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# Evaluation of the Colibrí from Copan for automated MALDI-TOF spotting

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## ABSTRACT:

The Colibrí is a new instrument from Copan that works in concert with WASPLab to further automate formerly manual microbiological processes. The Colibrí automates colony picking from culture plates and application to the target slides for MALDI-TOF MS identification. The aim of this study was to compare the performance MALDI- TOF MS spotting between the Colibrí and manual spotting in a multicenter study.

Colonies from routine culture plates of urines, wounds, bloods, and respiratory specimens plated and incubated in the WASPLab were assigned pickpoints after 18 to 24 hours of incubation. The culture plates were then transferred to the Colibrí for Vitek MALDI-TOF MS plate spotting. The Colibrí picked each of the pre-selected colony with a pipette tip, transferred the colony to the slide, and overlayed each spot with matrix. As the target slide was ejected from the instrument, the slide map was automatically transmitted to the MALDI instrument via Myla. The only manual intervention needed was to add the calibration spots. Manual spotting was then performed using the same colony whenever possible and 'like' colonies if the same colony was not available.

A total of 242 bacterial organisms were spotted in parallel by the Colibrí and manually by a trained operator. For Gram positives (GPs) 49 *Staphylococcus* spp., 34 *Streptococcus/Enterococcus* spp., and one organism from four different genera were tested. All GPs were identified by manual spotting and all but two of those were also successfully identified using the Colibrí (85/87). Concordance of identification was 100%. Two Colibrí spots (*Staphyloccocus* spp. and *Gemella* spp.) gave no identification. To evaluate Gram negatives (GNs) 132 Enterobacterales and 23 other organisms, including 18 *P. aeruginosa* were compared. Concordance of identification was 100%. One Colibrí spot was unsuccessful with a mucoid *K. pneumoniae* (154/155) but was successful upon repeat testing.

The Colibrí provides automation to a previously manual process with MALDI-TOF target spotting. From this initial study, a high level of concordance between manual and automated processing was observed. Use of the Colibrí instrumentation provides an opportunity to reallocate technologist time for more valuable tasks, will help to standardize spotting, and provides traceability from plating to organism identification.

## INTRODUCTION:

The introduction of matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI) has significantly changed how organism identifications are performed in the routine clinical microbioloy laboratory and is replacing routine biochemical identification methodologies. As we know that decreases in turn-around-times for the diagnosis of infective agents can greatly affect outcomes for patients, the rapid identifications of these organisms allowed by MALDI technology has further improved the microbiology laboratory's ability to assist with infectious disease patient management. Not only has MALDI allowed for significantly faster TAT in identifying infectious agents, but it is also much more accurate and less expensive. However, this new identification method is a manual system involving the 'picking' of a small amount of a colony growing on agar plates with a wooden stick or loop and then manually applying the colony to a small circle on MALDI target plate. After this step the addition of matrix is manually applied to each inoculated circle and allowed to dry before the plate is placed onto the MALDI instrument.

Copan has developed a semi-automated solution to the manual preparations necessary to process bacterial organisms for MALDI analysis, the Copan Colibrí System. The Colibrí is an in vitro diagnostic device composed of two modules: the Colibrí Vision System and the Colibrí Preparation Station. The Colibrí Vision System is a module for the digital image acquisition of culture plates showing bacteria growing on solid media that includes a workbench and image reading interface components which allow the user to read images of plates, select colonies, and assign appropriate workup. The Colibrí Preparation station includes a working area, a plate loading/unloading area, a PC, a color LCD monitor, and a computer keyboard (**Figure 1**). Together these two modules are used for automated picking of colonies designation by the operator using the WASPLab webapp, prepares MALDI TOF MS target slides using a pipetting system, and overlays the target spot with matrix. 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 analyzer. This system greatly eliminates the manual processing associated with MALDI organism preparation and reduces errors associated with this manual process.

The purpose of this study was to evaluate the ability of the Colibrí System to accurately prepare organisms for MALDI analysis as compared to the standard manual preparation of organisms at two different laboratory sites.

## MATERIALS AND METHODS:

Colonies for comparison were collected from routine culture plates (blood agar, MacConkey agar, and chocolate agar) of urines, wounds, and respiratory specimens, as well as from positive blood cultures plated and incubated in the WASPLab for 18-24 hours. From these plates, technologists assigned 'pick points' (**Figure 2**) which indicated to the Colibrí which colonies to sample for MALDI. Once pick points were assigned to the culture plates, they were transferred to the Colibrí for Vitek MALDI plate preparation (**Figure 3**). The Colibrí sampled each of the pre-selected colonies with a pipette tip (**Figure 4**), transferred the colony to a known place on the MALDI target slide (**Figure 5**), and then overlayed each spot with matrix. As the target slide was completed and ejected from the Colibrí, a slide map was automatically transmitted to the MALDI instrument via Myla middleware. The only manual intervention needed was to add the calibration spots to each automated prepared target plate prior to placing it on the MALDI instrument. Routine manual spotting was then performed using the same colony whenever possible or 'like' colonies (if the same colony was not available) for comparison purposes. A total of 240 bacterial organisms, 85 gram positive and 155 gram negative, were spotted in parallel by the Colibrí and manually by a trained operator for comparison.

**Figure 1:** Copan's Colibrí instrument


**Figure 2:** Pick points selected by technologists in the WASPLab Webapp.

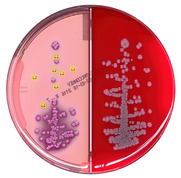


**Figure 3:** Canisters with pick point-culture plates are delivered to the Colibrí.

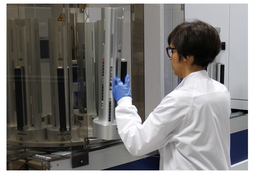


**Figure 4:** Colibrí samples the selected organisms from the culture plate.

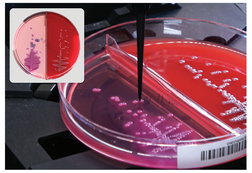
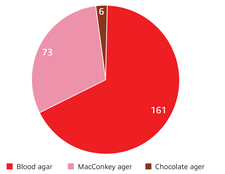


**Figure 5:** Colibrí applies the bacteria to the MALDI target plate and adds matrix.

**Figure 6:** Media types used in the study




## RESULTS:

85/87 Gram positive (GP) organisms were successfully identified using the Colibrí spotting. The concordance of identification was 100%. The Colibrí spot for 1 *Staphyloccocus haemolyticus* and 1 *Gemella haemolysans* species gave no identification. Only the *Staphyloccocus haemolyticus* was available for repeat testing and was successful upon repeat testing with the Colibrí (**Table 1**).

154/155 Gram negative (GN) organisms were successfully identified using the Colibrí spotting. The concordance of identification was 100%. The one Colibrí spot was unsuccessful with a mucoid K. pneumoniae but was successful upon repeat testing with the Colibrí (**Table 2**). A total of 240 organisms, including 19 different genera and 40 different species, were included in the study. Of these 240 organisms, 237 (98.8%) resulted in a correct identification, there were no (0%) mis-identifications, and only 3 organisms (1.2%) resulted in no identification (**Table 3**). The media used in the study for identifications included blood agar (161 isolates), MacConkey agar (73 isolates), and chocolate agar (6 isolates) (**Figure 6**).

## RESULTS CONTINUED:

**Table 1:** Colibrí spotting as compared to manual spotting with MALDI identification for GP bacteria.

| ORGANISM | # TESTED | COLIBRI CORRECT ID | COLIBRI INCORRECT ID | COLIBRI NO ID |
|---|---|---|---|---|
| *Staphylococcus aureus* | 22 | 22/22 | 0/22 | 0/22 |
| *Enterococcus faecalis* | 20 | 20/20 | 0/20 | 0/20 |
| *Staphylococcus epidermidis* | 11 | 11/11 | 0/11 | 0/11 |
| *Streptococcus agalactiae* | 5 | 5/5 | 0/5 | 0/5 |
| *Staphylococcus capitis* | 4 | 4/4 | 0/4 | 0/4 |
| *Staphylococcus lugdunensis* | 3 | 3/3 | 0/3 | 0/4 |
| *Staphylococcus saprophyticus* | 3 | 3/3 | 0/3 | 0/4 |
| *Staphylococcus simulans* | 3 | 3/3 | 0/3 | 0/3 |
| *Streptococcus haemolyticus* | 3 | 2/3 | 0/3 | 1/3a |
| *Streptococcus mitis/oralis* | 2 | 2/2 | 0/2 | 0/3 |
| *Streptococcus vestibularis/S. salivarius ssp salivarius/S. salivarius ssp thermophilus* | 2 | 2/3 | 0/2 | 0/2 |
| *Enterococcus faecium* | 1 | 1/1 | 0/1 | 0/1 |
| *Streptococcus anginosus group* | 1 | 1/1 | 0/1 | 0/1 |
| *Streptococcus gallolyticus ssp pasteurianus* | 1 | 1/1 | 0/1 | 0/1 |
| *Streptococcus parasanguinis* | 1 | 1/1 | 0/1 | 0/1 |
| *Streptococcus pyogenes* | 1 | 1/1 | 0/1 | 0/1 |
| *Gemella haemolysans* | 1 | 0/1 | 0/1 | 1/1b |
| *Cutibacterium acnes* | 1 | 1/1 | 0/1 | 0/1 |
| **TOTAL** | **85** | **83/85 (97.6%)** | **0/85 (0%)** | **2/85 (2.4%)** |

**Table 2:** Colibrí spotting as compared to manual spotting with MALDI identification for GN bacteria.

| ORGANISM | # TESTED | COLIBRI CORRECT ID | COLIBRI INCORRECT ID | COLIBRI NO ID |
|---|---|---|---|---|
| *Esherichia coli* | 61 | 61/61 | 0/61 | 0/61 |
| *Klebsiella pneumoniae* | 24 | 23/24 | 0/24 | 1/24c |
| *Pseudomonas aeruginosa* | 18 | 18/18 | 0/18 | 0/18 |
| *Enterobacter cloacae* | 7 | 7/7 | 0/7 | 0/7 |
| *Klebsiella aerogenes* | 6 | 6/6 | 0/6 | 0/6 |
| *Serratia marcescens* | 6 | 6/6 | 0/6 | 0/6 |
| *Proteus mirabilis* | 5 | 5/5 | 0/5 | 0/5 |
| *Klebsiella oxytoca* | 5 | 5/5 | 0/5 | 0/5 |
| *Morganella morganii* | 4 | 4/4 | 0/4 | 0/4 |
| *Citrobacter koseri* | 4 | 4/4 | 0/4 | 0/4 |
| *Citrobacter freundii* | 2 | 2/2 | 0/2 | 0/2 |
| *Enterobacter cloacae complex* | 2 | 2/2 | 0/2 | 0/2 |
| *Stenotrophomonas maltophilia* | 2 | 2/2 | 0/2 | 0/2 |
| *Moraxella catarrhalis* | 1 | 1/1 | 0/1 | 0/1 |
| *Leclercia adecarboxylata* | 1 | 1/1 | 0/1 | 0/1 |
| *Klebsiella variicola* | 1 | 1/1 | 0/1 | 0/1 |
| *Acinetobacter radioresistans* | 1 | 1/1 | 0/1 | 0/1 |
| *Citrobacter braakii* | 1 | 1/1 | 0/1 | 0/1 |
| *Citrobacter farmer* | 1 | 1/1 | 0/1 | 0/1 |
| *Neisseria flava/perflava/subflava* | 1 | 1/1 | 0/1 | 0/1 |
| *Raoultella ornithinolytica* | 1 | 1/1 | 0/1 | 0/1 |
| *Raoultella spp* | 1 | 1/1 | 0/1 | 0/1 |
| **TOTAL** | **155** | **154/155 (99.4%)** | **0/155 (0%)** | **1/155 (0.6%)** |

**Table 3:** Overall MALDI identifications obtained with Colibrí spotting as compared to manual spotting.

| TOTAL ORGANISMS TESTED | GENERA | SPECIES | CORRECT IDENTIFICATION | INCORRECT IDENTIFICATION | NO IDENTIFICATION |
|---|---|---|---|---|---|
| 240 | 19 | 40 | 98.8% | 0.0% | 1.2% |

## CONCLUSIONS:

The Colibrí from Copan provides highly accurate automation to the previously manual process of MALDI-TOF target plate spotting.

100% concordance between manual and automated processing was observed in both testing laboratories using multiple staff showing the excellent functionality of the Colibrí across study sites.

To prepare an entire slide for MALDI analysis can take a technologist ~ 15 minutes which can lead to hours a day spent on this manual process. The use of the Colibrí instrumentation will significantly decrease labor time and expense for identification of organisms by MALDI in the laboratory.

The Colibrí automates this repetitive task and provides an opportunity to reallocate technologists' time to more valuable assignments.

The Colibrí standardizes MALDI spotting and provides traceability from plating to organism identification.