Colibrí™ and Bruker MALDI-TOF: does the identification performance change when different chromogenic media are used for urine culture?

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Background

Urine is one of the most common specimen types submitted to the clinical microbiology laboratory; the use of chromogenic agar is one method that might speed up culture results and reduce hands-on time and materials required for urine culture analysis. A major advantage of most of the chromogenic agars is the ability to directly identify *E. coli* while for the other uropathogens an identification confirmation is required by the use of MALDI-TOF MS.

The COPAN Colibrí™ is a new automated system designed to automatically prepare the target for MALDI-TOF MS identification. Colibrí™ adopts pipettor tips to transfer colonies from agar plates to the target and to cover them with matrix, allowing standardization of target preparation and patient's sample traceability.

The objective of this study was to evaluate the performance of Colibrí™ target preparation and the following MALDI-TOF identification capability (Bruker Biotyper System) when uropathogen colonies grow on different chromogenic agar or on blood-agar-based media.

Material/Methods

One-hundred-thirteen urines were streaked by WASP™ (COPAN) with the 10 µl loop on one non selective media: Tryptic-Soy-Agar with 5% sheep blood (Decton Dickinson), and three chromogenic media: CPSE (Biomerieux), Orientation (Becton Dickinson), UTI (Thermo Fisher Scientific).

After 18 hours of incubation at 35±2°C digital images were acquired by WASPLab™ (COPAN) and examined for colonies growth. Images were analysed by PhenoMATRIX™ (COPAN) and samples were classified as:

- negative in presence of < 100 colonies
- positive in presence of one or two uropathogens with a load ≥10^4 CFU/ml.
- contaminated when ≥ 3 morphotypes grew at the load ≥10^4 CFU/ml.

For each positive sample and morphotype, two colonies were selected and the "Colibrì ID" (identification) Task was assigned in the WebApp (Graphical User Interface of WASPLab™). The media plates were loaded on Colibrí™ and the designed colonies were automatically picked for Bruker Biotyper target preparation (see fig. 1). Re-usable polished steel targets were used.

Identification results obtained from the chromogenic media were compared to those obtained on blood-agar-based media.

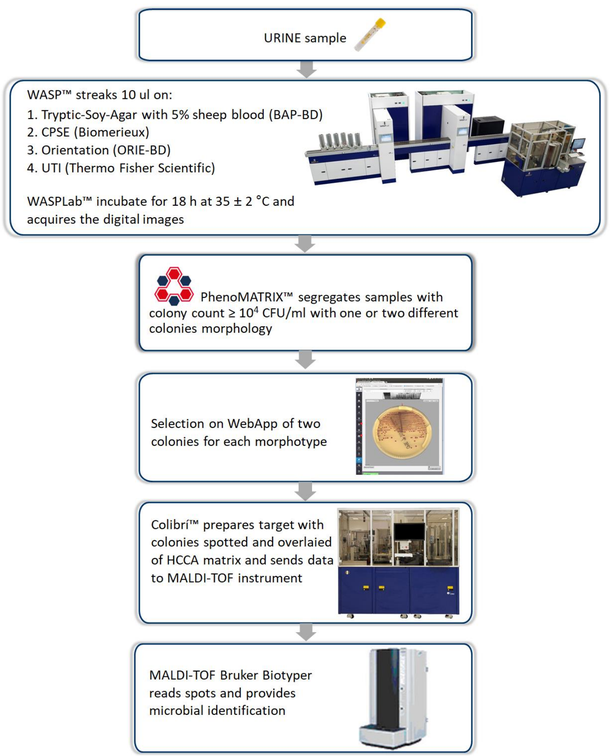

Figure 1: Schematic representation of the workflow

Results

Out of 113 samples, 44 were negative, 8 were classified as contaminated, and 61 were positive. In 57 samples a single uropathogen was present while two bacterial species grew in 4 samples (Fig. 2).

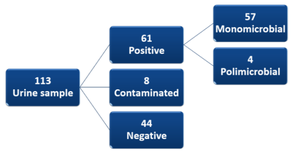

Figure 2: Primary classification of culture results according to Phenomatrix™

A total of 130 spots were processed from colonies on the CPSE agar and 128 from colonies on the other agars.

A 100% concordance was obtained for the identification of Gram-negative bacteria from all media used. In addition, the ID score value for Gram-negatives was ≥ 2.0 for all media evaluated (Table 1).

The identification performance was quite different among the media tested for *E. faecalis*, the only relevant Gram-positive bacteria isolated (Table 1). *E. faecalis* was best detected on CPSE agar with 91% of cases correctly identified. UTI and ORIE were equivalent with their identification performances because both allowed 50% of *E. faecalis* diagnosis. The mean ID value registered for *E. faecalis* identification was ≤ 2.0 for all media evaluated.

Table 1. Identification performance for the clinically relevant uropathogen detected on TSA+5% Sheep Blood and chromogenic agars with a score ≥ 1.7 (spot number and mean ID score value)

	BAP		CPSE		UTI		ORIE	
Microrganism isolated	N.SPOT	Mean ID score value	N.SPOT	Mean ID score value	N.SPOT	Mean ID score value	N.SPOT	Mean ID score value
Citrobacter koseri	4/4	2.545	4/4	2.494	4/4	2.500	4/4	2.483
Enterobacter cloacae	2/2	2.327	2/2	2.316	2/2	2.370	2/2	2.274
Enterococcus faecalis	16/20	1.832	20/22	1.980	10/20	1.875	10/20	1,819
Escherichia coli	84/84	2.441	84/84	2.456	84/84	2.442	84/84	2.446
Hafnia alvei	2/2	2.504	2/2	2.428	2/2	2.423	2/2	2.336
Klebsiella pneumoniae	4/4	2.426	4/4	2.486	4/4	2.464	4/4	2.464
Morganella morganii	2/2	2.548	2/2	2.561	2/2	2.619	2/2	2.524
Proteus mirabilis	10/10	2.537	10/10	2.504	10/10	2.546	10/10	2.484
No Identification / No peaks	4*	NA	2*	NA	10*	NA	10*	NA
TOTAL NUMBER OF SPOTS	128		130		128		128	

* No identification and no picks result were obtained for *E. faecalis* colonies; NA: not available

Supplementary evaluation of a new cycle for Gram positive bacteria

To overcome the critical identification results obtained with *E. faecalis* grown on UTI and ORIE an additional testing cycle with the addition of formic acid was performed. This procedure allowed to obtain the correct identification in 85% of *Enterococcus* spp. infections. 83 spots of *E.faecalis* and 8 spots of *E.faecium* were respectively identified with a mean score value of 2.194 and 1.9.

Results demonstrate that the formic acid aids the identification of Gram positive bacteria.

Conclusion

The present study demonstrated that Colibrí™ properly prepared MALDI-TOF targets allowing a correct identification of all Gram-negative uropathogens independently from the culture agars. Although *E.faecalis* spotting and identification were initially more critical from ORIE and UTI agars, a new cycle with the addition of formic acid was developed and evaluated and preliminary results are showing an increasing identification performance.

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