How to automatize the preparation of microbial suspensions for Antimicrobial Susceptibility testing (AST)

Laura Navarria, Francesca Maino, Mario Savarese
Copan Italia, Brescia, Italy

Laura Navarria
Laura.navarria@copangroup.com

COPAN
Innovating Together™

June 3rd
Poster N. 476

asm microbe 2017 JUNE 1–5 • NEW ORLEANS

Background:

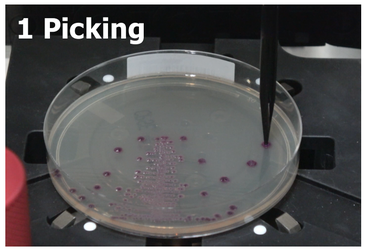
Automated Antimicrobial Susceptibility Systems provide reliable results improving clinical outcomes although the microbial suspension preparation is still completely manual. Colibrí® is a new automated system able to pick colonies from plates, re-suspend them in saline solution, check the turbidity of the suspension with the on-board nephelometer and adjust it if required.

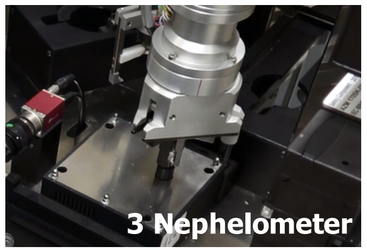
Objective:

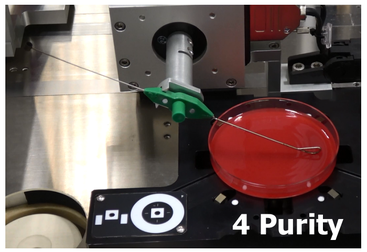
The aim of the study was to compare manual microbial suspensions preparation for Antimicrobial Susceptibility Testing (AST) to Colibrí® automated preparation.

Materials/methods:

Twenty-Two Gram negative and twenty-two Gram positive clinical strains were streaked on CPS®Elite (Biomerieux) agar plates using Copan WASP® and incubated in WASPLab® automation. Isolated colonies were manually picked and suspended in saline solution to obtain a 0.5 McFarland microbial concentration measured with the Biomerieux DensiCHECK Plus. The same plate was then processed by Colibrí®: selected bacteria colonies were automatically picked by the **pipettor** and transferred into a saline solution tube. The turbidity of the microbial suspension was verified by the **on-board nephelometer.** Manual and automated prepared microbial suspensions were used to perform the AST on the Biomerieux **Vitek2** (version 07.01) as per manufacturer instructions. Gram negative (*E. coli* (n=6), *E. cloacae* (n=5), *P. aeruginosa* (n=3) *S. marcescens* (n=2), *P. mirabilis* (n=3), *K. oxytoca* (n=1), *K. pneumoniae* (n=1). *C. freundii* (n=1)) were processed with AST-N204 card and Gram positive (S. epidermidis (n=6), S. aureus (n=8), E. faecalis (n=4), *E. faecium* (n=3), *E. gallinarum* (n=1)) were processed with AST-P592 card. The viable count of 0.5 McFarland suspensions was performed using the serial dilution method on both the manual and automated preparations.







Colibrí® is able to:

- Pick colonies using disposable tips (1)
- Release bacteria colonies in a saline solution tube (2)
- Check the microbial suspension turbidity using the on-board nephelometer (3)
- Adjust the microbial suspension adding colonies or saline to obtain a 0.5 MacFarland
- Prepare the microbial dilution needed for processing with AST system
- Prepare the purity plate from the microbial suspension (4)

Results:

The results were interpreted with Biomerieux Expert System EUCAST 2015:

- 318 antibiotic combinations were tested for Gram positive: 1 minor error (0.31%) and 1 major error (0.31%) were found.
- 306 antibiotic combinations were tested for Gram negative: 9 minor error (2.94%) and 1 very major error (0.32%) were found.
- Only 1 discrepant result could be related to the viable count where the manual preparation was 5-fold higher than Colibrí ® microbial preparation.

Five out of the 44 strains tested were defined as MDR organisms according to Magiorakos *et al* 2011 (1).

	Agreement	Minor error	Major error	Very major error
Gram positive (*n=318*)	*n=316* **(99.38%)**	*n=1* **(0.31%)**	*n=1* **(0.31%)**	*n=0*
Gram negative (*n=306*)	*n=296* **(96.74%)**	*n=9* **(2.94%)**	*n=0*	*n=1* **(0.32%)**

Conclusions:

The data generated in this study demonstrate Copan Colibrí®:

- is able to automatically prepare microbial suspensions from different bacteria colonies directly from agar plate culture.
- provides comparable results to those obtained with manual preparation
- standardizes the preparation of microbial suspensions for AST and purity plates eliminating the person to person variability.
- Reduce the exposure to pathogens improving staff safety

Reference: (1) Magiorakos *et al* 2011; Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance; European Society of Clinical Microbiology and Infectious Diseases