TECHNICAL NOTE

Identification of urinary pathogens by the RAPIDEC[™] ur system

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Summary. A commercial method (RAPIDEC[™] ur; bioMérieux UK Ltd, Basingstoke) and an in-house scheme based on conventional tests were compared for the identification of 179 commonly encountered gram-negative urinary pathogens. The methods identified correctly to genus level 97% and 84% of organisms, respectively. RAPIDEC ur was superior at identifying *Escherichia coli*, due to the detection of β -glucuronidase activity, but less cost-effective than the in-house scheme.

Urine specimens are among the commonest specimens submitted to hospital microbiology laboratories and the investigation of urinary tract infection (UTI) may place huge demands on laboratory resources. Non-cultural techniques based on visual appearance and the detection of leucocyte esterase, nitrite, blood or protein are becoming increasingly popular screening methods for predicting negative samples^{1,2} but only culture can provide epidemiological information about aetiological bacteria. The full identification of isolates is usually unwarranted and considered unnecessary, except for isolates from immunocompromised patients and multi-resistant strains. Moreover, a limited range of organisms cause uncomplicated UTIs and these are associated with highly predictable antimicrobial susceptibility patterns.³ Gram-negative bacilli are responsible for > 80% of UTIs⁴ and methods for their identification must be rapid, simple, inexpensive and give adequate information for presumptive antimicrobial therapy. Identification schemes for these organisms can be based on differential growth characteristics on selective and non-selective media⁵ and may be enhanced by the incorporation of media for the detection of enzymic activity associated with specific organisms. An example of this is the detection of β -glucuronidase activity, a characteristic of *Escherichia coli* and some shigellae; however, the latter are rarely isolated from the urinary tract.⁶ Recently, a single non-selective medium has been proposed which enables the detection of five biochemical characteristics useful in differentiating gram-negative urinary pathogens.⁷ In the present study, an in-house identification scheme based on conventional tests was compared with a

commercial method for the rapid characterisation of the most commonly encountered gram-negative urinary pathogens.

One hundred and seventy-nine gram-negative organisms isolated from consecutive urine cultures on Cysteine-Lactose-Electrolyte Deficient Medium (Oxoid) and horse-blood 5% agar (Oxoid) were identified by an in-house identification scheme. Lactose-fermenting (LF) or late lactose-fermenting coliform organisms were identified by a modification of a previously described scheme⁸ as *E. coli* (flat or mucoid colony, spot indole test positive), *Klebsiella* spp. (mucoid, spot indole test negative and ampicillin resistant) or coliforms (flat, spot indole test negative or mucoid, spot indole test negative and ampicillin sensitive). Non-lactose fermenters (NLFs) that swarmed on blood agar were identified as *Proteus* spp. and further differentiated by indole production into *P. mirabilis* and *P. vulgaris*. Non-swarming NLFs were identified as *Pseudomonas* spp. if oxidase positive. Other NLFs were classified by the spot indole test and 4-h tube tests for urease and *o*-nitro-phenyl- β -D-galactopyranosidase (ONPG) activity into *E. coli* (indole positive, ONPG positive, urease negative), *P. mirabilis* (indole negative, ONPG negative, urease positive) and *P. vulgaris* (indole positive, ONPG negative, urease positive). Urease- and ONPG-negative organisms were subjected to further biochemical testing and any potential *Salmonella* isolates were confirmed by serology. All other organisms were classified as coliforms.

The methodology of the RAPIDEC[™] ur test strip has been described in detail elsewhere.⁹ Briefly, after incubation for 2 h nine enzymes were detected by a colour or other change, directly or following addition of appropriate reagents. The enzymes included

Table. Identification results obtained with the three methods

API 20E	In-house scheme	RAPIDEC ur
73 <i>Escherichia coli</i>	61 <i>Escherichia coli</i> 9 coliforms 2 <i>Klebsiella</i> spp. 1 coliform	72 <i>Escherichia coli</i> 1 GNR, oxidase neg*
37 <i>Klebsiella pneumoniae</i>	34 <i>Klebsiella</i> spp. 2 <i>Escherichia coli</i> 1 coliform	37 Kleb/Enter/Citro†
37 <i>Proteus mirabilis</i>	37 <i>Proteus mirabilis</i>	37 <i>Proteus mirabilis</i>
15 <i>Pseudomonas aeruginosa</i>	15 <i>Pseudomonas</i> spp.	15 <i>Pseudomonas</i> spp.
4 <i>Enterobacter cloacae</i>	3 <i>Klebsiella</i> spp. 1 coliform	4 Kleb/Enter/Citro
4 <i>Proteus vulgaris</i>	4 <i>Proteus vulgaris</i>	4 Prot/Morg/Prov‡
3 <i>Citrobacter freundii</i>	3 coliforms	3 GNR, oxidase neg
2 <i>Acinetobacter</i> spp.	1 <i>Escherichia coli</i> 1 coliform	2 GNR, oxidase neg
1 <i>Morganella morganii</i>	1 <i>Proteus vulgaris</i>	1 Prot/Morg/Prov
1 <i>Providencia stuartii</i>	1 <i>Proteus mirabilis</i>	1 Prot/Morg/Prov
1 <i>Citrobacter diversus</i>	1 <i>Escherichia coli</i>	1 Kleb/Citro§
1 <i>Klebsiella oxytoca</i>	1 <i>Escherichia coli</i>	1 Kleb/Citro

*Gram-negative rod, oxidase negative.

†*Klebsiella pneumoniae*/*Enterobacter*/*Citrobacter*.‡*Proteus vulgaris*/*Morganella*/*Providencia*.§*Klebsiella oxytoca*/*Citrobacter diversus*.

l-alanine arylamidase; β -glucuronidase, phenylalanine deaminase, proline arylamidase, β -galactosidase, β -glucosidase, β -xylosidase, oxidase and catalase. Identification to species level, or into species or genus group, was made with the flowchart provided by the manufacturer.

The identity of all 179 test isolates was confirmed by API 20E (bioMérieux) and included: *E. coli* (73 isolates), *K. pneumoniae* (37), *P. mirabilis* (37), *Ps. aeruginosa* (15), *Enterobacter cloacae* (4), *P. vulgaris* (4), *Citrobacter freundii* (3), *Acinetobacter* spp. (2), *Morganella morganii* (1), *Providencia stuartii* (1), *C. diversus* (1) and *K. oxytoca* (1).

The results obtained by all three methods are shown in the table. In total, when compared with the API 20E, 151 (84%) of 179 organisms were correctly identified to at least genus level by the in-house method compared to 173 (97%) by the RAPIDEC ur system. However, the greater success of RAPIDEC ur was in part due to the grouping of genera under headings such as "*Klebsiella pneumoniae*/*Enterobacter*/*Citrobacter*" and "*Proteus vulgaris*/*Morganella*/*Providencia*". The in-house method failed to identify 12 of 73 *E. coli* isolates; three were ONPG-negative NLFs which could not have been identified without further tests, but a further six LF were spot indole test negative on primary testing but indole positive in the API 20E; false negative spot indole test results have been reported previously.¹⁰ The inclusion of a test for β -glucuronidase, highly specific for *E. coli* when used on isolates obtained from urine cultures, and included in RAPIDEC ur, would be a valuable addition to basic tests. Although only 89% of *E. coli* strains were β -glucuronidase positive, less than in previous studies,^{9,11} detection of these *E. coli* strains would improve the performance of the in-house identification method to > 90%.

RAPIDEC ur also enables the identification of gram-positive organisms and *Staphylococcus aureus*/*S. epidermidis* (19 isolates), *S. saprophyticus* (7), *Enterococcus* spp. (24) and *Candida albicans* (8) were identified correctly. It is of note that *S. saprophyticus* gives a positive reaction with the β -galactosidase substrate used in RAPIDEC ur, but not in the conventional ONPG test, with time-saving implications.

In identifying urinary isolates a compromise must be made between an appropriate level of accuracy, cost effectiveness and providing a rapid result. Both RAPIDEC ur and the in-house method identified most of the organisms encountered on the day of isolation. The consumables for the in-house method were cheap and technically undemanding to prepare; *p*-dimethylaminobenzaldehyde 5% in aqueous hydrochloric acid 10%, used for rapid detection of indole production, was stable and could be prepared in bulk. Well separated colonies on primary plating are necessary for both methods and up to five colonies may be needed to achieve an adequate inoculum for the RAPIDEC ur. The methods were similar in operator time and neither posed a significant hazard to the user. RAPIDEC ur removes some of the subjective judgement of colonial morphology, relied upon in the in-house system, although it is recommended that both antimicrobial susceptibility patterns and colonial morphology be taken into consideration when interpreting results.

The in-house method was more cost-effective although a direct comparison with the gram-negative component alone of RAPIDEC ur was not possible. The average cost of the in-house identification method, including preparation time, was £0.19 per isolate compared with £0.89 for each RAPIDEC ur. There is now a wide choice of good commercial identification

systems on the market, although at a substantial cost. With increasing financial restraints being imposed on laboratories the pressure to compare the cost effectiveness of commercial systems to in-house methods will be great. If in-house methods are used full attention to internal quality control must be employed to meet

both standards imposed by external accreditation bodies and to guarantee that patient management is not compromised. The present study showed that basic simple laboratory tests still have an important role to play in the identification of commonly encountered urinary pathogens.

References

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