Technical methods

Modified Grocott's methenamine silver nitrate method for quick staining of *Pneumocystis carinii*

R. L. PINTOZZI *University of Illinois Medical Center, 1853 West Polk Street, Chicago, Illinois 60612, USA*

A number of special staining methods for *Pneumocystis carinii* have been reported. Most of these methods give satisfactory results although they require varying amounts of diagnostic acumen. Three of the more popular stains for *P. carinii* are the toluidine blue 0 stain (Chalvardjian and Grawe, 1963), the Gram-Weigert stain (Rosen *et al.*, 1975), and Grocott's adaptation of Gomori's methenamine silver nitrate (MSN) stain (Grocott, 1955). The toluidine blue 0 stain reveals only the cyst form of the organism, but staining of background material in smears often makes identification of the organisms difficult. The Gram-Weigert stain permits identification of two morphological stages of the organism (cyst and sporozoite), but cysts may be indistinguishable from nonbudding yeast cells, overstained red blood cells, or stained nuclei of leucocytes. Grocott's MSN stain shows the greatest contrast between the organism and its environment. Staining the cyst stage dark brown and the background material light green, it is considered to be the best stain for identifying *P. carinii*.

In the past, the most common problems with Grocott's MSN stain have been the length of time needed for completion (2-3 hours) and the variability of the reduction step of the stain. The acute onset of *P. carinii* pneumonitis makes such a long and difficult stain impractical except for confirmation of diagnosis in many cases.

Two other modifications of Gomori's MSN stain have been reported. Smith and Hughes (1972) introduced a stain for smears while Churukian and Schenk (1977) introduced a stain for both smears and sections. Others have found the results of the former stain to be inconsistent (Churukian and Schenk, 1977). The latter stain, although still rather long, seems to stain the *P. carinii* cysts well, but variation in the reduction time has been noted.

In the following modification of Grocott's MSN stain, total staining time has been reduced to 10 minutes and the variation of incubation time for the MSN impregnation step (reduction step) of the stain has been minimised. Excellent results have been obtained with impression smears, frozen sections, and paraffin sections. Fungus seems to stain equally as well as *P. carinii*.

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Material and methods

PREPARATION OF REAGENTS

MSN stock solution is prepared by mixing 5 ml of 5% silver nitrate with 100 ml of 3% methenamine. The working solution is prepared by mixing 2 ml of 5% powdered borax with 25 ml of distilled water and 25 ml MSN stock solution. MSN working solution should be used only once in this quick method as re-use increases the amount of time for the MSN impregnation step. All MSN solutions should be kept refrigerated.

The light green stock solution is prepared by mixing 0·2 g light green SF, CI No. 42095 in 100 ml 0·2% glacial acetic acid. The working solution is prepared by diluting 10 ml of stock solution with 40 ml distilled water.

An 80°C waterbath is used in this procedure. Cracking of Coplin jars did not occur as long as the jars and their contents were at room temperature before immersion into the hot waterbath. Five minutes before starting the staining procedure, place two Coplin jars, one containing 5% chromic acid and the other containing MSN working solution (52 ml), into the 80°C waterbath.

Smears and cryostat sections should be fixed in 95% alcohol for 1-3 minutes before hydrating.

STAINING PROTOCOL

1 Place dehydrated smears, cryostat sections, or deparaffinised sections into hot 5% chromic acid for 2 minutes—oxidation step (in waterbath).
2 Dip slides in three changes of distilled water.
3 Place in 1% sodium bisulphite for 30 seconds.
4 Dip slides three times in fresh distilled water.
5 Place slides in heated MSN working solution for 5 minutes—reduction step (in waterbath).
6 Dip slides three times in fresh distilled water.
7 Place slides in 0·2% gold chloride for 10 seconds.
8 Dip three times in fresh distilled water.
9 Dip three times in 2% sodium thiosulphate.
10 Dip three times in distilled water.
11 Place slides in light green working solution for 30 seconds.

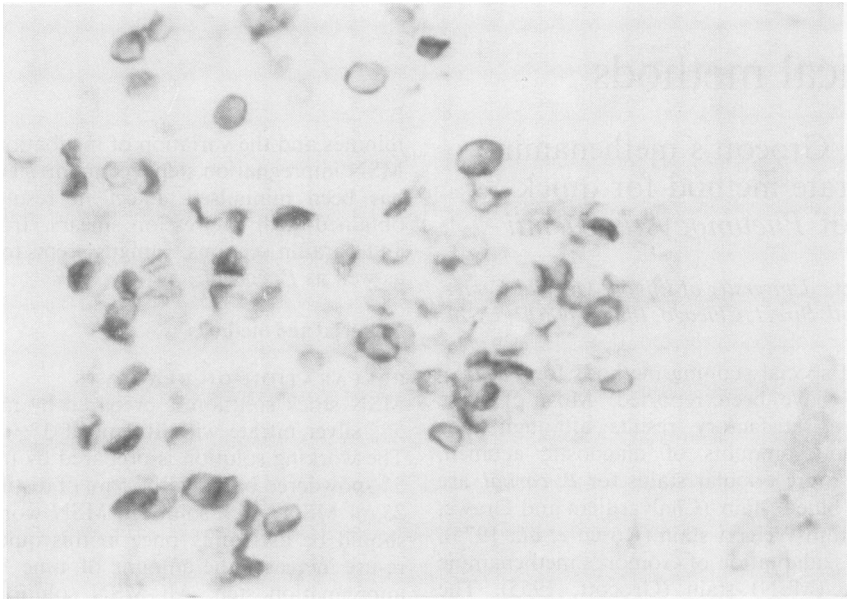

Fig. 1 *Paraffin-embedded lung section containing* Pneumocystis carinii *cysts stained with modified Grocott's silver stain. Note faintly staining RBCs in blood vessel on lower right.* × 1200.

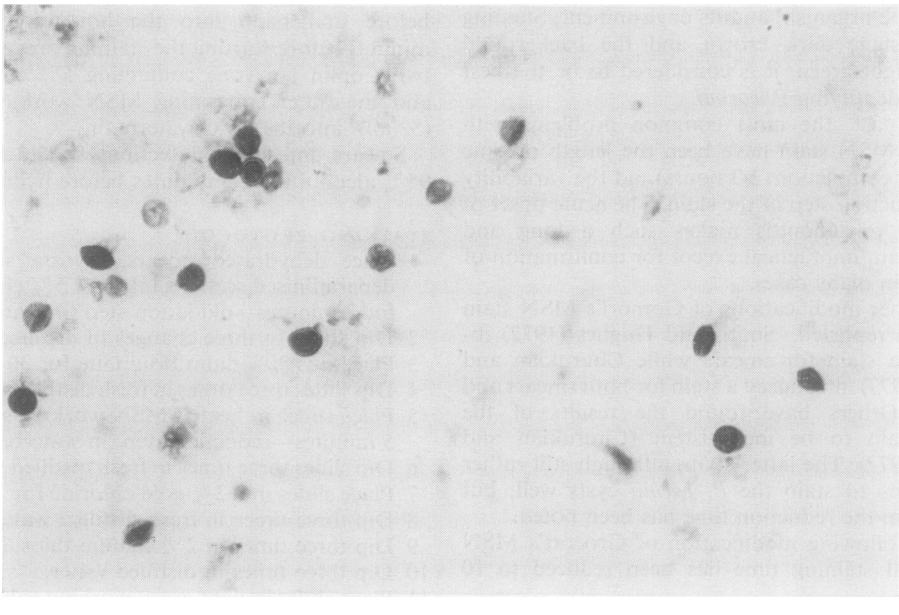

Fig. 2 *Lung impression smear stained with modified Grocott's silver stain.* × 1200.

12 Dip slides in three changes of distilled water.
13 Dehydrate in 95% alcohol and 100% alcohol, and clear in xylene.
14 Mount.

It is important to run a positive control along with your specimen. The control slide will look brown after 5 minutes in MSN working solution if the technique has been followed correctly.

Results

As can be seen in Figs 1 and 2, *P. carinii* cysts stain equally well in both smears and sections. Intracystic staining of sporozoites and parenthesis-like bodies, as seen by other authors (Kim *et al.*, 1972), were commonly found. The use of light green counterstain for smears was found to be unnecessary as far fewer tissue cells were present to pick up the stain.

Discussion

Hospitals maintaining patients at high risk for *P. carinii* infection would benefit most from this staining method. It is recommended that an 80°C waterbath be kept available for use by pathology residents and night technicians doing this technique.

The primary diagnostic tool for *P. carinii* infection being morphological identification of the organism in lung material, it is hoped that this modified Grocott's method will improve patient prognosis through speedy diagnosis.

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Requests for reprints to: R. L. Pintozzi, Department of Pathology, University of Illinois Medical Center, 1853 West Polk Street, Chicago, Illinois 60612, USA.