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. 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 granuloma, typical of tuberculosis 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 cultivable 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. 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.

We are grateful to the PHLS Laboratory, Dulwich, for identifying mycobacterial isolates.

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References


Syva MicroTrak stains: their use in a routine laboratory

We read with interest the article by Dr BJ Thomas et al in 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 was noticed.

We were interested, excited, and somewhat 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 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 a 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, 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
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detected with the iodine stain; but inclu-
sions (albeit smaller than usual) were
detected in the coverslips stained with the
cell culture confirmation stain.

As a result of these trials we have
replaced a traditional cell culture method
with the Syva MicroTrak direct smear test
for routine screening. The costs of this test
may appear prohibitive, but we have found
that 10–15 μl of reagent can be used, if
carefully applied to the smear, with no
apparent loss of sensitivity. This means that
the cost may be reduced to less that £1.00
per test. We believe that this system offers
considerable benefits to any laboratory
whose technical staff are prepared to
familiarise themselves with this technique.
We are currently evaluating two further
commercial direct smear reagents and hope
to report the results in the near future.

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1 Thomas BJ, Evans RT, Hawkins DA,
Taylor-Robinson D. Sensitivity of detecting
Chlamydia trachomatis elementary bodies in
smears by use of fluorescein labelled mono-
clonal antibody; comparison with conven-
tional chlamydial isolation. J Clin Pathol

Dr Taylor-Robinson and others reply as fol-
lows:

We know that there will be many inves-
tigators throughout the UK and elsewhere
who find, like Dr Francis and Dr Abbas,
that the use of fluorescein conjugated
chlamydial monoclonal antibodies is a
rapid and sensitive way of detecting
chlamydial elementary bodies directly in
genital or conjunctival smears. We were
and still are confident that what we see in
smears are chlamydial elementary bodies
not just because of their distinctive appear-
ance but because of the excellent correla-
tion between the results of this direct test
and those of culture.1 We realise that no
matter in whose hands, the sensitivity of
culture, as Dr Francis and Dr Abbas men-
tion, is not optimal from time to time.
Many other factors may also lead to failure
in isolating chlamydiae and we note that Dr
Francis and Dr Abbas recorded 60% of
their positive results by smear alone. While
this may well have been due to a relative
failure to culture, it does raise the general
question of the extent to which false posi-
tive results might also arise, a question
which is likely to have even greater force if
monoclonal antibodies are used that are
less specific than those of Syva. A spurious
epidemic of disease attributed to
chlamydiae as a result of false identification
is not an idle thought and we have already
expressed apprehension concerning this possi-
ibility.2 Stained smears travel well and
it seems to us that thought should be given
to the setting up of a central referral system
for quality control and for help and gui-
dance on difficult cases. This would go a
long way to avoiding a calamity of the kind
we mention.

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Chlamydia trachomatis elementary bodies in
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tional chlamydial isolation. J Clin Pathol
2 Jones BR, Taylor-Robinson D. Observations
on and future trends in chlamydial research.

Erythrocyte acetylcholinesterase in
Hirschsprung’s disease

Erythrocyte acetylcholinesterase activity in the agang-
lionic segment of bowel in Hirschsprung’s
disease is increased.1 Boston2 and She3 have also found
increased erythrocyte acetylcholinesterase activity in Hirsch-
spurgn’s disease and have suggested that
this might be of value diagnostically. We
have measured erythrocyte acetylcholin-
esterase in children with confirmed
Hirschsprung’s disease and in control
patients of similar age.

Patients and methods

Erythrocyte acetylcholinesterase activity
was measured in blood from eight children
with Hirschsprung’s disease (age range 18
days–27 months) and in 53 children with-
out the disease (age range 1 day–36 months).
Seven of the patients with
Hirschsprung’s disease had already under-
gone colostomy or a definitive procedure,
but in no case had more than half of the
aganglionic bowel been resected. Thirty
two of the controls had gastrointestinal
disease, mainly congenital; of these, 18 pre-
sented with small or large bowel obstruct-
tion. The remaining children had a variety
of surgical and medical conditions, but
patients with liver disease, haematological
disorders, or a history of recent blood
transfusion were excluded.

Erythrocyte acetylcholinesterase was assay-
ed by Ellman’s colorimetric method,5 as modified by Lewis.6
Heparinised blood (0.5 ml) was centrifuged within
2 h of collection. The red cells were resus-
pended in a roughly equal volume of saline
(9 g/l) and the packed cell volume was
measured. The red cells were stored at
−20°C until assay. Each sample was assayed
in triplicate.

Results were analysed by Student’s t test.

Results

Initial studies showed that the enzyme was
stable in unseparated heparinised blood at
room temperature for 6 h. Red cells stored at
−20°C for one month showed no loss of
erthrocyte acetylcholinesterase activity.
There was 3.6% and 11.8% loss of activity
after two and three months’ storage respec-
tively. The between batch coefficient of
variation for erythrocyte acetylcholinester-
ase was 5.5%.

There was no significant difference in
erythrocyte acetylcholinesterase activity
between controls and patients with
Hirschsprung’s disease (Fig. 1) (p > 0.05).6

Erythrocyte acetylcholinesterase activity
related to age in both controls and patients
is shown in Fig. 2. Erythrocyte acetyl-
cholinesterase activity is 52% of the adult
value in the first month of life, and reaches
the adult value by the age of 3 months.

Discussion

The diagnosis of Hirschsprung’s disease,
particularly in neonates, may be difficult,
and an additional non-invasive diagnostic
test would be of value. Measurement of
erythrocyte acetylcholinesterase activity
has been proposed as such a test. In this
study erythrocyte acetylcholinesterase
activity was measured in controls and
patients with Hirschsprung’s disease be-
tween 0 and 36 months of age. All but two
Syva MicroTrak stains: their use in a routine laboratory.

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