Figure Malignant transitional cells in urine. (Haematoxylin and eosin $\times 62$)

filters have sometimes become opaque, because of further drying out and air becoming trapped within the pores.

Comment

This simple modification of the filter technique produces cellular preparations with good preservation of morphological detail unobscured by background staining. The method enables large numbers of urine samples to be screened more easily. Using this method over the past eight years, between 5000 and 6000 urines have been examined. Many have been from surgical patients with recurrent papillary tumours. Of the 1000 industrial wine specimens examined so far, one positive imprint has been found, and the presence of a bladder tumour has been confirmed by cystoscopic biopsy.

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Comparison of radioimmunoassay and enzyme immunoassay for detecting hepatitis B surface antigen in serum from freshly donated blood and selected blood products

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Engvall and Perlmann (1971, 1972) and van Weemen and Schuur (1971, 1972) pioneered work on enzyme immunoassay (EIA). Solid phase EIA techniques now offer an attractive alternative to the established ^{125}I or fluorescein-labelled-antibody methods for detecting microbial antigens and antibodies.

For some time the solid phase radioimmunoassay (RIA) has been widely accepted as the most sensitive practical assay for HBsAg in the serum of patients and blood donors. Recent reports indicate, however, that a solid phase EIA (Hepanostika) marketed by Organon Teknika may be of comparable sensitivity to the solid phase RIA (Ausria-II) marketed by Abbott Laboratories (Wolters *et al.*, 1976; Ukkonen *et al.*, 1977). Hepanostika is a microplate double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (Voller *et al.*, 1976) in which microplates coated with antibody to HBsAg are reacted with the test samples thought to contain antigen. The HBsAg becomes fixed to such plates and is indicated by means of a reagent consisting of enzyme-labelled antiserum to HBsAg. This reagent converts a colourless substrate to a coloured product, which can be assessed visually.

We have made a direct comparison of Ausria-II and Hepanostika for detection of HBsAg in freshly donated blood, using a coded panel including strongly and weakly positive sera, and factor VIII concentrates previously found to be weakly RIA positive and confirmed by specific neutralisation. Ausria-II was performed, as recommended by the manufacturers, using a Pentawash gun and Filamatic dispenser for bead washing. ^{125}I activity was monitored with a Nuclear Enterprises NE1600 (16-channel) gamma counter. Hepanostika was performed, as recommended by the manufacturer, using semiautomatic washing apparatus and an 11-channel pipette supplied by Organon Teknika. Results (colour change) were read with the naked eye.

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Results

The coded panel consisted of HBsAg positive sera, subtype D and subtype Y, diluted in pooled (antigen and antibody negative) normal human serum. The 'titration sensitivity' of both assays was comparable for subtype D, but that of Hepanostika appeared marginally more sensitive for subtype Y (Table 1). Both assays were considerably more

Table 1 Results of testing coded HBsAg panel by Ausria-II, Hepanostika, and Hepatest

Code	HBsAg/ADW	Ausria-II	Hepanostika	Hepatest
5	Diluent	—	—	—
2	1/20	+	+	+
6	1/40	+	+	+
1	1/80	+	+	—
7	1/160	+	+	—
4	1/320	+	+	—
8	1/640	+	+	—
3	1/1280	+	+	—
11	1/2560	(+)	(+)	—
10	1/5120	—	—	—
9	1/10240	—	—	—
<i>HBsAg/AYW</i>				
A	Diluent	—	—	—
B	1/20	+	+	+
E	1/40	+	+	—
K	1/80	+	+	—
C	1/160	+	+	—
J	1/320	+	+	—
D	1/640	(+)	+	—
G	1/1280	—	+	—
I	1/2560	—	(+)	—
F	Diluent	—	—	—
H	Diluent	—	—	—

Diluent = pooled (HBsAg/HBsAB negative) normal human serum;
+ positive result; (+) weakly positive result; — negative result

sensitive than Hepatest, the reverse passive haemagglutination assay marketed by Wellcome Reagents. The results obtained from 415 fresh blood donations, among which were several positives of varying concentration, confirmed that, for serum at least, the sensitivity of Ausria-II and Hepanostika was similar. Both assays detected the known positive sera, but Hepanostika gave initial weakly positive reactions with two additional donor sera (Table 2), both of which were negative by Hepanostika upon repeat. Ausria-II was the test of choice for the factor VIII concentrates since two batches, confirmed as weakly RIA positive, were negative by Hepanostika (Table 3).

Comment

The data indicate that EIA (Hepanostika) is comparable to RIA (Ausria-II) for detection of

Table 2 Results obtained from testing blood donations by Ausria-II and Hepanostika

No. of new donations tested	No. of Ausria-II false positive	No. of Hepanostika false positive
415	0	2* (0.48%)

*Negative on re-test

Table 3 Results of testing IF VIII concentrates by Ausria-II and Hepanostika

	Ausria-II	Hepanostika
IF VIII Batch		
2a	—	—
2b	—	—
3	(+)	—
4	(+)	—
5	—	—
6	—	—
7	—	—
8	+	+

+ positive reaction; (+) weak positive reaction; — negative reaction

HBsAg in serum, a finding in agreement with previous reports by Wolters *et al.* (1976), Ukkonen *et al.* (1977), and Vandervelde *et al.* (1977). Of 415 donor sera tested, two (0.48%) gave initial weak positive reactions with EIA, which were negative upon re-testing. No such false reactions were recorded by RIA. The performance of EIA was somewhat disappointing with regard to the factor VIII blood product. The reason for this is not immediately obvious, but it may be possible to correct the apparent lack of sensitivity by adjusting the conditions of the initial incubation step.

No difficulty was experienced in reading EIA directly, as negative wells remained completely colourless. The alternative to visual inspection is to employ spectrophotometry. With conventional equipment this is laborious when large numbers of samples are being read. A recent development is a semi-automated EIA plate reader (MSE), which quantitates colour-change in the well and prints out the readings.

EIA has the advantage over RIA that the reagents (enzyme-antibody conjugate) are more stable, and, as there are no radioactive isotopes, expensive monitoring equipment is not essential. The 11-channel pipette was found necessary to avoid reagent contamination between wells, which could be a source of misleading reactions. EIA requires an additional step, namely, addition of substrate, which increases both time and risk of technical error. Hepanostika requires five hours from addition of test samples to reading results, compared to three hours for Ausria-II. In the latter, however,

considerably more than two hours may be spent in counting a large number of samples.

An important drawback of Hepanostika relates to the presentation of the solid phase. Each kit (550 tests) contains five rigid plastic trays, each possessing 110 antibody-coated wells. While this arrangement was no doubt designed with the large blood transfusion laboratory in mind, donations do not always arrive in multiples of 110 for hepatitis testing. This arrangement will also prove inconvenient for those laboratories wishing to test only a few samples at a time. The individual coated beads of Ausria-II allow considerably greater flexibility of application.

In 1973 the WHO expert committee on viral hepatitis recommended that blood donations should be tested for HBsAg by a method with sensitivity comparable to counter-immunoelectrophoresis. In 1975 the WHO recommended the use of a test with sensitivity comparable to reverse passive haemagglutination. In 1977 the recommendation remained unaltered, but the potential of EIA was acknowledged. The potential of EIA versus RIA was raised in a letter to the editor of *Lancet* recently by Kato *et al.* (1977), in which the point was made that while RIA is routinely used to measure femtomole (10^{-15} mol) amounts of hormones, EIA has been used to detect 30 attomoles (1 attomole = 10^{-18} mol) of ornithine aminotransferase. If a 100- to 1000-fold improvement in sensitivity for detection of HBsAg is achieved as a result of improved EIA technology (eg, use of a more sensitive enzyme-substrate combination), the gap between test sensitivity and sample infectivity could be greatly reduced.

Despite the fact that RIA is well established in a number of laboratories, many others may find EIA better suited to their particular requirements.

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