Automatic preparation of MALDI_TOF targets with Copan Colibrí®

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Background:

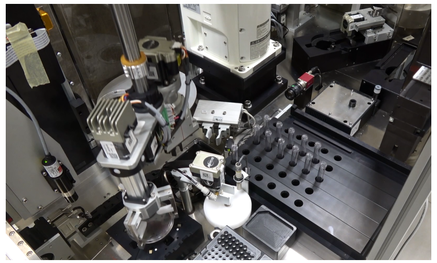
Microbial identification using MALDI-TOF Mass Spectrometry Technology is a necessary tool to speed up the diagnosis process. Despite the rapidity and the simplicity of the method, the preparation of the sample and the managing of patient information are still time consuming and subject to human errors. Copan Colibrí® is a new automated system for the preparation of targets for Mass Spectrometry, microbial suspension for Antimicrobial Susceptibility testing, purity and subculture plates.

Objective:

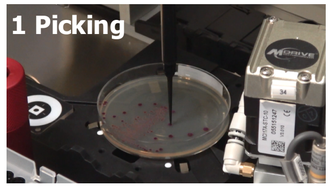
The objective of this study was to evaluate the performance of Colibrí® for targets preparation to be analyzed with MALDI-TOF Mass Spectrometry Technology.

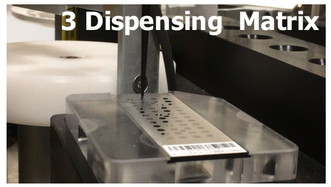
Materials/methods:

Clinical strains (n=326) were plated on TSA+5% sheep blood (BD), Orientation CHROMagar plate (BD), PVX, CPS Elite, Mac Conkey (Biomerieux). Isolated colonies were spotted on target by Colibrì® and processed on Vitek MS (Version 5.0.1, Biomerieux). In this study, clinical strains were tested in duplicate and only 1 colony was deposited in each spot of the target. Clinical isolates included: *Escherichia coli* (n=26), *Enterobacter aerogenes* (n=20), *Citrobacter koseri* (n=8), *Acinetobacter baumanii* (n=16), *Eikenella corrodens* (n=12*), Klebsiella oxitoca* (n=102), *Klebsiella pneumoniae* (n=56), *Kingella kingae* (n=4), *Moraxella catarrhalis* (n=4), *Neisseria gonorrhoeae* (n=4), *Haemophilus influenzae* (n=4), *Stenotrophomonas maltophilia* (n=8), *Staphylococcus capitis* (n=4), *Staphylococcus haemolyticus* (n=8), *Serratia marcescens* (n=8), *Salmonella spp* (n=16), *Vibrio cholera* (n=8), *Staphylococcus epidermidis* (n=30), *Pseudomonas aeruginosa* (n=156), *Staphylococcus aureus* (n=51), *Enterococcus faecalis* (n=53) and *Enterococcus faecium* (n=54).



Planar view of the Colibrì®:
The agar plate is released on the picking station





Colibrí® is able to:

- Pick bacteria colonies with disposable tips (1)
- Spot bacteria colonies on the target (2)
- Dispense the matrix on the spot (3)

Results:

Results obtained by Vitek MS were categorized as:

- **Good discrimination** when a single identification is displayed with a confidence value of 60 to 99.9.
- **Low discrimination** when more than one significant organism was displayed or no identification was found for the acquired spectrum.
- **No discrimination** due to insufficient picks detected by the instrument.

Displayed results:

- **Good discrimination:** 571 spots
- **Low discrimination:** 29 spots included *Enterococcus faecalis* (5/53), *Enterococcus faecium* (9/54), *Klebsiella pneumoniae* (3/56), *Pseudomonas aeruginosa* (5/156), *Staphylococcus epidermidis* (4/30), *Staphylococcus aureus* (3/51).
- **No discrimination:** 52 spots included *Enterococcus faecalis* (3/53), *Enterococcus faecium* (27/54), *Klebsiella pneumoniae* (2/56), *Pseudomonas aeruginosa* (15/156), *Staphylococcus epidermidis* (3/30), *Staphylococcus aureus* (2/51).

	Good discrimination	Low discrimination	No discrimination
Gram negative (n=452)	n=427 **(94%)**	n=8 **(2%)**	n=17 **(%)**
Escherichia coli (n=26)	n=26 **(100%)**	n=0	n=0
Enterobacter aerogenes (n=20)	n=20 **(100%)**	n=0	n=0
Citrobacter koseri (n=8)	n=8 **(100%)**	n=0	n=0
Acinetobacter baumanii complex (n=16)	n=16 **(100%)**	n=0	n=0
Eikenella corrodens (n=12)	n=12 **(100%)**	n=0	n=0
Klebsiella oxitoca (n=102)	n=102 **(100%)**	n=0	n=0
Klebsiella pneumoniae (n=56)	n=51 **(91%)**	n=3 **(5%)**	n=2 **(4%)**
Kingella kingae (n=4)	n=4 **(100%)**	n=0	n=0
Moraxella catarrhalis (n=4)	n=4 **(100%)**	n=0	n=0
Neisseria gonorrhoeae (n=4)	n=4 **(100%)**	n=0	n=0
Pseudomonas aeruginosa (n=156)	n=136 **(87%)**	n=5 **(3%)**	n=15 **(10%)**
Haemophilus influenzae (n=4)	n=4 **(100%)**	n=0	n=0
Stenotrophomonas maltophilia (n=8)	n=8 **(100%)**	n=0	n=0
Serratia marcescens (n=8)	n=8 **(100%)**	n=0	n=0
Salmonella spp (n=16)	n=16 **(100%)**	n=0	n=0
Vibrio cholerae (n=8)	n=8 **(100%)**	n=0	n=0
Gram positive (n=200)	n=144 **(72%)**	n=21 **(10%)**	n=35 **(18%)**
Enterococcus faecalis (n=53)	n=45 **(85%)**	n=5 **(9%)**	n=3 **(6%)**
Enterococcus faecium (n=54)	n=18 **(33%)**	n=9 **(17%)**	n=27 **(50%)**
Staphylococcus epidermidis (n=30)	n=23 **(77%)**	n=4 **(13%)**	n=3 **(10%)**
Staphylococcus aureus (n=51)	n=46 **(90%)**	n=3 **(6%)**	n=2 **(4%)**
Staphylococcus capitis (n=4)	n=4 **(100%)**	n=0	n=0
Staphylococcus haemolyticus (n=8)	n=8 **(100%)**	n=0	n=0
Total *(n=652)*	n=571 **(88%)**	n=29 **(4%)**	n=52 **(8%)**

Conclusions:

- Bacterial strains selected for the evaluation included a substantial number of bacterial strains with challenging morphological characteristics.
- Colibrí® generated 88% of reliable identifications and 12% of poor or no discrimination.
- Poor or no discrimination was associated to *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* mucoid phenotypes and *Enterococcus faecalis*, *Enterococcus faecium* and *Staphylococcus epidermidis with s*mall and dry colonies.
- Given these results, Copan will implement the possibility to pick and transfer more than 1 colony for each spot.
- Colibrí® can be used as a target preparator associated to a Copan WASPLab® to standardized the process in the microbiology laboratory.

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