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Research Note

A Selective Silver Staining Method for Identification of *Pneumocystis carinii* in Histologic Sections

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Many methods have been developed for the purpose of staining *Pneumocystis carinii* in paraffin sections, including Grocott-Gomori methenamine-silver nitrate (Gomori 1946 ; Grocott 1955), modified Grocott's methenamine-silver nitrate (Smith and Hughes 1972), toluidine blue O(Chalvardjian and Grawe 1963), cresyl echt violet (Bowling *et al.*, 1973), and ammoniacal silver nitrate (Senba 1983, 1984). Among these staining techniques, the most popular is the methenamine-silver nitrate method, which can stain fungi easily but is difficult to stain *Pneumocystis carinii* constantly and to obtain stable results. It was found that more stable and satisfactory results could be obtained by the ammoniacal silver nitrate method previously reported by the author (Senba 1983, 1984). The present paper further describes a modified method which is simpler and more specific than the author's previous one ; the improved method stains *Pneumocystis carinii* selectively without staining reticulum fibers. In the improved procedure, periodic acid is used as the oxidizing reagent instead of potassium permanganate and ten steps are omitted from previous procedures (Senba 1983, 1984).

The lung specimens from autopsy cases of *Pneumocystis carinii* infection were used. The materials were fixed in formalin and embedded in paraffin. The steps involved in the modified silver staining technique are as follows: (1) Deparaffinize and hydrate to distilled water. (2) Treat with 0.5 % periodic acid solution for 20 min. (3) Wash in distilled water. (4) Treat with ammoniacal silver nitrate solution and keep in at 60°C water bath for 30 min. Ammoniacal silver nitrate solution : To 20 ml of 10 % silver nitrate solution add 0.4 g sodium hydroxide, and add dropwise 28 % ammonium hydroxide, until only a few granules of the resulting precipitate remain on the bottom of the cylinder. And add distilled water to make 100 ml. Dilute 1 part ammoniacal silver nitrate solution with 4 parts distilled water for use. Store in the refrigerator and use as needed. (5) Wash in distilled water. (6) Treat with 0.5 % gold chloride solution for 3 min. (7) Wash in running water. (8) Treat with 10 % sodium thiosulfate solution for 5 min. (9) Wash in running water. (10) Treat with nuclear fast red solution for 5 min. Nuclear fast red solution : Dissolve 0.1 g nuclear fast red in 100 ml of 5 % solution of aluminum sulfate with aid of heat. Cool, filter, and add grain of thymol as a preservative. (11) Wash in running water. (12) Dehydrate, clear and mount.

Pneumocystis carinii and fungi are stained black (Figs. 1 and 2). Nuclei are stained red. In the modified ammoniacal silver method, reticulum fibers and nuclei of host cells are not stained with the silver particles. *Pneumocystis carinii* are stained with ammoniacal silver nitrate solution at 60 °C for 30 min, but not at room temperature. The exfoliation of sections from the slide glass due to diffuse bleeding, can be prevented by the use of 2 % ferric ammonium sulfate solution for 2 min after 3 of this procedure and was-

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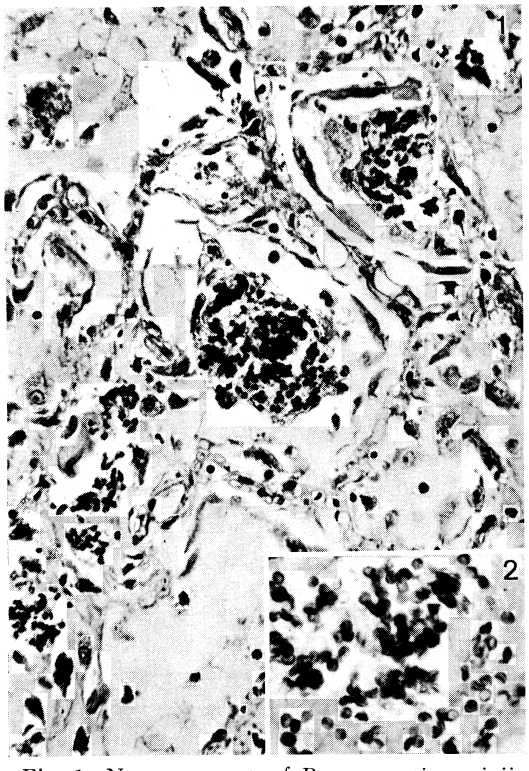

Fig. 1 Numerous cysts of *Pneumocystis carinii* stained with ammoniacal silver nitrate in the alveolar space. Note the typical round, oval and crescent forms. Original magnification ×200.

Fig. 2 Highter magnification of figure 1. Original magnification ×400.

hed in running water. In such cases, the reticulum fibers are stained black. The ammoniacal silver nitrate solution has been used for the routine staining of reticulum fibers. The used ammoniacal silver nitrate solution has a risk of explosion due to the formation of the blackish silver fulminate (AgONC) in the solution. Such explosion could be prevented by the addition of sodium thiosulfate, which accelerates the formation of stable $AgS_2O^-_3$, Ag $(S_2O_3)_2^{3-}$, and Ag $(S_2O_3)_3^{5-}$.

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References

1) Bowling, M. C., Smith, I. M. and Wescott, S. L. (1973): A rapid staining procedure for *Pneumocystis carinii*. Am. J. Med. Technol., 39, 267-268.
2) Chalvardjian, A. M. and Grawe, L. A. (1963): A new procedure for the identification of *Pneumocystis carinii* cysts in tissue sections and smears. J. Clin. Pathol., 16, 383-384.
3) Gomori, G. (1946): A new histochemical test for glycogen and mucin. Am. J. Clin. Pathol., 10, 177-179.
4) Grocott, R. G. (1955): A stain for fungi in tissue sections and smears using Gomori's methenamine-silver nitrate technic. Am. J. Clin. Pathol., 25, 975-979.
5) Japanese Society of Chemistry. (1973): Handbook of Chemistry. Maruzen, Tokyo, 1152 (in Japanese).
6) Senba, M. (1983): A reliable silver staining method for *Pneumocystis carinii* and reticulum fibers in histologic sections. Acta Histochem. Cytochem., 16, 169-171.
7) Senba, M. (1984): A reliable silver staining method for identification of *Pneumocystis carinii* in histologic sections. Tohoku J. Exp. Med., 143, 397-404.
8) Smith, J. W. and Hughes, W. T. (1972): A rapid staining technique for *Pneumocystis carinii*. J. Clin. Pathol., 25, 269-271.

短　報　　**特異的な *Pneumocystis carinii* の組織内における染色法**

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Pneumocystis carinii の染色には，一般にメテナミン銀を使用した染色法が行われている．この染色法では真菌を染めるのは容易であるが，*Pneumocystis carinii* を常に満足できるように染色することは難しい．著者はかってアンモニア銀を使用する方法を報告した．今回著者は前回と同じアンモニア銀を使用して，細網線維が染まらず *Pneumocystis carinii* が特異的に染め出し，しかも手技を簡単にした方法を開発したので報告した．