β -glucuronidase (PGUA) and β -xylosidase (ONPX) disc tests for rapid identification of urinary coliforms

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The reporting of urine cultures usually entails a compromise between accuracy and speed in the clinical laboratory. Most conventional biochemical tests and commercial systems such as API 20E (API Laboratory Products Ltd) require overnight incubation. Even the so called rapid systems may require four hours of incubation, so that results are not available until late in the day. An "instant" method has been proposed in which lactose fermenters are differentiated as *Escherichia coli* or *Klebsiella* sp or *Enterobacter* sp by the pattern of their colony growth on MacConkey agar and the spot indole reaction.¹ The commonest non-lactose fermenter occurring in urine cultures is also *E coli*,² and therefore other means of identification are required.

An alternative to conventional biochemical tests is the detection of specific enzyme activities of a non-multiplying organism by hydrolysis of defined chromogenic substrates. Kilian and Bülow developed tube tests for bacterial glycosidases,³ in which dense suspensions of organisms in buffered substrate solutions were incubated at 37°C for four hours. β -glucuronidase activity (PGUA) was specific for *E coli*, the commonest cause of urinary tract infections, and *Shigella* spp. which are only rarely found in urine. β -xylosidase-activity (ONPX) was confined to strains of the *Klebsiella* or *Enterobacter* group and *Yersinia* spp.³ The incubation period of the tube tests may be reduced to as little as one hour.⁴ To eliminate this extra incubation period we devised a method in which paper discs impregnated with a substrate are applied to urine culture plates at the time of inoculation, and any colour change is observed after overnight incubation.

Material and methods

p -nitrophenyl β -D glucopyranosiduronic acid (PGUA) (0.1 g) was dissolved in 10 ml of 0.2 M Tris-hydrochloric acid buffer (pH 8.5) to give hydrochloric acid a final concentration of 1% (w/v). o -nitrophenyl β -D-xylopyranoside (ONPX) (0.1 g) was dissolved in 0.5 ml dimethyl sulphoxide: 1/15M phosphate buffer (pH 8.0) was added to a total volume of 10 ml, giving a final concentration of 1% (w/v). The solutions were

sterilised by filtration. Antibiotic assay discs (Whatman AA discs), 6 mm in diameter, were sterilised in a high vacuum autoclave. Each was then impregnated with 0.03 ml of one of the substrate solutions, delivered by a pasteur pipette. The discs were dried at 37°C and then stored in screw capped universal containers at 4°C until use.

For the disc test, a fresh subculture of the test organism was inoculated into peptone water to produce a faint opacity, and a loopful of the suspension placed on Isosensitest agar was spread evenly with a cotton wool swab. PGUA and ONPX discs were then placed on opposite halves of the plate. After incubation at 37°C for 18 hours, a positive reaction was shown by a yellow colouration of the disc, which if strong, stained the surrounding growth for up to 2–3 cm in diameter.

Ninety one stored strains of coliforms previously identified by API 20E and examined by the PGUA and ONPX tube tests were tested by the disc method described above. After reading the tests a loopful of culture from the periphery of the plate remote from the discs was used to repeat the tube tests.

A further collection of 105 coliforms freshly isolated from urine specimens was similarly tested by the disc and tube methods. These were selected as ampicillin and cephalothin resistant and thus likely to include a high proportion of *Klebsiella* or *Enterobacter* strains.

Finally, the PGUA disc test was carried out on a series of routine urine cultures. A PGUA disc was applied to the centre of an Isosensitest agar plate inoculated with urine, with up to six antibiotic discs placed peripherally. After overnight incubation at 37°C the PGUA disc test was read at the same time as the culture was evaluated on accompanying media, strains giving a positive PGUA reaction being reported directly as *E coli*. A loopful of growth from the sensitivity plate was subsequently tested by the PGUA and ONPX tube methods to confirm the disc reaction. A total of 385 unselected urine specimens producing considerable growths of coliforms were tested in this way.

PGUA and ONPX disc tests were carried out on the following media in parallel: Isosensitest agar (the reference medium), Columbia agar, blood agar, MacConkey medium, and CLED medium (all Oxoid). The 33 test strains included 18 PGUA disc positive strains and eight ONPX disc positive strains. The reproducibility of the disc tests was determined for 26 PGUA disc positive and 15 PGUA disc negative strains of *E coli* on Isosensitest agar. Duplicate PGUA discs were placed on inoculated plates, which were incubated at 37°C overnight. Growth taken well away from the region of a disc (so as to avoid any possibility of enzyme induction) was then used to inoculate a further plate, and the test repeated. In this way the disc

Technical methods

test was repeated on each strain for four consecutive days.

Results

The detailed results are shown in the table. Of the 196 bacterial strains tested, 112 gave a positive result by the PGUA tube test, and 104 (93%) of these were PGUA disc positive. Forty two strains gave a positive result by ONPX tube test, and 41 (98%) of these were ONPX disc positive. One strain gave a false positive ONPX disc test result initially, but was negative on retesting.

Of the 385 direct urine cultures tested, 324 gave a positive PGUA tube test result, of which 296 were PGUA disc positive (91%). Eleven cultures that were PGUA disc positive, PGUA tube positive, and ONPX tube positive were found on subculture to consist of mixtures of *E coli* with *Klebsiella* or *Enterobacter* or other organisms. Strains that were initially disc negative and tube positive for a substrate were still found to be disc negative when retested. The overall concordance between the disc and tube tests for the whole investigation was 533 of 581 or 92%.

Identical results were obtained on both Isosensitest and Columbia agars. None of the strains gave a positive disc reaction on blood agar or MacConkey medium. The results on CLED agar were variable: 12 strains that were either PGUA or ONPX disc positive on Isosensitest agar were disc negative on CLED agar, even when retested. Lactose fermenting coliforms that gave positive disc reactions (PGUA or ONPX) on CLED agar developed a large zone of "inhibition of

lactose fermentation" around the positive yellow disc, but not around the negatively reacting disc. This phenomenon could not be ascribed to the maintenance of an alkaline pH in the vicinity of the disc, as both positive and negative discs contained buffer (pH 8.0 or 8.5). Galactosidase activity seemed to be inhibited in the presence of β -glucuronidase or β -xylosidase activity. The disc tests were highly reproducible, no strain showing any change in reaction on subculture.

Discussion

Several commercial systems incorporating enzyme substrates are now available for the rapid identification of *E coli*. Two of these, the Rapid Identification Method (RIM, Austin Biological Laboratories, Houston, Texas), and the Rapid Detect *E coli* (RDE, General Diagnostics, Morris Plains, New Jersey, USA) have been assessed,⁷ and seem to be satisfactory. Both require an incubation time of up to one hour, and the PGUA activity is detected by exposure to an ultraviolet light source.

Kilian and Bülow also devised an agar medium for urine culture which contained enzyme substrate and kaolin and permitted the instant recognition of PGUA positive colonies in mixed cultures.⁵ A commercial medium is now available for multipoint inoculation of urine specimens (Mast Laboratories Ltd), incorporating an enzyme substrate (pH 7.6). An evaluation of this medium showed that only 87% of *E coli* isolates produced detectable enzyme.⁶ This reduction in sensitivity, compared with that of the original tube test,

Table Comparison of disc and tube tests for detection of β -glucuronidase (PGUA) and β -xylosidase (ONPX)

Group	PGUA disc	PGUA tube	ONPX disc	ONPX tube	No of strains	Concordance	Identification by API 20E
1 Stored, previously identified strains (Total 91)	+	+	-	-	46	} 83 (91%)	<i>E coli</i>
	-	-	+	+	17		<i>Klebsiella/Enterobacter</i>
	-	-	-	-	20		<i>E coli</i> , <i>Citrobacter</i> (7), <i>Proteus</i> (8), <i>Morganella</i> (1)
	- (-)	+	+	-	6	}	<i>E coli</i>
	-	-	- (-)	+	1		<i>K oxytoca</i>
	-	-	+	- (-)	1		<i>C freundii</i>
2 Freshly isolated coliforms (Total 105)	+	+	+	+	55	} 99 (94%)	
	-	-	+	+	24		
	-	-	-	-	20		
	+	+	-	-	3	}	<i>E coli</i>
	- (-)	+	+	-	2		<i>E coli</i>
	-	-	- (-)	+	1		<i>K pneumonia</i>
3 Routine urine specimens (Total 385)	+	+	-	-	279	} 351 (91%)	
	-	-	-	-	61		
	+	+	+	+	11		<i>E coli</i> + <i>Klebsiella/Enterobacter</i> (7); <i>E coli</i> + NLF (4)
	- (-)	+	+	-	28	}	
	+	+	-	-	6		

(-) result of retesting

NLF = unidentified non-lactose fermenter

was ascribed to the lower pH of the medium necessary for growth, but suboptimal for colour development.³ In the disc test a local alkalinity is produced, and the resulting bright yellow colour is readily seen against the white paper background.

The disc tests discriminate between most coliforms when urine cultures are being assessed after overnight incubation. A single substrate disc may be conveniently applied to the centre of the plate carrying the antibiotic sensitivity discs. If there is space for only one substrate disc the PGUA disc should be chosen as it specifically identifies *E coli*, the coliform most commonly encountered in routine urine specimens.

Generally the PGUA and ONPX reactions are mutually exclusive. Thus Kilian and Bülow found that only one of 113 strains of *E coli* was ONPX positive, and that strains of the *Klebsiella* or *Enterobacter* group were invariably PGUA negative.³ We also have occasionally encountered strains of *E coli* that are both PGUA and ONPX positive. If both PGUA and ONPX discs are positive for a particular urine culture, however, a mixture of *E coli* and *Klebsiella* or *Enterobacter* is usually present. The disc reactions do not seem to be affected by the presence of other genera in mixed culture, and are detectable under a spreading growth of *Proteus*. They provide the basis of a system of identification that does not depend on lactose fermentation as follows:

The spot indole test is usefully retained, as a positive reaction confirms the identification of *E coli* and identifies ONPX positive organisms with reasonable certainty as *Klebsiella oxytoca*.

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