Pneumocystis carinii in bronchoalveolar lavage

We read with interest the paper on diagnosis of *Pneumocystis carinii* in bronchoalveolar lavage specimens by Young *et al.*¹ and we agree with the conclusions drawn. *P. carinii* can be identified in smears after routine Papanicolaou staining, but the diagnosis must be confirmed by showing the presence of the organism with a silver impregnation technique. In the cases we have seen we have not been struck by the biphasic staining described but found the frothy spongy aggregates of cysts and mucus very distinctive. Although we agree that specimens obtained by thorough bronchoalveolar lavage give excellent results most consistently, it is worth noting that good results may be obtained in patients with acquired immune deficiency syndrome, but not in immunosuppressed patients, by examining the sputum.²

Grocott's methenamine silver method is, as suggested, a rather long technique and we now no longer use it but rely routinely on Pintozzi's modification.³ We found that a slightly modified form of this method gave consistent results. The modifications introduced by one of us (JMcC), are the inclusion of 0.1% dimethylsulphoxide in the silver solution and the reduction of the incubating temperature of this solution from 80°C to between 70 to 75°C. Addition of DMSO at this low concentration gives a more precise deposition of silver on to the organisms without the presence of the precipitate mentioned by Mahan and Sale.⁴ Reducing the incubating temperature, even by a few degrees, ensures a greater degree of control during staining and does not unduly prolong the staining time.

Gram staining is also useful for showing the presence of cysts and sporozoites of *P. carinii*, but we found that the Weigert Gram technique gives superior results to the conventional Gram method, cited by Young.¹ Using the Weigert Gram method, cysts and sporozoites stain purple and contrast well with the red background (figure).⁶⁻⁸ Locating areas of interest at $\times 100$ magnification is greatly facilitated by the striking colour contrast which is absent in conventional Gram staining of *Pneumocystis*. The conventional Gram method uses acetone-alcohol as the differentiating agent, and this is rapid in action and difficult to control. The Weigert Gram method calls for differentiation of the crystal violet in aniline oil-xylene, and this is an easily controlled process which avoids the risk of overdifferentiation.

As the number of patients at risk from *P. carinii* pneumonia is rising, effective and rapid diagnostic methods will become increasingly important, and we are delighted to see this paper dealing with the various techniques currently available.

GM CAMPBELL

J MCCORRISTON

Department of Cytology,
Royal Infirmary,
Glasgow G4 0SF,
Scotland

References

- 1 Young JA, Stone JW, McGonigle RJS, Adu D, Michael J. Diagnosing pneumonia by cytological examination of bronchoalveolar lavage fluid: report of 15 cases. *J. Clin Pathol* 1986; 39:945-9.

Figure *Pneumocystis carinii*. Mature cysts and sporozoites are shown. Bronchoalveolar lavage fluid. (Gram Weigert stain.) Original magnification $\times 500$.

- 2 Pitchenik AE, Ganjei P, Torres A, Evans EA, Rubin E, Baier H. Sputum examination for diagnosis of *Pneumocystis carinii* pneumonia in the acquired immune deficiency syndrome. *Am Rev Respir Dis* 1986; 133:226-9.
- 3 Pintozzi RL. Modified Grocott's methenamine silver nitrate method for quick staining of *Pneumocystis carinii*. *J Clin Pathol* 1978;31:803-5.
- 4 Mahan CT, Sales GE. Rapid methenamine silver stain for *Pneumocystis* and fungi. *Arch Pathol Lab Med* 1978;102:351-2.
- 5 Macher AM, Shelhamer J, Maclowry J, Parker M, Masur H. *Pneumocystis carinii* identified by Gram stain of lung imprints. *Ann Intern Med* 1983;99:484-5.
- 6 Pintozzi RL, Blecka LJ, Nanos S. The morphologic identification of *Pneumocystis carinii*. *Acta Cytol (Baltimore)* 1979;23:35-9.
- 7 Orenstein M, Webber CA, Heurich AE. Cytological diagnosis of *Pneumocystis* infection by bronchoalveolar lavage in acquired immune deficiency syndrome. *Acta Cytol (Baltimore)* 1985;29:727-31.
- 8 Gaurner J, Robey SS, Gupta PK. Cytological detection of *Pneumocystis carinii*: a comparison of Papanicolaou and other histochemical stains. *Diagn Cytopathol* 1986;2:133-7.

Analysis of odontogenic keratocyst fluids for preoperative diagnosis

We were interested to read the recent report¹ of a protein in fluid aspirates from odontogenic keratocysts, which may have potential as a marker for preoperative diagnosis.

There have now been several reports²⁻⁵ of proteins thought to be unique to odontogenic keratocyst fluids. The relation between the proteins reported in these studies is still not clear, although it is possible that some at least may be related to keratin. Southgate *et al.*⁵ showed an anodal electrophoretic mobility for their protein, and we have noted that fluids from keratocysts in patients with Gorlin's syndrome often seem to show strong protein staining in this region. Interestingly, an antiserum raised to keratocyst fluid¹ showed strong staining of a protein in Gorlin's syndrome cyst fluids, suggesting a possible association. The epithelial origin of these proteins suggests that they could have potential as markers for keratocysts, but further studies are required to confirm this. In particular, the possible presence of the proteins in other odontogenic cysts that show areas of keratinisation in their epithelial linings requires investigation.

The analysis of total soluble protein con-

Letters to the Editor

centration, the ratio of albumin to globulins, and the presence of epithelial squames seem to be reliable in preoperative diagnosis of keratocysts.⁶ Various studies have shown that keratocysts contain lower concentrations of total soluble protein than other odontogenic cyst fluids and that the proportion of albumin to globulins is higher in the keratocyst.

The very wide range of protein concentrations in cyst fluids reported by Douglas and Craig¹ seems to be at variance with these other studies, and perhaps questions the validity of their conclusion that protein assay is unreliable for preoperative diagnosis. The analysis of protein concentration in odontogenic cyst fluids requires careful sampling techniques and suitable assays.⁶ The assay of protein by the method of Lowry, as used by Douglas and Craig, produces sampling problems because of the sensitivity of the assay. Sample volumes of the order of one microlitre or dilution of the cyst fluids about one hundred times are required for the Lowry assay, both of which are inherently troublesome because of the mucinous and highly viscous nature of some fluids. We thus advocate the use of the bromocresol green dye binding assay for albumin and the glyoxylic acid method for globulins, together with the use of positive displacement pipettes for accurate sampling of the fluids.⁶ Quantitation of protein values in fluids stored for prolonged periods at -20°C requires caution because of possible concentration of protein components by freeze drying. We have found that small blood tubes with rubber O-ring sealing gaskets are beneficial for storage and seem to reduce the freeze drying by providing an effective seal.

We look forward to further confirmation of the presence of unique proteins in keratocyst fluids, but at present, the combination of protein analysis and showing the presence of epithelial squames offer the most accurate preoperative diagnosis of the odontogenic keratocyst. The analysis of total soluble protein concentration alone may partially account for false negative diagnosis of keratocysts,¹ and the need to combine such determinations with assessment of the relative proportions of the different serum proteins and the showing of epithelial squames should be emphasised.

AJ SMITH

RM BROWNE

Department of Oral Pathology,
Dental School,
University of Birmingham,
St Chad's Queensway,
Birmingham, B4 6NN

References

- 1 Douglas CWI, Craig GT. Recognition of proteins apparently specific to odontogenic keratocyst fluids. *J Clin Pathol* 1986;39:1108-5.
- 2 Kuusela P, Hormiam M, Tuompo H, Ylipaavalniemi P. Demonstration and partial characterisation of a novel soluble antigen present in keratocyst. *Oncodevelopmental Biology and Medicine* 1982;3:283.
- 3 Kuusela P, Ylipaavalniemi P, Thesleff I. The relationship between the keratocyst antigen (KCA) and keratin. *J Oral Pathol* 1986;15:287-91.
- 4 Davies JD, Southgate J, Whicher JT, Matthews RW. Novel antigenic marker of squamous epithelium: keratocyst anodal protein. *J Pathol* 1984;142:29-30.
- 5 Southgate J, Whicher JT, Davies JD, O'Reilly DSJ, Matthews RW. A protein of squamous keratinising epithelium from odontogenic keratocyst fluid. *Virchows Arch (Pathol Anat)* 1986;409:705-13.
- 6 Smith AJ, Smith G, Browne RM. Analysis of odontogenic cyst fluid aspirates. *IRCS Med Sci* 1986;14:304.

Simple reagent sparing approach to immunocytochemical staining of haematological and other samples with APAAP technique

The use of immunocytochemical techniques for detecting leucocyte differentiation antigens in the diagnosis and classification of haematological malignancies has become an attractive and practical proposition for haematology laboratories.¹ The immunalkaline phosphatase (APAAP) technique² is simple, does not require special equipment, and lends itself to routine laboratory

use. A major drawback of this technique has been the high cost of the reagents required, in particular the commercially available monoclonal antibodies and, to a lesser extent, the APAAP complexes.

One way to minimise reagent costs is to use cytopsin preparations or buffy coat smears to concentrate the cells of interest, but there are often advantages in practice in using simple smears of blood or bone marrow. We have found that the method works very well on routine smears when these are "flooded" with reagent, but that reducing reagent volumes leads to drying during incubation and poor localisation of reagent over the site of interest. This results in background staining, loss of morphological detail, impaired antigen antibody binding, and patchy staining.

We have used a simple technique to allow the use of small volumes of reagent without introducing drying artefacts. Simple "chambers" were constructed using 50×75 mm glass slides and two coverslips mounted on each with neutral medium (XAM improved white, BDH) (figure). During staining slides are placed face downwards with the area of interest over the well between the two coverslips. The slides to be stained will be wet and therefore adhere to the coverslips allowing the desired reagents to be run into the well. The size of the chamber can be varied to allow different volumes of reagent to be used. We have found that $50-70 \mu\text{l}$ of reagent is quite adequate when the coverslips are $1.0-1.5$ cm apart, and that this spacing is usually sufficient to cover the area of interest on the slides. The chamber can be rinsed in distilled water and dried while test slides are being washed.

Using a 30 minute incubation in a moist chamber at room temperature we saw no evaporation of fluid, and when we attempted

Figure Staining "chamber".