Letters to the Editor


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. This was performed using Bacteriuretest strips (Mast Laboratories) and the manufacturer’s recommendations: 20 colonies equivalent to 10⁴ organisms/ml (significant bacteriuria) and 10–20 colonies equivalent to 10¹–10² organisms/ml (doubtful significance). Ideally, we would like to have included a reference method such as the pour plate technique, or calibrated loops, although it should be noted that this latter method can have an error rate as high as 5% due to operator variability.

We concede that it was unclear that the urinary screening 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 in a similar population.

Of the urines tested, only six fell within the “doubtful significance” range of 10¹–10³ 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 as existing data from a number of workers still support the criterion of ≥10³ 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. 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. 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 Chlamydiae is similar to that of MikroTrak. We found that Chlamydiae 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. Indeed, Jones and Taylor-Robinson 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 upon antigen
detection tests alone should be viewed with caution, particularly if they are being used in situations in which they have not been formally evaluated.6

Workers in diagnostic laboratories are under considerable pressure to provide their clinical colleagues with a chlamydial diagnostic service. They will naturally tend to select the test best suited to the population being studied and the workload and technical expertise of the department. They must ensure, however, that the test does not mislead. The first indication of problems may be a report from the clinicians that the laboratory results are at variance with the clinical picture. Such discordant results will indicate the need for careful re-evaluation of the test being used, and if possible, cross testing with a different technique should be performed. Although cell culture remains the method of choice, in many laboratories an antigen detection test may be all that is available.

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References


Drs Taylor-Robinson and Thomas comment: It seems inappropriate to reiterate the points made in our paper, but the comments of Dr Ridgway et al provide us with an opportunity of clarifying one issue and raising others that might be helpful in improving the sensitivity of the enzyme immunoassay system. We must point out that in evaluating the Chlamydiazyme assay by examining serial dilutions of stock laboratory strains of chlamydiae, we used the Chlamydiazyme transport fluid for the first dilution and the dilution buffer for subsequent dilutions. We did not use chlamydial growth medium or medium containing fetal calf serum, both of which Dr Ridgway et al suggest would lead to false negative results when the assay is compared with immunofluorescence. This does not explain, therefore, the discrepancy between the results of this evaluation procedure carried out in our separate laboratories.

We are in complete agreement, however, that there must be stricter quality control of all tests for detecting chlamydiae, whether based on culture, immunoassay, immunofluorescence or anything else, and that all of them have their pitfalls. This can lead to spurious conclusions about the qualities of a particular test, simply because the procedure with which it is compared is less or even more efficiently performed than it might be in the hands of other workers. It seems to us beyond question that antigen detection based on immunoassay systems is less sensitive than that achieved by the best culture procedures or by direct immunofluorescence. It is possible that antibody of poor avidity, the inadequate solubilisation of antigen, and the fact that only a small proportion of the antigen available for testing is used in the immunoassays may lead to lack of sensitivity. It may never be possible to increase the sensitivity of chlamydial immunoassays to a level comparable with that of, for example, direct immunofluorescence, but greater attention to the issues mentioned, together with the possibility of increasing antigen concentration by multiple swabbing, may increase the sensitivity of immunoassays to a point where they are clinically acceptable.

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Management of women with abnormal cervical smears: supplement to Terminology in Gynaecological Cytology

The necessity for standard terminology in cytology is clear, but the publication of advisory reports on medical management, such as that by the Working Party of the British Society of Clinical Cytopathology1 is unusual in British medicine and is open to strong debate. Such a report can quickly inflict a "usual custom and practice" norm on the profession with a medico-legal corollary of potential negligence claims if mishaps occur following major deviation from recommendations. The scientific basis for recommendations must be fully proved, and recommendations should not be made in scientifically grey areas.

The original report from the BSCC on terminology2 is already proving to be highly successful. The latest and extremely brief daughter paper on the management of the abnormal smears, however, is disappointing.

The recommendation that women with negative smears should be recalled according to the "screening policy for Well Women" was a golden lost opportunity to place further pressure on the government to reduce the currently recommended five year recall period.

The paper initially states expectations that careful characterisation of cytological changes should in many cases correlate with histological classification. This is not accepted with reference to minor abnormalities, however, and the group join the runaway bandwagon that cytology correlates poorly with histopathology. This may eventually be accepted as the case, but studies propagating this view have displayed poor attendance to their own cytology/histopathology reporting error. The table directs that koilocytosis warrants a repeat smear at three months. As the incidence of koilocytosis may be as high as 86% in some areas,3 such repeats will prove an onerous laboratory task. Persisting koilocytic, borderline, and mild dyskaryotic changes are regarded as an indication for colposcopy, but as the term persisting is not defined, no consensus seems to have been reached by the group on its meaning.

The paper seems to support the increasing view that cervical cytology should be regarded as no more than a simple selection tool for referral for colposcopy, temporarily forgetting, however, the financial and staffing impossibility of currently instituting the comprehensive NHS colposcopy service which is necessary. If cervical cytology proves to be such a crude diagnostic tool, the future for biochemical screening tests must be optimistic.4 In the short term, attempted attention to all minor abnormalities should not swamp and hinder the urgent management of moderate and severe dyskar- yosis. If the recommendations do represent the theoretical ideal there is certainly no hope.
Enzyme immunoassay for detecting genital tract Chlamydia trachomatis.
G L Ridgway, G Mumtaz and J D Oriel

*J Clin Pathol* 1988 41: 113-114
doi: 10.1136/jcp.41.1.113-b

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