Head-space gas liquid chromatography for rapid detection of *Escherichia coli* and *Proteus mirabilis* in urine

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SUMMARY Measuring ethanol formed from arabinose by *Escherichia coli* and dimethyl disulphide formed from methionine by *Proteus mirabilis* is a rapid way of detecting these organisms in the urine. Ethanol and dimethyl disulphide are detected by head-space gas-liquid chromatography. Since sedimented organisms and not whole urine are used the method is not subject to interference from compounds within the urine. When it was tested on 122 samples of urine, 94 from patients with suspected urinary infection and 28 supposedly uninfected control specimens, the results correlated well with viable counts by a conventional bacteriological method. As well as being rapid (results available in four hours) the method is reliable, easy to use, and could be automated. It now requires extensive testing in a hospital laboratory.

Rapid screening methods for detecting urinary infections have been sought for years. Accuracy is necessary because urinary tract infection (UTI) may be asymptomatic (Constable, 1975) and persist undiagnosed for a long period (Freeman and Sindu, 1974). Laboratory methods for detecting bacteriuria are hampered by the contamination of urine that invariably happens during voiding. To overcome this Kass (1956) described a criterion for UTI based on the quantitative estimation of bacteria in midstream specimens of urine. Nevertheless, most screening methods indicate only the presence of organisms and not their number. More recent culture methods such as the use of dip-slides (Craig and Kunin, 1972) do show the number of organisms present, but they are only marginally faster than routine culture methods and cannot be called rapid.

This paper describes a rapid way of quantifying *Escherichia coli* and *Proteus mirabilis* in urine by a method based on those of Coloe (1978) and Hayward *et al.* (1977).

Material and methods

The method was tested on 122 urine samples, of which 94 were from patients with suspected UTI and 28 were control samples from healthy persons with no recent history of UTI. Care was taken to ensure

that all were midstream specimens. Only the predominant organism was studied in detail provided it was well in excess of the contaminants that were often present.

To sediment all the organisms 60 ml urine was centrifuged at 1000 *g* in a swing-out-bucket rotor of a Mistral 4L centrifuge for 10 minutes at 4°C. The supernatant was discarded and the sediment resuspended in 6 ml yeast-extract peptone water enriched with arabinose and methionine. The suspension was transferred to a 28-ml screw-capped bottle and incubated with shaking at 37°C for four hours. The resulting culture was examined for ethanol and dimethyl disulphide by means of head-space gas-liquid chromatography as described by Coloe (1978).

Ethanol and dimethyl disulphide produced from bacterial cultures were measured by comparing the retention times of peaks with peaks from standard aqueous solutions of ethanol and dimethyl disulphide. They were confirmed as ethanol and dimethyl disulphide by mass spectrometry. Yields of ethanol and dimethyl disulphide were expressed as peak area in cm², the area under the peak on the gas-liquid chromatograph chart.

Peptone water was prepared from Bacteriological Peptone (Oxoid) as described by Cowan and Steel (1965). Peptone water enriched with 0.3% yeast extract, 0.05 M arabinose (BDH), and 0.1 M methionine (BDH) was prepared in 100 ml lots, adjusted to pH 7.4, and sterilised by filtration through a sintered

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glass filter (Gallenkamp bacteriological filter, porosity 5).

Viable counts were made by the method of Miles *et al.* (1938). Dilutions were prepared in 1% peptone water and one drop of each dilution was placed on each of eight MacConkey agar plates and one blood-agar plate. For urine samples from patients with infections other than enterobacteriae viable counts were made on nine blood-agar plates.

Results

URINE SAMPLES CONTAINING *E. COLI*

Forty-five samples with counts of 10^5 or more *E. coli*/ml were tested. Cultures of all of them yielded ethanol. The average peak area was 440 cm², the maximum 1200 cm², and the minimum 95 cm² (Fig. 1). Forty-two cultures formed a small amount of

Fig. 1 Scatter plot of ethanol yields detected by head-space gas-liquid chromatography of urine cultures grown in medium enriched with arabinose and methionine.

Fig. 2 Scatter plot of dimethyl disulphide yields detected by head-space gas-liquid chromatography of urine cultures grown in medium enriched with arabinose and methionine.

dimethyl disulphide. The average peak area was 94 cm² and the maximum was 429 cm² (Fig. 2). No methylmercaptan was detected in any of the cultures.

Sixteen samples with 10^4 or fewer *E. coli*/ml were tested (Fig. 1). One culture, from a urine with a viable count of 6.9×10^4 *E. coli*/ml, yielded ethanol with a peak area of more than four of the cultures from urines with significant counts. All other cultures formed only small amounts of ethanol and none was detected from three of them. The second highest yield was a peak area of 18.0 cm² and the average was only 16 cm². Nine of the cultures formed small amounts of dimethyl disulphide. The highest was 26 cm² and the average only 8.0 cm² (Fig. 2).

URINE SAMPLES CONTAINING *KLEBSIELLA AEROGENES*

Four urine samples with counts of 10^5 or more *K. aerogenes*/ml were tested (Fig. 1). Cultures from all of them yielded ethanol. The average peak area was

1407 cm², the maximum 2550 cm², and the minimum 195 cm². These yields are as large as those from cultures of urine containing *E. coli*. Three of the four cultures produced dimethyl disulphide. The average yield was 78 cm² (Fig. 2). Methylmercaptan was not detected in any of the cultures.

Only one urine sample with a count of 10⁴ or less was tested. Culture from it yielded a very small amount of ethanol but no dimethyl disulphide (Figs 1, 2).

URINE SAMPLES CONTAINING EITHER *P. MIRABILIS* OR *P. VULGARIS*

Seven samples containing 10⁵ or more *P. mirabilis*/ml and one containing 10⁵ *P. vulgaris*/ml were tested. Ethanol was detected in only two cultures, both from samples containing *P. mirabilis*. The larger amount formed was 45 cm², less than from any culture of urine with a significant count of *E. coli* (Fig. 1). Large amounts of dimethyl disulphide were formed (Fig. 2). The average peak area was 1563 cm², the maximum 2200 cm², and the minimum 570 cm². This minimum was considerably larger than the yield from any culture of urine containing *E. coli* or *K. aerogenes*. Methylmercaptan was always present but it eluted so quickly that it was impossible to calculate the area of the peak.

Three urine samples that contained 10⁴ or fewer *P. mirabilis*/ml were tested. Only two cultures produced any ethanol, the most being 21 cm² (Fig. 1). All three cultures produced some dimethyl disulphide but the maximum peak area of 355 cm² was less than from any culture of urine with a significant count of *P. mirabilis* or *P. vulgaris* (Fig. 2). Methylmercaptan was detected in only one of the cultures.

URINE SAMPLES CONTAINING OTHER BACTERIAL SPECIES

Five urine samples with 10⁵ or more *Streptococcus*

faecalis per ml, two with 10⁵ or more *Staphylococcus epidermidis* per ml and two with less than 10⁵ *Staphylococcus epidermidis* per ml were tested. None of the cultures formed any ethanol, dimethyl disulphide, or methylmercaptan.

URINE SAMPLES CONTAINING BOTH *E. COLI* AND *P. MIRABILIS*

Eight urine samples that contained 10⁵ or more per ml of both *E. coli* and *P. mirabilis* were tested (Table). All cultures produced some ethanol and some dimethyl disulphide. The amount of ethanol in seven of the cultures was compatible with the count of *E. coli* in the urine. The yield of ethanol from the eighth culture was so small that the count of *E. coli* in the urine would have been mistaken for one that was not significant. The amount of dimethyl disulphide in six of the cultures was compatible with the count of *P. mirabilis* in the urine and methylmercaptan was detected in all of these. However, the yields of dimethyl disulphide from two cultures were less than would have been expected from the counts of *P. mirabilis*. Methylmercaptan was not detected from either of these cultures.

URINE SAMPLES STERILE ON CULTURE

Mid-stream specimens of urine, collected under aseptic conditions from 28 men yielded no organisms on culture. Neither ethanol nor dimethyl disulphide was detected.

Discussion

The organisms were sedimented and then resuspended in the test medium to ensure that stimulating or inhibitory substances were not carried over into the test medium to influence the result. These substances may be in the urine only temporarily. Consequently they have very little effect on the bacterial count of the

Table Results from urine samples containing *E. coli* and *P. mirabilis*

Viable organisms (No./ml)	Ethanol produced (peak area in cm ²)	Dimethyl disulphide produced (peak area in cm ²)	Methyl-mercaptan present
<i>E. coli</i> 1.5 × 10 ⁶	600		+
<i>P. mirabilis</i> 1.3 × 10 ⁶		1215	
<i>E. coli</i> 3.0 × 10 ⁶	460		—
<i>P. mirabilis</i> 2.4 × 10 ⁷		460	
<i>E. coli</i> 1.5 × 10 ⁶	825		+
<i>P. mirabilis</i> 1.5 × 10 ⁶		1490	
<i>E. coli</i> 2.0 × 10 ⁶	330		+
<i>P. mirabilis</i> 5.2 × 10 ⁶		1430	
<i>E. coli</i> 1.7 × 10 ⁶	300		+
<i>P. mirabilis</i> 2.9 × 10 ⁶		2110	
<i>E. coli</i> 3.0 × 10 ⁶	554		+
<i>P. mirabilis</i> 3.4 × 10 ⁶		1950	
<i>E. coli</i> 3.6 × 10 ⁶	360		+
<i>P. mirabilis</i> 4.0 × 10 ⁶		910	
<i>E. coli</i> 3.0 × 10 ⁶	30		—
<i>P. mirabilis</i> 1.8 × 10 ⁶		135	

urine but possibly considerable effect on the result of any test in which whole urine is used. When antibiotics are present and active in urine the centrifugation and subsequent inoculation into fresh medium may be enough to reduce the concentration of the antibiotic below the minimum inhibitory level. The result would possibly differ from that of routine culture but false-positive results are preferable to false-negatives.

The method seems superior to other screening tests because it is easy to perform, rapid, identifies *E. coli* and *P. mirabilis*, and indicates whether they are present in significant numbers. Everything from collecting the sample to reporting the results can be performed in 4½ hours. The time may be shortened to 3½ hours, but then organisms present in smaller numbers might be missed. The time could not be shortened much more because the specific products do not appear until the organisms are in the early logarithmic phase of growth. That is why four hours was selected for the test.

The trial with 122 urine samples showed that the method will determine whether 10^5 or more or less than 10^5 *E. coli* or *P. mirabilis* per ml of urine are present. If, for the instrument used, a cut-off point of a peak area of 90 cm² of ethanol is chosen to indicate urine infected with *E. coli* all urine samples with 10^5 or more *E. coli*/ml and one with 6.9×10^4 *E. coli*/ml would have been diagnosed as infected. The test is consistent with the preference for false positives rather than false negatives. If a cut-off point of a peak area of 500 cm² of dimethyl disulphide is chosen to indicate *P. mirabilis* infection all urine samples with 10^5 or more *P. mirabilis*/ml would have been diagnosed as infected and no urine with less than 10^5 /ml would have been classed as infected.

Among the 122 urine samples were eight containing significant numbers of *E. coli* and *P. mirabilis*. Only six of the eight would have been identified correctly, one would have been identified as infected only with *E. coli*, and one would have been called uninfected. In the case of the negative result, however, there was evidence that the patient had begun unauthorised antibiotic therapy before the sampling. If the test has any major failings they are likely to be related to cases of mixed infection. The method is well suited for use in a diagnostic laboratory. The medium is simple, its components should be readily available, it is easily prepared, and may be stored for a long period. No special technical expertise is required in preparing, inoculating, or analysing the culture. Ethanol eluted on the gas chromatograph used after 1.5 minutes and dimethyl disulphide after 2.6 minutes. Allowing for a settling down period of about three minutes, samples could be analysed every six minutes.

To reduce the time needed to analyse a large number of samples a dual-column, dual-detector gas chromatograph fitted with two recorders or several gas chromatographs could be used. Alternatively, if only one machine were available the analysis could be performed at a temperature higher than 100°C, or by using a column with less holding capacity than carbowax. Under these conditions all components would elute more quickly. It would then be helpful to have the recorder directly attached to a computer. The method lends itself to automation, especially with a machine such as the Perkin-Elmer F42 automated head-space gas chromatograph. If such a machine could be attached to a computer the method would be almost completely automated other than the preparation of the culture. Under these conditions it could function 24 hours a day.

Antibiotic sensitivity testing could also be incorporated into the test if a gas chromatograph with a sensitive flame ionisation detector were used. The urine samples, after centrifugation and inoculation into the test medium, could be divided into four before incubation. One of the four would be incubated as the test sample and the other three would each be supplemented with one of the antibiotics used for treating urinary infection and then incubated in the same way as the test sample. If the test sample showed that there were 10^5 or more organisms/ml in the urine the antibiotic sensitivities could be determined by analysing the antibiotic-supplemented media. If ethanol or dimethyl disulphide was not detected in an antibiotic-supplemented medium it would indicate that the organism causing the infection was sensitive to that antibiotic.

The method is not perfect as a screening technique but it is better than most. At present it is not envisaged as replacing conventional techniques but rather as detecting infections due to *E. coli* and *P. mirabilis* rapidly. The method would also be useful as a routine screening technique for detecting most asymptomatic urinary infections in pregnant women and in school children since, especially in the latter group, *E. coli* is the organism mainly responsible (Freeman and Sindhu, 1974).

Although the method can distinguish *E. coli* from *P. mirabilis* in urine it does not differentiate *E. coli* and *Klebsiella* spp. It probably does not differentiate the various *Proteus* spp, nor does it detect *Pseudomonas* spp, *Strep faecalis*, or *Staph epidermidis*. Tests for these organisms within the confines of the method are desirable.

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