To MALDI or Not to MALDI: Specificity of Chromogenic Agars for Identifying *Escherichia coli* from Urine Cultures

eP485
ECCMID 2014

B. M. Willey, O. Leung, I. Manji, S. Kim, A. Tsang, E. Ramoutar, S. Tharmaradinam, P. Lo, S. M. Poutanen
Mount Sinai Hospital/University Health Network, University of Toronto, Toronto, Ontario, Canada

ABSTRACT

Objectives: Renewed interest in the specificities of chromogens in urine agars arose as a potential way to reduce the number of isolates requiring MALDI-TOF testing from urine cultures. 'Burgundy-pink' (BP) colonies on urine chromogenic agars are claimed by manufacturers to distinguish *E. coli,* the most common urinary pathogen, from other bacteria. This prospective study aimed to determine whether development of BP colonies was sufficiently specific for *E. coli* such that further identification could be eliminated.

Methods: To reduce inoculation variability, the Copan WASP sequentially pipetted 1uL from 2500 consecutive urines to five agars: 5% Sheep Blood (BA), MacConkey w/out crystal violet (MAC), Brilliance UTI Clarity (UTIC; Oxoid), CPS 4 ChromID (CPS4; bioMérieux) and Colorex Orientation (CORI; Alere). Growth was interpreted following clinical laboratory BA/MAC definitions of significant (SG), non-SG (NSG) or mixed (MG) cultures. Cultures were read at 16-20h by persons blinded to each others' results. Quantities, colours and sizes of all colony types were documented in Access. Isolate identification was by MALDI-TOF (bioMérieux VITEK-MS Plus). Analyses included all BP-coloured isolates and all *E. coli* regardless of colony colour, whether from SG or inadvertently from NSG or MG cultures. As MALDI-TOF cannot distinguish *E. coli* from *Shigella*, *Shigella* was ruled out in non-BP, non-lactose-fermenting *E. coli* using the Wellcolex Colour Shigella kit (Oxoid). Statistics were calculated using www.graphpad.com.

Results: Of 2584 isolates identified from all agars, 814 were BP-coloured of which 100% were *E. coli* (CPS4: n=282; 249 SG; 33 NSG/MG; UTIC: n=270; 246 SG; 24 NSG/MG; CORI: n=262; 236 SG; 26 NSG/MG). This resulted in a BP-specificity of 100% (95%CI: 99.7-100) for all chromogenic agars combined. Of 282 *E. coli* identified on CPS4, all were BP (CPS4 BP-sensitivity: 100%; 95%CI: 98.4-100). Of 280 *E. coli* identified on UTIC, all were BP except 10 (3.6%) which were 'cream-translucent' (CRM) (UTIC BP-sensitivity: 96.4%; 95%CI: 93.5-98.1%). Of 286 *E. coli* identified on CORI, all were BP except 4 (1.4%) that were CRM and 20 (7%) that were light-BP (CORI BP sensitivity: 91.6%; 95%CI: 87.8-94.3%). The proportion of *E. coli* detected as BP on CPS4 (100%) was significantly higher compared to UTIC (96.4%; P=0.0009) and CORI (91.6%; P<0.0001)

Conclusions: This study demonstrated that BP colour on all urine chromogenic agars tested was 100% specific for *E. coli* and that BP colour alone may be considered sufficient for species-level identification of *E. coli* from urine cultures without the need for additional confirmatory testing. While use of any of these chromogenic agars will dramatically reduce the workload associated with *E. coli* identification from urines when implementing MALDI-TOF, CPS4 had an advantage as it detected a significantly higher proportion of *E. coli* as BP (100%) compared to UTIC (96.4%) and CORI (91.6%).

INTRODUCTION

Most clinical microbiology laboratories are changing from traditional biochemical identification methodologies to more rapid and cost-effective protein-based MALDI-TOF mass spectrometry (MS). As identification by MS is applicable to all bacteria, large laboratories are attempting to reduce the number of organisms submitted for MALDI-TOF so as to streamline workflow and better utilize MS technology.

When considering MS for identification of urinary pathogens, it is clear that there would be a large volume of organisms requiring identification. Since *E. coli* are the most common opportunistic pathogen associated with urinary tract infections, and account for roughly 40-50 percent of significant isolates reported, it would improve the turn-around-times for MS-identification of other urine pathogens and pathogens from other specimen types if MS of urinary *E. coli* could be circumvented.

Chromogenic agars for urine culture have been available for many years. Many laboratories did not adopt their use due to early issues with poor Gram-positive growth and high costs. As new formulations reputedly improved performance and costing is more reasonable, their use is again being considered especially since claims that chromogenic substrate cleavage by b-galactosidase of *E. coli* produces highly-specific "burgundy-pink" colonies.

OBJECTIVES

The aim of this prospective study was to determine whether the observation of the development of "burgundy-pink" coloured colonies of three differently formulated chromogenic urine agars would be sufficiently sensitive and specific for *E. coli* grown from urines to enable other methods of identification to be eliminated.

MATERIALS & METHODS

This 3 month prospective study utilized 2,500 unselected, semi-consecutive urine specimens plated in parallel to the current laboratory algorithm of Oxoid's 5% sheep blood (BA) and MacConkey without salt or crystal violet (MAC) agars to study agars using the Copan Walk-Away Specimen Processor (WASP).

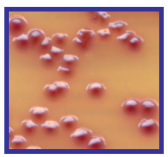
The WASP inoculated and streaked 1ul aliquots of each specimen to the following agars: BA, MAC, Brilliance UTI Clarity (Oxoid), CPS4 chromID (bioMérieux), and Colorex Orientation (Alere). Plate orders were not varied since the WASP incinerates the inoculation loop between each aliquot, thereby eliminating inoculation variables often associated with agar comparison studies.

Agars were examined independently after 16-20h incubation at 37°C, at which time quantities, colours and sizes of all colony types were documented. Any "burgundy-pink" colony observed on chromogenic agars, regardless of the significance of growth, was subjected to MALDI-TOF identification using the VITEK MS PLUS system (bioMérieux); any non-burgundy-pink isolate grown in significant quantities was similarly identified by VITEK MS PLUS. On laboratory agars, *E. coli* were identified using conventional laboratory methods that included detection of b-glucuronisidase and indole production, and if negative, a VITEK 2 Gram Negative Identification card (bioMérieux) was used.

Results of chromogenic agars were documented into individual queries within an Access study database to maintain blinding between plate examiners. Results obtained by the clinical laboratory were imported into the database from with LIS and discrepancies were resolved on a weekly basis.

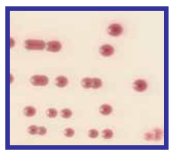
After study completion, sensitivity and specificity of "burgundy-pink" was determined, and statistical analyses were calculated using the on-line QuickCalcs program at www.graphpad.com.

Study agars growing *E. coli*



Brilliance UTI Clarity agar from Oxoid

Colorex Orientation agar from Alere



CPS4 chromID agar from bioMérieux

RESULTS

From the 2500 urines cultured, a total of 2584 isolates were subjected to VITEK MS from all chromogenic study agars combined. Of the 814 (31.5%) "burgundy-pink" coloured isolates identified, 282 were from CPS4 chromID, 270 from Brilliance UTI, and 262 from Colorex Orientation agars.

Without exception, every burgundy-pink organism tested by VITEK MS was identified as *E. coli,* resulting in a "burgundy-pink" specificity of 100% (95% CI: 99.7-100%) for all chromogenic agars combined.

While all 282 (100%) *E. coli* on CPS4 chromID produced "burgundy-pink" colonies, 10 (3.6%) of 280 *E. coli* on Brilliance UTI Clarity produced "cream-translucent" colonies, and 4 (1.4%) and 20 (7%) of 286 *E. coli* on Colorex Orientation produced "cream-translucent" and 'light-pink' colonies, respectively (see TABLE 1 below for sensitivities and 95% confidence intervals).

The proportion of *E. coli* producing 'burgundy-pink" colonial morphology was significantly higher on CPS4 chromID than on Brilliance UTI Clarity (P=0.0009) or Colorex Orientation (P<0.0001) agars.

TABLE 1: Performance statistics of urine chromogenic agars for producing "burgundy-pink" colonies with *Escherichia coli*

	Brilliance UTI Clarity	Colorex Orientation	CPS4 chromID
Sensitivities (95% CI)	96.4% (93.5-98.1%)	91.6% (87.8-94.3%)	100% (98.4-100%)
Specificities (95% CI)	100% (97-100%)	100% (97-100%)	100% (97-100%)

CONCLUSIONS

•This study found "that the burgundy-pink" colour of colonies on three different chromogenic urine agars produced by the enzymatic degradation of the chromogenic substrate by b-galactosidase was sufficiently specific (100%) for the identification of *E. coli* so as to enable the elimination of other methods for the identification of *E. coli* isolated from urines.

•As some *E. coli* produced clear or "light-pink" rather than "burgundy-pink" colonies, sensitivities varied significantly between agars: CPS4 chromID was most sensitive at 100%, followed by Brilliance UTI Clarity at 96.4% (P=0.0009) and Colorex Orientation at 91.6% (P<0.0001). In such cases, identification by MALDI-TOF or conventional methods would still be required.

Acknowledgements

We extend our gratitude to Alere and bioMérieux for providing student support and chromogenic agars, and to Oxoid for the provision of chromogenic agars and all other plates and supplies, without which this study would not have been possible.

bwilley@mtsinai.on.ca