a single microtube did not exceed one drop and the thickness of the cell block after centrifugation was less than 2 mm. In cases where the cell block exceeded 2 mm, the specimen had to be subdivided and processed in more than one microtube. Attempts at processing larger cell blocks always resulted in poor penetration.

Discussion

The method outlined has been used successfully in our laboratory to process more than 50 specimens and has been found to be consistent and reliable. Sufficient numbers of cells were recovered, even in those cases where the yield of aspirated material was relatively scanty. Furthermore, the overall quality of the ultra-thin sections obtained from the blocks was good, and morphological details were well preserved (Fig. 2).

The role of electron microscopy in the field of surgical pathology is now well established. Using the technique described in this report, electron microscopy can now be extended to fine-needle aspiration biopsy specimens. We believe that, in selected cases, electron microscopy of fine-needle aspiration biopsy specimens may provide valuable information, enabling the pathologist to make a much more precise diagnosis than is possible with light microscopy alone.

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An economical, simplified haemagglutination test for mass syphilis screening

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Until recently the only fully automated syphilis antibody screen tests were those employing Venereal Disease Research Laboratory (VDRL) carbon antigen on mass blood grouping machines like the Technicon BG 15 AutoAnalyzer (Technicon Instruments, Basingstoke, Hants) and the Groupamatic 360C (Kontron, Roche Bio-Electronics, St Albans, Herts). We present a semi-automated modification of the commercially available Fujizoki *Treponema pallidum* haemagglutination test (TPHA) marketed by Diamed Diagnostics Ltd (Bootle, Merseyside).

This test uses sheep red cells sensitised with *Treponema pallidum* extracts. Other authors¹ have presented similar methods. However, our modification is based on extensive dilution of the test cells² and the use of a semi-automated mass sampling device³ for diluting donor sera 1 in 8 in one step, in microtitration plates; with this device we can easily transfer 96 samples into microtitre plates using metal loops that can be rinsed between runs. The sera are diluted to a final volume of 25 μ l in the absorption diluent provided in the TPHA kit (with sheep serum added to a final 2% concentration). Extra diluent separate from the kits can be purchased from Diamed. Then 25 μ l of a 0.1% suspension of TPHA test cells in distilled water (with azide, if desired) are added. The plates are kept at room temperature for 15 minutes, centrifuged at 260 g for 1½ minutes, and then sloped at 70°. After approximately 10 minutes positive samples have remained as 'buttons', whereas the negative samples have formed 'streaks'.² Serum samples are used because plasma samples tend to give false-positive results. Inactivation is unnecessary. Screen positive samples are checked by titration with test and control cells and absorbed with control cells if necessary and re-titrated.

To date we have tested 36 500 donor serum samples by both the 0.1% TPHA and the carbon antigen test as used with AutoAnalyzers. Only one or two 0.1% TPHA screen positives are found per plate. Ninety-five per cent of these are quickly verified by titration with test and control cells. The remainder require a repeat titration after absorption. Fifty-five (1 in 700)

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Reference Laboratory results on 55 0.1% TPHA 'positive' samples

	VD Ref Lab confirmed 'positive'				VD Ref Lab found 'negative'			
Reciprocal titre*	8	16	32	> 32	8	16	32	> 32
No. at that titre	2	6	9	22	4	9	3	0

*Serum dilutions.

samples tested were 'positive' after titration (≥ 1 in 8 serum dilution) and sent to the VD Reference Laboratory, Whitechapel, to be checked. The exact cut-off dilution adopted will determine the level of 'false positives' detected by the method (and the level of 'false negatives'). A serum dilution of 1 in 20 would approximate the cut-off in the standard Fujizoki test. By this criterion 34 of the 55 TPHA positives might warrant follow-up (Table). In the same period, only four (1 in 9000) samples were carbon antigen positive on the AutoAnalyzer, and two of these were assessed as biological false positives (BFPs) by the VD Reference Laboratory. Of the 55 TPHA positives, nine were carbon antigen positive when tested manually. Thus seven of these nine were negative on the AutoAnalyzer. However, comparison of carbon antigen results with TPHA (preponderantly weak) positives would magnify even a small difference between manual and automated carbon antigen sensitivities. The two AutoAnalyzer BFPs were negative by TPHA. Thirty-nine (71%) of our putative positives were confirmed as having serological evidence of past treponemal infection by the VD Reference Laboratory by one or more of the following tests: TPHA, VDRL, fluorescent antibody, and treponemal immobilisation. The titres are given in the Table.

Comparison of both the standard and modified methods by parallel titrations of a panel of 40 positives (confirmed by the Reference Laboratory) showed that in the cut-off region the 0.1% TPHA was approximately three times more sensitive than the standard method when serum dilutions were considered (Figure). All of 24 carbon antigen positive samples from the panel of verified 'positives' were detected by the 0.1% TPHA. However, none of eight carbon antigen positive samples classified as BFPs by the Reference Laboratory was positive by 0.1% TPHA.

Standard TPHA has been recommended for syphilis screening by several authors^{4 5} although the lack of automation and, more importantly, the high cost made TPHA screening unattractive. The modified TPHA test described here may avoid those problems. It streamlines the syphilis testing, especially when combined with semi-automated sampling of the same sera for other microtitre-based tests, for example, HBsAg screens. At roughly 2p per test

Comparative titres (serum dilutions) by standard and 0.1% TPHA using the same batch of cells: no. tested = 40, slope = 0.8. Sensitivity increment by 0.1% is between two- and fourfold. Standard method starting dilution is 1 in 20.

(including microtitre plates and screen titrations) it is no more expensive than the carbon antigen test on AutoAnalyzers. Apart from this, the 0.1% TPHA is more specific than the carbon antigen test but does detect many more past infections of doubtful infectivity. We are continuing a large-scale assessment of 0.1% TPHA screening to follow our preliminary work. So far we have no evidence that the test fails to detect early primary syphilis (although we have no accurate data concerning 'false negatives'). The checking of any positives found by the initial 1 in 8 TPHA screen with a carbon antigen test should further reduce the risk of this. The modified test may have considerable scope both in the Blood Transfusion Service and for special clinics.

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Letters to the Editors

Immunoperoxidase techniques and controls

As we have considerable experience of immunoperoxidase techniques we were most interested to read Dr Heyderman's article in your journal (*J Clin Pathol* 1979;**32**:971-8). We think, however, that many of her comments regarding immunohistochemical techniques are appropriate only for laboratories that are able to prepare affinity-purified labelled antibodies. Most clinical histopathology laboratories would prefer a sensitive, reliable, and convenient immunoperoxidase method which does not entail any special immunological purification procedures.

Such a method, the peroxidase-antiperoxidase (PAP) technique, used after

treatment of formalin-fixed sections with proteolytic enzymes such as trypsin^{1,2} and pronase,³ has been dismissed lightly by Dr Heyderman. Pre-treatment with trypsin ensures reliable detection of protein antigen⁴ while the PAP method provides a high degree of sensitivity.⁵⁻⁹ Although the amount of treatment with trypsin may vary a little with the degree of fixation, surgical biopsies, which have been fixed for similar periods, will require the same amount of treatment with trypsin to ensure consistent results.^{10,11} An important feature of this particular immunoperoxidase method is that the primary antibody may be used at considerably higher dilutions than those required for either the indirect or the direct methods, thus reducing the possi-

bility of staining due to cross-reactions.

Dr Heyderman has made some important comments on the use of immunological controls, and there is no doubt that absorption methods are the most satisfactory¹² but, as many laboratories do not have access to purified antigens, blocking controls are more readily performed and will ensure immunological specificity.¹³ The use of antisera raised in differing animal species and obtained from different commercial sources overcomes the likelihood of a significant contaminating antibody occurring in both test and blocking antisera.

The technique (Table) is now well established for the routine assessment of intracellular protein components in lymph

Trypsin-immunoperoxidase (PAP) technique

1	Deparaffinise sections in xylol and take to alcohol	10 min × 2
2	Inhibit endogenous peroxidase by treating with freshly prepared 0.5% H ₂ O ₂ in methanol	10 min
3	Wash well in tap water	
4	Equilibrate temperature of slides in distilled water at 37°C	10 min
5(a)	For intracellular immunoglobulins and other protein antigens Treat with 0.1% trypsin in 0.1% CaCl ₂ (adjust to pH 7.8 with N/10 NaOH) at 37°C	15-30 min* (usually 20 min)
(b)	For extracellular immunoglobulins and other protein antigens 0.05% trypsin solution in 0.05% CaCl ₂ (adjust to pH 7.8 with N/10 NaOH) at 37°C	30-60 min* (usually 40 min)
6	Rinse in cold distilled water with agitation and transfer to moist chamber	2-3 min
7	Wash in Tris buffered saline (TBS) (0.5 M Tris/HCl buffer, pH 7.6, diluted 1/10 with saline)	10 min × 2
8	Normal swine serum, diluted 1/5 with TBS	10 min, drain off
9	Rabbit anti-human Ig sera (usually diluted 1/1000 in TBS)†	30 min
10	TBS wash	10 min × 3
11	Swine anti-rabbit IgG (usually diluted 1/100 in TBS)†	30 min
12	TBS wash	10 min × 3
13	PAP (peroxidase/rabbit antiperoxidase), usually diluted 1/200†	30 min
14	TBS wash	10 min × 3
15	Demonstrate peroxidase with 5 mg 3,3' diaminobenzidine tetra-HCl dissolved in 10 ml 0.2 M Tris/HCl buffer (pH 7.6), to which 0.1 ml fresh 1% H ₂ O ₂ has been added immediately before use ¹⁴	10 min
16	Wash in TBS followed by a wash in running tap water	5 min
17	Counterstain with haematoxylin, differentiate, blue, dehydrate, clear, and mount in DPX	

*Time may vary with batch of trypsin and degree of fixation.

†Determined by titration.