mg/l. The addition of larger amounts of proteinaceous material would probably have also adversely affected the GCC broth. Undigested vegetable matter could have impaired the action of both selective antibiotics, again permitting the growth of facultative and obligate anaerobic enteric organisms. Several of these organisms have been shown to inhibit the growth of *C difficile* in vitro.[5]

All of these factors, to a greater or lesser extent, probably simultaneously play a part in reducing the efficiency of GCC broth when a large inoculum is used. It is important that workers in routine clinical laboratories are aware that it is possible to "overload" selective enrichment broths containing antibiotics. Accordingly, therefore, we would recommend that the maximum inoculum size for 10 ml of GCC broth should be either 0·1 ml or 0·2 ml of a 25% suspension of stool.

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Urease activity of *Campylobacter pylori*

Campylobacter pylori, first cultured in 1982, is increasingly being associated with gastritis and peptic ulceration.[1] Unlike most campylobacters, it possesses a powerful extracellular urease activity.[2] In acute *C pylori* gastritis stomach juice urea falls and ammonia rises,[3] with an accompanying rise in pH.[4] The cytopathic effect observed in gastric epithelial cells[1] may be mediated by high local concentrations of ammonia. We decided to investigate this enzyme activity.

Colonies of *C pylori* were scraped off blood agar plates, suspended in phosphate buffered saline, then centrifuged. The supernatant was used as the source of urease activity. Aliquots were incubated with urea in buffer, and liberated ammonia measured colourimetrically by the method of Berthelot.

A pH profile showed two pH optima in each of three strains examined, at 5 and at 8. Activity was irreversibly inhibited at and below pH 4·5. The low pH activity was inhibited by phosphate ions, considerably at 10 mM, and almost completely at 250 mM, leaving the pH 8 activity almost unaffected. Both activities were inhibited by low concentrations of acetohydroxamic acid; kinetics suggested non-competitive inhibition of each.

We suggest the existence of two extracellular isoenzymes of urease produced by this organism. The low pH one may represent a partial adaptation to the acid environment of the healthy stomach. Hydroxamic acid derivatives have been used therapeutically in man[5]; perhaps they may have some role in controlling the gastritis associated with *C pylori* infection, particularly if newer derivatives escape the suspicion of tetragenecity and carcinogenicity suggested with older ones.

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Dipstick screening for bacteriuria

Boreland and Stoker recently reported the results of a valuable study of the use of dipstick analysis to screen urines from children for bacteriuria.[1] After studying their report we cannot agree with their conclusion that the method described is suitable for routine use.

The reference culture method was the screening technique using blotting paper strips described by Leigh and Williams.[2] This was originally shown to be suitable for screening large groups of patients who may have asymptomatic infections such as pregnant women. It has not been shown to be suitable for use with specimens from symptomatic patients. In these cases a clinically confined method should be used—for example, one using calibrated loops.[3] Boreland and Stoker did not indicate that their population was predominantly asymptomatic: 12% of their urines yielded significant growth compared with 5% of those studied by Leigh and Williams.

When strips yielded between five and 30 colonies Leigh and Williams recommended that repeat specimens of urine should be examined, as over 40% of the repeat specimens they tested contained more than 10^5 organisms/ml. Boreland and Stoker gave no consideration to the problem of urines with borderline colony counts.

The screening methods using dipstick analysis showed good predictive values for a negative result, but a positive result in a child's urine is important because of the possible consequences of urinary tract infection in childhood. Of the 700 specimens examined, 14 yielded positive cultures but were negative by the reagent strip methods. Thus 17% of culture positive specimens were not detected by dipstick screening. Concentrating on developing a method which detects negative urines well, the authors seem to have overlooked the clinical importance of paediatric bacteriuria.

The need for bacteriological examination of urine does place a large burden on laboratories, but in attempting to relieve this burden the importance of the results of such examination must not be overlooked.

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Drs Boreland and Stoker comment:

In our evaluation of the Ames reagent strip tests, shortage of manpower meant that only a comparison of the dipstick method with our routine screening method—the Leigh and Williams blotting paper technique could be made.[1] This was performed using Bacteriuritest strips (Mast Laboratories) and the manufacturer's recommendations: 20 colonies equivalent to 10^5 organisms/ml (significant bacteriuria) and 10–20 colonies equivalent to 10^4–10^5 organisms/ml (doubtful significance). Ideally, we would like to have included a reference method such as the pour plate technique,[2] or calibrated loops,[3] although it should be noted that this latter method can have an error rate as high as SEM 50% due to operator variability.[4]

We concede that it was unclear that the urines screened were from predominantly asymptomatic patients. The prevalence of 5% reported by Leigh and Williams referred to the presence of significant bacteriuria in pregnant women; the 12% prevalence in our paediatric population compared with that found by Cannon *et al*[5] in a similar population.

Of the urines tested, only six fell within the "doubtful significance" range of 10^4–10^5 organisms/ml, and 50% of these were detected by the Ames reagent strips. In our laboratory we normally request repeat samples in these cases. For the purpose of our evaluation, however, we strictly adhered to Kass's criteria,[6] as existing data from a number of workers[7–10] still support the criterion of $\geqslant 10^5$ organisms/ml for the diagnosis of significant bacteriuria in asymptomatic patients.

With the Ames dipsticks, the method of reading them influenced the number of false negative results obtained. Visual reading of the strips resulted in 19% false negative results; photometrically read strips yielded 7%. This may be regarded as an unacceptably high false negative rate, but no urine screening method is 100% accurate. All our urines giving a positive (true and false) strip result are subjected to culture and microscopy before a report is issued to the clinician. We have also encouraged our clinicians to specify those urines from symptomatic patients which require culture and microscopy without screening.

With urine specimens comprising up to 40% of the workload of clinical microbiology laboratories, most are forced to use some form of screening procedure. We consider the dipstick analysis to be as accurate a screening method as those currently in use, and its ability to screen out clinically insignificant urine samples rapidly reduces the number of specimens to be cultured. This rapid screening method also enables same day reporting of negative urine specimens to be made.

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Enzyme immunoassay for detecting genital tract *Chlamydia trachomatis*

The paper by Taylor-Robinson, Thomas, and Osborn provides interesting data on the use of an enzyme immunoassay (EIA) to detect *Chlamydia trachomatis* in clinical specimens.[1] As the authors point out, these results are at variance with the work of seven other cited groups, including our own findings previously reported in this Journal.[2] Clearly, there is cause for concern because EIA tests are now widely used.

Quite rightly, Taylor-Robinson *et al* emphasise the importance of quality control. We agree that quantitative testing of a diluted suspension of the organisms is important, but our results (unpublished) indicate that the sensitivity of Chlamydiazyme is similar to that of MikroTrak. We found that Chlamydiazyme gave a positive result when the titrated sample contained fewer than four elementary bodies by MikroTrak testing. The dilution medium is also important. Diluting the stock strain with growth medium, or with medium containing fetal calf serum, or chlamydial antibodies, reduces the optical density (OD) reading three to five fold compared with EIA buffer alone. This would lead to false negative results with EIA compared with immunofluorescence. We agree that problems of reproducibility can occur with EIA. For example, on one occasion we found that our washing water was heavily contaminated with Gram negative organisms; readings of OD were obtained which were above the cut off level, giving false positive results. Problems of this kind should be detected by strict batch quality control.

Antigen detection tests are still in their infancy, and the study of Taylor-Robinson *et al* is a timely reminder of their potential fallibility. Immunofluorescence tests are a case in point. In the hands of experienced workers the tests perform well, but the literature contains reports of unexpectedly high numbers of chlamydiae when assessed by immunofluorescence alone.[3,4] Indeed, Jones and Taylor-Robinson[5] have expressed concern that the widespread use of these tests for the detection of elementary bodies could lead to a rise in the number of cases wrongly attributed to chlamydiae.

What is the ideal test for the diagnosis of chlamydial infection? The fact of the matter is that all existing procedures have drawbacks and may give misleading results unless there is strict quality control in the laboratory and a careful comparison of the clinical and microbiological findings. Even cell culture, widely regarded as the "gold standard", may give misleading results because of variations in technique and cell sensitivity and lack of technical expertise. Antigen detection tests, whether EIA or immunofluorescence, require careful assessment before marketing, and again before use in the diagnostic laboratory. We believe that verification requires the use of a fully evaluated and reliable cell culture system. At present, the results of prevalence or treatment studies which are based on antigen