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.

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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-