Identification of *Pneumocystis carinii* by Quick Hematoxylin and Eosin Smear

Roberto Orozco-Florian* and Alberto Trillo, Department of Pathology, Victoria General Hospital, 1278 Tower Road, Halifax, Nova Scotia, Canada B3H 2Y9

Abstract

Intraoperative identification of *Pneumocystic carinii* generally involves the use of special stains, which are time consuming and require expertise. The hematoxylin-eosin (H & E) stain readily demonstrates the organism in smears but not in tissue sections. The method described affords identification of these organisms within 5-10 min. A smear obtained from bisected lung biopsy tissue is immediately fixed with cytosol spray, dried for a few sec, and stained with H & E. When slides are examined with either high dry or oil immersion objectives, the smear shows foamy amorphous clusters of organisms, macrophages, and epithelial cells. The method stains both the cysts and the trophozoites, and the results correlate with those from special stains on tissue sections. It is rapid, simple to perform, low in cost, and easy to interpret. (*The J Histotechnol* 14:179, 1991)

Key words: hematoxylin-eosin, lung biopsy, *Pneumocystis carinii*, pneumonia, smear, special methods

Introduction

Pneumocystis carinii pneumonia is a common opportunistic infection in immunocompromised patients (1,2). Presently, it is the most frequently recognized cause of death in patients with AIDS (3). If diagnosed and treated early, its fatal outcome can be prevented, but there is no rapid and reliable method for identifying the organisms in lung biopsy tissue (2). There are methods, such as those that use indirect fluorescent antibodies, that are sensitive and specific, but they are time consuming, technically difficult, and unavailable in most laboratories (4,5).

The need for a simple procedure becomes more critical in the frozen section lab where experienced technical assistance may not be available. This problem prompted us to search for such a method. We describe a method of processing smears from freshly cut lung biopsy tissue that utilizes the traditional smear technique and a modification to the routine hematoxylin-eosin (H & E) staining. This method is fast, simple, easy to interpret, and does not introduce any unfamiliar background staining.

* Author to whom offprint requests are addressed.

Materials and Methods

Preparation of smear

1. Wear gloves, mask, goggles and gown.
2. Have cytospray ready at hand.
3. Cut open biopsy tissue.
4. Gently scrape cut surface of tissue with one end of a glass slide.
5. Smear scraped tissue onto a clean glass slide.

Quick H & E stain

1. Fix slide with cytospray immediately.
2. Blow dry for a few sec.
3. Place slide in Harris alum hematoxylin for 20 sec.
4. Wash in tap water for 2-3 sec.
5. Place in Scott tap water substitute (sodium bicarbonate, magnesium sulfate, and tap water) for 10 sec.
6. Wash in tap water for 2-3 sec.
7. Place in 2% aqueous eosin for approximately 10 sec.
8. Wash in tap water for 2-3 sec.
9. Dehydrate with absolute denatured ethyl alcohol.
10. Wash slide with xylene.
11. Coverslip using DePex mountant medium (BDH Ltd, Poole, England).
12. Examine slide under high power or oil immersion.

Results

The result is the familiar H & E staining of tissue with the suggestive intraalveolar accumulation of amorphous eosinophilic material. The cysts (Figure 1) and the intracystic trophozoites of the organism are clearly seen (Figure 2). This stain also allows evaluation of underlying or concurrent lung pathology.

Discussion

There is an increase in the number of *P. carinii* pneumonias seen in patients with AIDS (5). Recent advances have been made in large centers in the identification of the organism, especially in samples obtained from bronchoalveolar lavage, transbronchial biopsies, and induced or spontaneous