 |
| Ciprofloxacin | *Non-fermenters* | 81 | 81 | 100% | 28 | 28 | 100% | 81 | 80 | 98.8% | 51 | 18 | 0 | 0 | 1 |
| | *Staphylococcus* | 48 | 48 | 100% | 6 | 6 | 100% | 48 | 48 | 100% | 33 | 12 | 0 | 0 | 0 |
| | *Enterococcus* | 48 | 48 | 100% | 30 | 30 | 100% | 48 | 47 | 97.9% | 27 | 12 | 0 | 0 | 1 |
| Clindamycin | *Streptococcus* | 90 | 90 | 100% | 2 | 2 | 100% | 90 | 89 | 98.9% | 54 | 33 | 0 | 0 | 1 |
| Ertapenem | *Enterobacterales* | 186 | 186 | 100% | 22 | 22 | 100% | 186 | 184 | 98.9% | 102 | 81 | 0 | 0 | 2 |
| Erythromycin | *Staphylococcus* | 48 | 48 | 100% | 9 | 9 | 100% | 48 | 47 | 97.9% | 21 | 24 | 0 | 0 | 1 |
| | *Enterococcus* | 48 | 48 | 100% | 15 | 15 | 100% | 48 | 48 | 100% | 6 | 30 | 0 | 0 | 0 |
| | *Streptococcus* | 90 | 90 | 100% | 29 | 29 | 100% | 90 | 90 | 100% | 54 | 36 | 0 | 0 | 0 |
| Gentamicin | *Enterobacterales* | 186 | 186 | 100% | 40 | 40 | 100% | 186 | 185 | 99.5% | 108 | 66 | 0 | 0 | 1 |
| | *Non-fermenters* | 81 | 81 | 100% | 27 | 27 | 100% | 81 | 81 | 100% | 54 | 21 | 0 | 0 | 0 |
| | *Staphylococcus* | 48 | 48 | 100% | 6 | 6 | 100% | 48 | 48 | 100% | 27 | 21 | 0 | 0 | 0 |
| Imipenem | *Non-fermenters* | 105 | 104 | 99.0% | 54 | 54 | 100% | 105 | 105 | 100% | 72 | 33 | 0 | 0 | 0 |
| Levofloxacin | *Enterobacterales* | 186 | 186 | 100% | 47 | 47 | 100% | 186 | 183 | 98.4% | 69 | 99 | 0 | 0 | 3 |
| | *Non-fermenters* | 81 | 81 | 100% | 63 | 63 | 100% | 81 | 79 | 97.5% | 42 | 27 | 0 | 0 | 2 |
| | *Staphylococcus* | 33 | 33 | 100% | 14 | 14 | 100% | 33 | 32 | 97.0% | 27 | 6 | 0 | 0 | 1 |
| | *Enterococcus* | 48 | 48 | 100% | 41 | 41 | 100% | 48 | 47 | 97.9% | 27 | 6 | 0 | 0 | 1 |
| | *Streptococcus* | 90 | 90 | 100% | 90 | 90 | 100% | 90 | 90 | 100% | 87 | 0 | 0 | 0 | 0 |
| Linezolid | *Staphylococcus* | 48 | 48 | 100% | 48 | 48 | 100% | 48 | 48 | 100% | 48 | 0 | 0 | 0 | 0 |
| | *Enterococcus* | 48 | 48 | 100% | 48 | 48 | 100% | 48 | 47 | 97.9% | 45 | 0 | 0 | 0 | 1 |
| | *Streptococcus* | 90 | 90 | 100% | 0 | 0 | N/A | 90 | 90 | 100% | 90 | 0 | 0 | 0 | 0 |
| Meropenem | *Enterobacterales* | 186 | 186 | 100% | 26 | 26 | 100% | 186 | 184 | 98.9% | 111 | 72 | 0 | 0 | 2 |

| Agent | Organism group | Total tested | # EA | % EA | Total Evalu-able | # EA of Evalu-able | % EA of Evalu-able | Total cat. | # CA | % CA | # S | # R | # vmj | # maj | # min |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Non-fermenters* | 105 | 105 | 100% | 64 | 64 | 100% | 105 | 105 | 100% | 72 | 27 | 0 | 0 | 0 |
| Moxifloxacin | *Staphylococcus* | 48 | 48 | 100% | 12 | 12 | 100% | 48 | 48 | 100% | 39 | 6 | 0 | 0 | 0 |
| Nitro-furantoin | *Enterobacterales* | 186 | 186 | 100% | 134 | 134 | 100% | 186 | 184 | 98.9% | 51 | 90 | 0 | 0 | 2 |
| | *Staphylococcus* | 48 | 48 | 100% | 2 | 2 | 100% | 48 | 48 | 100% | 48 | 0 | 0 | 0 | 0 |
| | *Enterococcus* | 48 | 48 | 100% | 27 | 27 | 100% | 48 | 47 | 97.9% | 24 | 15 | 0 | 0 | 1 |
| Oxacillin | *Staphylococcus* | 48 | 48 | 100% | 13 | 13 | 100% | 48 | 48 | 100% | 21 | 27 | 0 | 0 | 0 |
| Penicillin (Benzyl-penicillin) | *Enterococcus* | 48 | 48 | 100% | 38 | 38 | 100% | 48 | 48 | 100% | 33 | 15 | 0 | 0 | 0 |
| | *Streptococcus* | 90 | 90 | 100% | 25 | 25 | 100% | 90 | 90 | 100% | 90 | 0 | 0 | 0 | 0 |
| | *Staphylococcus* | 48 | 48 | 100% | 11 | 11 | 100% | 48 | 48 | 100% | 12 | 36 | 0 | 0 | 0 |
| Piperacillin / Tazobactam | *Enterobacterales* | 177 | 177 | 100% | 29 | 29 | 100% | 177 | 176 | 99.4% | 60 | 111 | 0 | 0 | 1 |
| | *Non-fermenters* | 105 | 105 | 100% | 52 | 52 | 100% | 105 | 103 | 98.1% | 57 | 30 | 0 | 0 | 2 |
| Quinupristin / Dalfopristin | *Staphylococcus* | 48 | 48 | 100% | 16 | 16 | 100% | 48 | 48 | 100% | 45 | 3 | 0 | 0 | 0 |
| Tetracycline | *Enterobacterales* | 186 | 186 | 100% | 54 | 54 | 100% | 186 | 184 | 98.9% | 90 | 87 | 0 | 0 | 2 |
| | *Staphylococcus* | 48 | 48 | 100% | 7 | 7 | 100% | 48 | 48 | 100% | 30 | 18 | 0 | 0 | 0 |
| | *Enterococcus* | 48 | 48 | 100% | 0 | 0 | N/A | 48 | 48 | 100% | 15 | 33 | 0 | 0 | 0 |
| | *Streptococcus* | 42 | 42 | 100% | 0 | 0 | N/A | 42 | 42 | 100% | 39 | 3 | 0 | 0 | 0 |
| Tigecycline | *Enterobacterales* | 186 | 186 | 100% | 104 | 104 | 100% | 186 | 183 | 98.4% | 144 | 18 | 0 | 0 | 3 |
| | *Staphylococcus* | 33 | 33 | 100% | 0 | 0 | N/A | 33 | 33 | 100% | 33 | 0 | 0 | 0 | 0 |
| | *Enterococcus* | 21 | 21 | 100% | 0 | 0 | N/A | 21 | 21 | 100% | 21 | 0 | 0 | 0 | 0 |
| | *Streptococcus* | 90 | 90 | 100% | 2 | 2 | 100% | 90 | 90 | 100% | 90 | 0 | 0 | 0 | 0 |
| Tobramycin | *Enterobacterales* | 186 | 186 | 100% | 42 | 42 | 100% | 186 | 185 | 99.5% | 51 | 108 | 0 | 0 | 1 |
| | *Non-fermenters* | 81 | 81 | 100% | 1 | 1 | 100% | 81 | 80 | 98.8% | 57 | 24 | 0 | 0 | 1 |
| Trimetho-prim / Sulfa-methoxazole | *Enterobacterales* | 186 | 186 | 100% | 3 | 3 | 100% | 186 | 186 | 100% | 63 | 123 | 0 | 0 | 0 |
| Vancomycin | *Staphylococcus* | 48 | 48 | 100% | 35 | 35 | 100% | 48 | 48 | 100% | 45 | 0 | 0 | 0 | 0 |
| | *Enterococcus* | 48 | 48 | 100% | 18 | 18 | 100% | 48 | 48 | 100% | 30 | 18 | 0 | 0 | 0 |
| | *Streptococcus* | 90 | 89 | 98.9% | 65 | 65 | 100% | 90 | 90 | 100% | 90 | 0 | 0 | 0 | 0 |

EA = essential agreement; CA = category agreement; S = susceptible; R = resistant; vmj = very major error; maj = major error; min = minor error; * one major error (false resistance) occurred for the preparation of *P. aeruginosa* with Cefepime. The preparation of this strain was repeated manually and with the Copan Colibrí System three times. Both preparations generated a susceptible result with all MICs within one two-fold dilution. This major error was therefore likely to be due to random error.

5. Carry-Over:

The ability of the on-board incinerator to properly sterilize the spreader after preparation of a purity plate was analyzed using positive samples (suspensions of *Geobacillus stearothermophilus* prepared at a turbidity equal to a 2 McFarland turbidity standard) and negative samples (saline, aqueous 0.45% NaCl, pH 4.5-7.0; the same solution used in Secondary Tubes). *G. stearothermophilus* was selected as a representative strain due to it resistance to heat. Eight replicates of positive and negative samples were alternatively processed using three Colibrí Systems to prepare purity plates on Chocolate agar. Purity plates were incubated at 55°C±2°C for 48 hours and growth was observed. No growth was observed on any purity plates prepared from negative samples.

The data demonstrate that the on-board incinerator can effectively sterilize the spreader and avoid carry-over between purity plates and is acceptable.

## B Other Supportive Instrument Performance Characteristics Data:

*Pipettor Trueness and Precision*

The trueness and precision of the onboard pipettor was determined using three different Colibrí System pipettors. Four volumes representing 5% (50 µL), 10% (100 µL), 50% (500 µL), and 90% (900 µL) of the nominal volume of the 1000 µL tip used for AST preparations were tested on each instrument. For each device and each target volume, 10 replicates were performed using saline solution (aqueous 0.45% NaCl, pH 4.5 to 7.0, the same solution required for microbial suspension preparation for the VITEK 2 AST procedure). The actual dispensed volume was calculated from the net weight of the liquid.

For each target volume, systematic and random errors were calculated for each instrument and for overall performance. To estimate trueness, the systematic error (SE) was calculated as the deviation of the mean true volume from the target volume. To estimate precision, the random error (RE) was expressed as the coefficient of variation (CV) representing the ratio between the sample standard deviation and the mean volume. A summary of results for all samples and instruments, including acceptance criteria, is shown in **Table 10**.

**Table 10. Summary of Results for Pipettor Trueness and Precision**

| Target Volume (µL) | Average Volume (µL) | Standard Deviation (µL) | Acceptable Deviation (Systematic Error) | Trueness (% bias) | Acceptable CV (Random Error) | Precision (CV) |
|---|---|---|---|---|---|---|
| 50 | 51.9 | 0.714 | ≤ 5.1% | 3.8% | ≤ 2.5% | 1.4% |
| 100 | 102.2 | 2.019 | ≤ 3.5% | 2.3% | ≤ 2.5% | 2.0% |
| 500 | 501.5 | 2.345 | ≤ 2.2% | 0.3% | ≤ 2.5% | 0.5% |
| 900 | 902.5 | 3.214 | ≤ 2.0% | 0.3% | ≤ 2.5% | 0.4% |

CV = Coefficient of Variation

For each tested volume, systematic and random errors were within the acceptance criteria for each instrument and overall. Therefore, the trueness and precision of the pipettor were deemed acceptable.

*Accuracy of the Onboard Nephelometer*

Nephelometer calibration verification was performed to demonstrate that the on-board nephelometer that is part of the Colibrí system can be consistently and properly calibrated and will provide turbidity measurements, in the form of McFarland values, that will accurately estimate the concentration of bacteria present in microbial suspensions within the calibration range. Three different Colibrí systems and three lots of turbidity standards were used. Isolated colonies of *E. coli* ATCC 25922 grown on non-selective medium were used to manually prepare suspensions at determined concentrations (0.25, 0.5, 1.0, 2.0, 3.0, McFarland). Twenty suspensions were prepared by three operators for each concentration and the turbidity of each

suspension was measured using the on-board nephelometer. Suspensions were then diluted, plated, and colonies were counted to determine the initial tube concentration.

A total of 300 suspensions were prepared. The ability of Colibrí on-board nephelometer to accurately prepare microbial suspensions was measured calculating their concentration from colony enumeration and comparing it to the expected concentration based on the nominal turbidity value (calculated based on the assumption that a suspension with turbidity equivalent to 0.5 McFarland contains approximately 1-2x$10^8$ CFU/mL for *E. coli* ATCC25955 according to CLSI M07). To be acceptable, at least 98% of suspensions should contain the expected concentration of *E. coli*. Data is summarized in **Table 11**, including between-instrument one-way ANVOA comparison to assess statistical differences between instruments. No statistically significant differences were observed.

**Table 11. Average Turbidity Values and Suspension Concentrations**

| Colibrí System | Target Turbidity (McF) | Expected Concentration (CFU/mL) | Acceptance Range (CFU/mL) | Average McF Readings | Avg. Calculated Concentration (CFU/mL) | p-value (one-way ANOVA) |
|---|---|---|---|---|---|---|
| #1 | 0.25 | 7.5 x $10^7$ | 5 x $10^7$ – 1 x $10^8$ | 0.258 | 7.63 x$10^7$ | 0.08 |
| #2 | | | | 0.255 | 8.32x$10^7$ | |
| #3 | | | | 0.258 | 7.86 x$10^7$ | |
| #1 | 0.5 | 1.5 x $10^8$ | 1 x $10^8$ – 2 x $10^8$ | 0.517 | 1.57 x$10^8$ | 0.09 |
| #2 | | | | 0.512 | 1.60 x$10^8$ | |
| #3 | | | | 0.522 | 1.47 x$10^8$ | |
| #1 | 1 | 3.0 x $10^8$ | 2 x $10^8$ – 4 x $10^8$ | 1.019 | 2.99 x$10^8$ | 0.23 |
| #2 | | | | 1.021 | 3.11 x$10^8$ | |
| #3 | | | | 1.025 | 3.04 x$10^8$ | |
| #1 | 2 | 6.0 x $10^8$ | 4 x $10^8$ – 8 x $10^8$ | 2.007 | 5.95 x$10^8$ | 0.84 |
| #2 | | | | 2.018 | 6.07 x$10^8$ | |
| #3 | | | | 2.003 | 6.03 x$10^8$ | |
| #1 | 3 | 9.0 x $10^8$ | 6 x $10^8$ – 1.2 x $10^9$ | 3.002 | 9.06 x$10^8$ | 0.96 |
| #2 | | | | 3.004 | 9.13 x$10^8$ | |
| #3 | | | | 3.009 | 9.08 x$10^8$ | |

All the suspensions (100%) prepared with the Colibrí on-board nephelometer contained an acceptable microbial concentration. Therefore, the nephelometer can provide McFarland values that accurately estimate the concentration of bacteria in microbial suspension and is acceptable.

*E. coli Suspensions Preparation Verification Study*

The ability of the Colibrí System to prepare primary tubes at various concentrations with the expected number of microorganisms was assessed using *E. coli* ATCC 25922. Three Colibrí systems run by three different operators were included in the study. A variable number of colonies ranging from 1 to 13 were selected from Trypticase Soy Agar + 5% sheep's blood plates grown aerobically at 35◦C for 14-24 hours to create suspensions at increasing turbidity values.

Primary tubes with a turbidity value inside the working range (0.25 McFarland – 1.75 McFarland) were processed automatically to prepare secondary tubes. Primary tubes with turbidity values outside the working range were automatically moved to the back table of the Colibrí instrument. Primary tubes moved to the back table were discarded. Primary tubes that were processed were ten-fold diluted and enumerated in triplicate. The turbidity value after nephelometer check and colony count obtained from each suspension of processed primary tubes were recorded. To correlate turbidity measurements with colony counts after enumeration, it was assumed that the 0.5 McFarland has a nominal content of 1-2 × $10^8$ CFU/mL for *E. coli* (as described in CLSI guidance M07, 11$^{th}$ edition, 2018).

A total of 144 suspensions were prepared (16 per operator on 3 Colibrí systems with 3 different lots of turbidity standards). Results were deemed acceptable if colony counts were within the expected concentration ranges (with the lower range estimating a nominal content of 1 × $10^8$ CFU/mL for *E. coli* and the upper range estimating a nominal content of 2 × $10^8$ CFU/mL for *E. coli* in a suspension with a 0.5 McFarland turbidity reading). For all combined instruments, ≥98% of suspensions should exhibit colony counts within the acceptance criteria. **Table 12** shows the percentage of acceptably prepared suspensions (number of suspensions within the expected concentration range / the total number of suspensions prepared).

**Table 12. Summary of Suspensions Prepared at the Correct Concentration**

| Colibrí Instrument | N° of Suspensions within the Expected Range/ Total Suspensions Prepared |
|---|---|
| #1 | 42/42 (100%) |
| #2 | 43/43 (100%) |
| #3 | 43/43 (100%) |

100% of suspensions over the entire working range contained the expected number of colonies. Therefore, the ability of the Colibrí System to automatically pick colonies and generate Primary Tubes with expected turbidity values using the on-board nephelometer and with the expected number of microorganisms is acceptable.

*Purity Plate Growth*

To additionally evaluate the potential for cross-contamination between purity plates due to incomplete/improper sterilization of the on-board spreader by the incinerator, the totality of purity plate preparation results across all analytical studies provided in K220546 were evaluated. **Table 13** below lists the number of purity plates analyzed in each analytical study and shows the percentage of purity plates that exhibited monomicrobial growth.

**Table 13. Purity Plates Prepared during Analytical Studies**

| Analytical Studies | Number of purity plates that showed monomicrobial growth | Total number of purity plates tested | % of purity plates that showed monomicrobial growth |
|---|---|---|---|
| Challenge Test | 480 | 480 | 100% |
| Colony Picking Test | 261 | 261 | 100% |
| Quality Control | 165 | 165 | 100% |
| Reproducibility Test | 1458 | 1458 | 100% |

| Analytical Studies | Number of purity plates that showed monomicrobial growth | Total number of purity plates tested | % of purity plates that showed monomicrobial growth |
|---|---|---|---|
| **Total** | **2364** | **2364** | **100%** |

Of the strains analyzed, a minimum of 27 replicates of ATCC strains (*Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Streptococcus pyogenes*) and a minimum of three replicates of clinical strains (*Acinetobacter baumannii, Citrobacter freundii, Citrobacter koseri, Klebsiella (Enterobacter) aerogenes, Enterobacter cloacae, Enterococcus spp., E. coli, Klebsiella spp., Morganella morganii, Proteus mirabilis, Proteus vulgaris, P. aeruginosa, Raoultella planticola, Salmonella ser. Typhimurium, Serratia marcescens, Staphylococcus spp.,* and *Streptococcus spp.*) were included. All ATCC strains (426/426, 100%) and clinical strains (1938/1938, 100%) exhibited monomicrobial growth after purity plate preparation. Both bi-plates and whole plates were used for colony picking prior to preparation of purity plates. Purity plates from all bi-plates (99/99, 100%) and all whole plates (2265/2265, 100%) exhibited monomicrobial growth. Based on the totality of evidence, carry-over and cross-contamination risk was deemed to be acceptably low.

*Reagent Hold Time*

This study verifies that on the Preparation Station deck of the Colibrí instrument, the environmental conditions (specifically, the temperature) are within the recommended stability conditions of the Secondary Tubes. The open-vial stability of the primary tubes was assessed during real time stability evaluation shelf-life studies. This reagent hold time study was conducted with the Secondary Tubes. Secondary Tubes were manually filled with the standard saline solution (aqueous 0.45% to 0.50% NaCl, pH 4.5-7.0). The temperature inside the Colibrí Preparation Station was measured using a datalogger over the course of 10 days during standard use of the instrument and recorded temperature values were compared to that recommended by the manufacturer of the Secondary Tubes and saline solution. A second data logger outside of the instrument was used to provide the reference temperature of the room. The study was conducted using one Colibrí instrument. The mean, minimum, and maximum recorded temperatures for both the tube carousel and the reference temperature of the room are summarized in **Table 14**.

**Table 14. Temperatures of Colibrí Tube Carousel and Room Reference**

| Probe Location | $T_{mean}$ | $T_{min}$ | $T_{max}$ |
|---|---|---|---|
| Tube Carousel Temp (◦ C) | 22.1 | 20.8 | 23.3 |
| Room Temp (◦ C) | 21.7 | 20.4 | 23.0 |
| Net Difference (◦ C) | 0.4 | 0.4 | 0.3 |

Data suggest the presence of electronic components in the instrument only minorly contribute to the temperature inside the Colibrí Preparation Station; the internal temperatures follow the same trend of the room temperature, and the temperature never exceeds the storage temperature recommended for the secondary tubes and saline solution. The environmental conditions of the deck of the Colibrí Preparation Station do not appear to affect the stability of the saline solution and/or the secondary tube and are acceptable.

## VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

## IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.