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Recognition of *Pneumocystis carinii* by Gram Stain in Impression Smears of Lung Tissue

T. P. FELEGIE,[1] A. W. PASCULLE,[1,2]* AND A. DEKKER,[1,2]

Department of Pathology, Presbyterian-University Hospital, Pittsburgh, Pennsylvania 15213,[1] and University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261[2]*

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In 12 of 20 (60%) biopsy-proven cases of *Pneumocystis carinii* pneumonia, the diagnosis was first suggested by examination of routine Gram stains of impression smears made from infected lung tissue and later confirmed by methenamine-silver staining. The cysts appeared as 5- to 7-μm unstained spheres, each containing six to eight intracystic gram-negative bodies (sporozoites). Although the Gram stain does not appear to be as sensitive as more traditional staining techniques for the detection of *P. carinii*, clinical microbiologists should be aware of the morphology of this organism in gram-stained specimens because this relatively simple procedure gives quick results.

Pneumocystis carinii produces a rapidly progressive and often fatal pneumonitis in a variety of immunocompromised patients, including transplant recipients and patients with various hematological malignancies (10). Infection with this organism has also been found to occur among homosexuals, drug abusers, and others suffering from acquired immunodeficiency syndrome (9).

Traditionally, diagnosis of this infection has been made by finding the organism in either sections or impression smears of lung tissue. The cyst form may be detected by staining with the Grocott methenamine-silver stain or one of its modifications (3, 4, 8), the Giemsa stain (2), or the Gram-Weigert stain (6, 12). Although it has been reported (1) that the organism can be stained by the Gram stain techniques which are used for sections of fixed tissue, it was believed until recently that this organism could not be routinely visualized by gram-staining impression smears (7).

During routine processing of a lung tissue specimen from a patient with acquired immunodeficiency syndrome, we discovered that the cyst form of *P. carinii* was stained by the routine Gram stain procedure used in our laboratory. The organism could also be seen in impression smears stained with a modified Ziehl-Neelsen stain. Over the next 20 months, of 20 biopsy-proven cases of *P. carinii* infection, 12 (60%) were initially diagnosed by the routine Gram stain. This report discusses the microscopic morphology of *P. carinii* stained by the Gram method so that others may recognize this organism under similar circumstances.

All patients involved in this study were immunosuppressed due to underlying disease or organ transplantation. Among the patients, 16 were renal transplant recipients, 1 was a heart transplant recipient, and 2 were suffering from acquired immunodeficiency syndrome. The 20 specimens included in this study were collected as part of our routine workup for pneumonitis in immunocompromised patients (5). Of the 20 specimens, 19 consisted of lung tissue collected by either open lung (18 specimens) or transbronchial (1 specimen) biopsy. The remaining specimen consisted of bronchial brushings collected with a protected bronchial brush.

Impression smears of each specimen were made and stained by the routine Gram stain procedure used in our laboratory. The duration of staining with crystal violet, iodine, and safranine was 1 min each, and a 50% (vol/vol) solution of acetone-ethanol was used for destaining. To attempt to increase the number of organisms seen with the Gram stain, we increased the staining time with safranine to 5 min for a duplicate slide of one specimen. We also used a carbolfuchsin counterstain (11) on duplicate slides of several specimens.

In the 12 specimens that were positive by Gram stain, the number of *P. carinii* cysts seen was very small (typically one to five per slide), and thus it was often necessary to examine the slides thoroughly (15 to 20 min) to find a representative cyst form.

In gram-stained tissue impression smears, *P. carinii* cysts appeared as spherical gram-negative structures with a diameter of 5 to 7 μm. Most cysts contained eight gram-negative intracystic bodies and appeared to be similar in morphology to *P. carinii* cysts stained by the Giemsa technique. The most easily recognized form of *P. carinii* contained a rosette of the eight intracystic bodies (Fig. 1A). Many of the cysts lacked this characteristic arrangement, but could still be seen to contain six to eight irregularly arranged intracystic bodies (Fig. 1B). The morphology of the organism when stained by the modified Ziehl-Neelsen stain was identical to that seen with the Gram stain. Organisms stained by this method appeared to be non-acid fast, with blue intracystic bodies.

All 20 specimens were found to contain *P. carinii* when stained with the Grocott methenamine-silver stain. The number of organisms seen in the silver-stained preparations was always much higher in specimens which were also Gram stain positive than in those which were Gram stain negative. These results suggest that the Gram stain is much less sensitive for the detection of *P. carinii*. Increasing the staining time with safranine to 5 min did not increase the number of identifiable organisms. Although the substitution of carbolfuchsin for safranine as a counterstain did not increase the number of organisms seen, carbolfuchsin did stain the organisms more intensely, making them easier to see. We have since adopted the use of carbolfuchsin as a counterstain for this procedure.

Our results suggest that the Gram stain may be useful in the diagnosis of infections caused by *P. carinii*. A thorough

* Corresponding author.

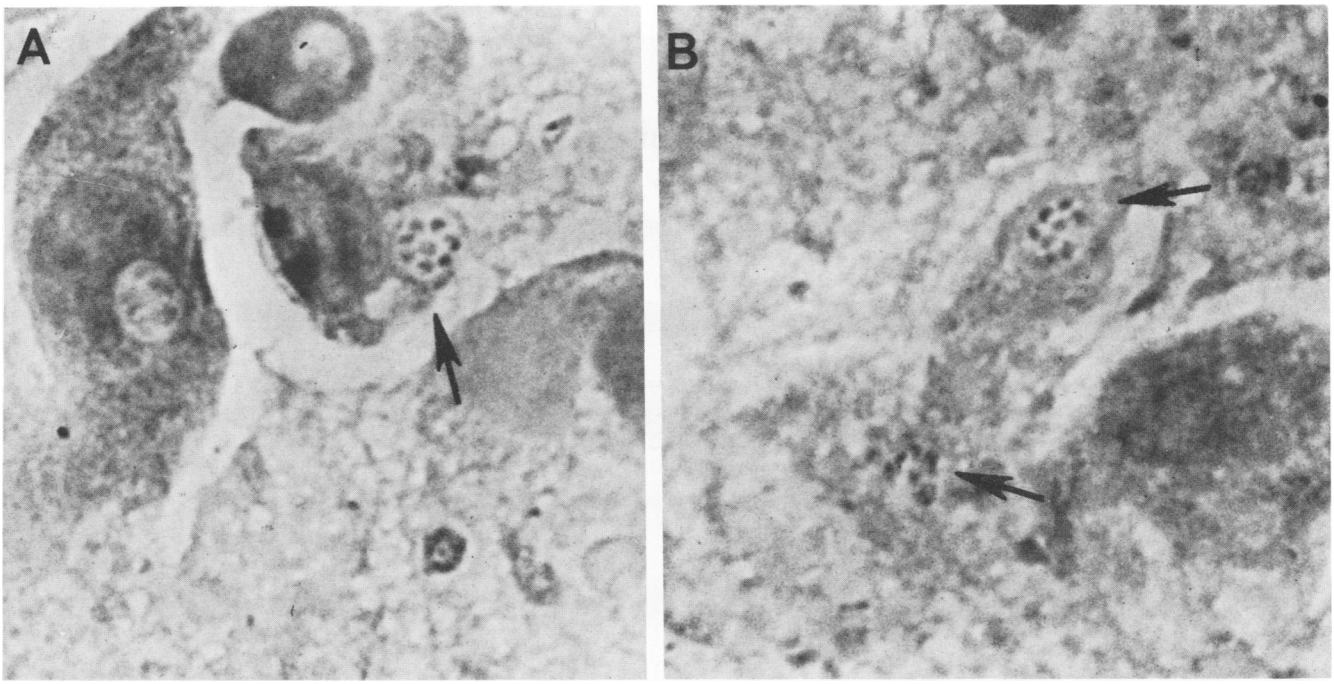

FIG. 1. *P. carinii* cysts in gram-stained lung impression smear counterstained with safranine. (A) The most recognizable form appears as a 5- to 7-μm spherical cyst containing a rosette of eight intracystic bodies. (B) Another are of the same specimen containing less characteristic but still recognizable cysts (×970).

(15-min) examination of the gram-stained slide is usually necessary to detect the characteristic cysts in samples from patients with a compatible clinical history and roentgenographic features. The Gram stain alone is not sufficiently sensitive for the routine detection of *P. carinii*. However, clinical microbiologists should be aware of the morphological appearance of *P. carinii* in gram-stained materials since the results of this examination are often available long before those of the stains traditionally used to detect *P. carinii*.

LITERATURE CITED

1. **Frenkel, J. K.** 1976. Pneumocystosis, p. 303–307. *In* C. H. Binford and D. H. Connor (ed.), Pathology of tropical and extraordinary diseases. Armed Forces Institute of Pathology, Washington, D.C.
2. **Gajdusek, D. C.** 1957. *Pneumocystis carinii*—etiological agent of interstitial plasma cell pneumonia of premature and young infants. Pediatrics **19**:543–565.
3. **Gomori, G.** 1946. A new histochemical stain for glycogen and mucin. Am. J. Clin. Pathol. (Tech. Bull.) **16**:177–179.
4. **Grocott, R. G.** 1955. A stain for fungi in tissue sections and smears. Am. J. Clin. Pathol. **25**:975–979.
5. **Haverkos, H. W., J. N. Dowling, A. W. Pasculle, R. L. Myerowitz, D. B. Lerberg, and T. R. Hakala.** 1983. Diagnosis of pneumonitis in immunocompromised patients by open lung biopsy. Cancer (Philadelphia) **52**:1093–1097.
6. **Le, T.-V., A. M. Cochard, T.-D. Vu, and W. Solanar.** 1963. Diagnostic "in-vivo" de la pneumonie á "pneumocystis." Arch. Fr. Pediatr. **20**:773–792.
7. **Macher, A. M., J. Shelhamer, J. MacLowry, M. Parker, and H. Masur.** 1983. *Pneumocystis carinii* identified by Gram stain of lung imprints. Ann. Intern. Med. **99**:484–485.
8. **Mahan, C. T., G. E. Sale.** 1978. Rapid methenamine silver stain for *Pneumocystis* and fungi. Arch. Pathol. Lab. Med. **102**:351–352.
9. **Masur, H., M. A. Michelis, J. B. Greene, I. Onorato, R. A. Vande Stouwe, R. S. Holzman, G. Wormser, L. Brettman, M. Lange, H. W. Murray, and S. Cunningham-Rundles.** 1981. An outbreak of community-acquired *Pneumocystis carinii* pneumonia. N. Engl. J. Med. **305**:1431–1438.
10. **Waltzer, P. D., D. P. Perl, D. J. Krogstead, P. G. Rawson, and M. G. Shultz.** 1974. *Pneumocystis carinii* in the United States. Epidemiologic, diagnostic, and clinical features. Ann. Intern. Med. **80**:83–93.
11. **Weaver, R. E., and J. C. Feeley.** 1979. Cultural and biochemical characterization of the Legionnaires disease bacterium, p. 20–25. *In* G. L. Jones and G. A. Hebert (ed.), Legionnaires: the disease, the bacterium, and methodology. Department of Health, Education and Welfare, Atlanta.
12. **Weigert, C.** 1887. Uber eine neue Methode zur Farbung von Fibrin und von Microorganisms. Fortschr. Med. **5**:228–232.