Ziehl-Neelsen staining of urine deposits in the diagnosis of genitourinary tuberculosis

Microscopy of centrifuged urine deposits for acid-alcohol fast bacilli may give misleading results due to contamination by environmental saprophytic mycobacteria.^{1,2} This has not been the experience at this hospital, where over the past 10 years Ziehl-Neelsen staining of smears has been a useful adjunct to urine culture in the early diagnosis of tuberculosis.

During this period urine from 3000 patients was examined for evidence of genitourinary tuberculosis. Three early morning specimens were collected on consecutive days and the urine allowed to settle at 4°C. The resulting sediments were pooled to a total volume of 25 ml and centrifuged at 2000 g for 20 min. Acid-alcohol fast bacilli were then sought in smears taken from the centrifuged deposits. The deposits were decontaminated by adding an equal volume of molar sodium hydroxide and mixed for 25 min before culturing on glycerol and pyruvic acid egg media.

In seven specimens acid-alcohol bacilli were seen in stained smears. In four of these *Mycobacterium tuberculosis* was eventually cultivated from the urine. The remaining three smear positive specimens failed to grow on culture. Records of two of these three patients showed that a clinical diagnosis of pulmonary tuberculosis was confirmed by growing *M. tuberculosis* from their sputum samples. One of these patients died shortly after admission, and at necropsy renal tuberculosis was found.

The third patient had been referred because antituberculosis chemotherapy had not eradicated acid-alcohol fast bacilli repeatedly seen in urine smears. At subsequent partial nephrectomy, histology disclosed caseating granulomata, typical of tuberculous disease.

Mycobacteria were cultured from 27 specimens of urine, although in 23 of these, no acid-alcohol fast bacilli were seen on stained smears. Seventeen of the 23 were ultimately identified as *M. tuberculosis*, one as *M. bovis*, and five as other cultivatable mycobacteria of no clinical importance. The number of clinically irrelevant mycobacteria found in urine, as compared with true mycobacterial pathogens, may finally depend on the prevalence of tuberculosis in the particular community.

These data show that the small number of urine smears which were positive for acid-alcohol fast bacilli (about 0.2%) were all associated with active tuberculosis. A reputed high incidence of false positives

has not been confirmed here or elsewhere in West London.³ Even in the rare cases where culture fails to verify the presence of mycobacteria, identifying acid-alcohol fast bacilli in stained smears taken from centrifuged urine specimens is of importance in the early diagnosis of tuberculosis.

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ALISON WEBSTER

DJM WRIGHT

Charing Cross Hospital
London W6

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Syva MicroTrak stains: their use in a routine laboratory

We read with interest the article by Dr BJ Thomas *et al*¹ describing the MicroTrak systems for the detection of *Chlamydia trachomatis* elementary bodies in smears and inclusions in cell cultures. These systems have been in use for some months in this laboratory, and our findings are broadly in agreement with those reported. We think that the following information may be of interest to other routine laboratories who wish to offer a service for the recognition of *C. trachomatis*.

Before the introduction of these monoclonal antibodies our routine culture procedure had used McCoy cells treated with 5-iodo-2'-deoxyuridine, followed by iodine staining after 48 h incubation. This staining method was changed to the Syva MicroTrak culture confirmation method as prolonged testing in parallel with iodine staining showed certain advantages of the MicroTrak stain. The technical staff were pleased with the reduction in time and fatigue inevitable when screening some 3000 specimens a year while an increase in the number of positive isolates were noticed.

We were interested, excited, and some-

what sceptical of a test which claimed to demonstrate *C. trachomatis* in smears taken directly from the patient, but tests with the Syva product were both convincing and impressive. The results of testing 156 specimens by both cell culture and direct smear tests are shown in the Table. All

Results of testing 156 specimens by both cell culture and direct smear tests

	No of specimens (%)
Culture - Smear -	118 (75.6)
Culture + Smear +	11 (7.1)
Culture + Smear -	4 (2.6)
Culture - Smear +	23 (14.7)

+ = positive; - = negative.

specimens, which included urethral, cervical and eye swabs, were obtained from the Department of Genitourinary Medicine, Rotherham District General Hospital. No attempt was made to classify the patients by clinical presentation, but specimens were submitted on the basis of a high probability of chlamydial infection.

If cultures alone had been performed 15/156 (9.7%) of specimens would have been recorded as positive; 34 (21.8%) would have shown positive results if direct smear only had been examined; and 38 (24.4%) would have been reported as positive if both tests had been carried out. We would agree that the threshold of 10 inclusions or elementary bodies seems overcautious, and any number of stained elements was assessed as positive in either test.

In contrast to the previous report,¹ we were able to recognise inclusions after 18-20 h incubation, using the culture confirmation test. When 23 known positive specimens were examined at 18-20 h and 45-48 h after inoculation, 22/23 showed inclusions after 18-20 h and all 23 cell cultures were positive by iodine and MicroTrak culture confirmation stains after 45-48 h. The culture which was negative at 18 h showed only one inclusion with iodine stain and two inclusions by MicroTrak culture confirmation tests after 48 h incubation.

A further phenomenon became apparent when earlier trials were being carried out. Most workers are familiar with the occasional batches of cell cultures which lose their sensitivity and appear generally unhealthy. When such a batch of cells, which appeared normal when inoculated, was infected with a number of known positive samples, no inclusions could be