Technical methods

the screen. The titres of these sera are shown in Table II.

Comment

Absorption of non-specific inhibitors from sera by kaolin was chosen in preference to the newer methods described by Cooper, Matters, Rosenblum, and Krugman (1969) or Liebhaver (1970) because kaolin was specific in our hands, highly reproducible, and did not have disadvantages the other methods showed.

In this laboratory, serological tests for syphilis are made on about 140 antenatal sera each week. To perform an additional screen test on these sera involves no extra labour in bleeding patients and separating clots. The rubella screen test is carried out twice a week, which is not an undue delay for this type of investigation, but an immediate titration of serum can always be made when indicated.

Sera are dealt with in batches of about 70, titrations being performed at the same time as the next batch of screens. It is found that the total technician time involved is less than five technician hours a week for screens, while the further treatment and titration of sera takes an additional two hours a week. The work load has been absorbed by fragmentation of the procedure into stages which can be fitted into re-organized schedules without disruption. A few routines which experience shows no longer produce results commensurate with the effort involved have been discarded, but this is a constant feature of any laboratory with an expanding work load.

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A rapid staining technique for *Pneumocystis carinii*

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Pneumocystis carinii pneumonia is an acute, severe, rapidly progressive and generalized pulmonary infection occurring almost exclusively in patients receiving immunosuppressive therapy for organ transplantation, leukaemia, or other malignancies and in premature or debilitated infants. Early therapy with pentamidine isethionate may be life-saving. However, diagnosis requires the demonstration of *P. carinii* in lung tissue or fluid obtained by biopsy or percutaneous needle aspiration. The organism can be clearly demonstrated by the methenamine silver nitrate impregnation technique of Gomori. Although the preparations by this method are satisfactory, there are practical disadvantages to the technique. The Gomori method is unduly long, requiring two to three hours to complete and the skill of an experienced technician. Clinically, such a delay in diagnosis hours may impede successful treatment. If patients present during off-duty hours for laboratory personnel, more prolonged delays can be expected.

These considerations were the basis for the development of a technique described below, which is both rapid and simplified, can be performed in the routine clinical laboratory, and retains the basic principles and specificity of Gomori's method. The procedure can be completed in five minutes.

Method

The technique for aspiration of pulmonary fluid has been described (Johnson and Johnson, 1970). Usually 0.1 ml or less of the aspirate is dropped onto a clean slide and allowed to dry.

SOLUTIONS REQUIRED

Five percent chromic acid, 1% sodium bisulphite, working methenamine silver nitrate (Grocott), dimethyl sulphoxide (DMSO), working light green (Grocott), 95% ethyl alcohol, absolute ethyl alcohol, and xylene

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PROCEDURE

- 1 Heat slide for five seconds for fixation.
- 2 Cover slide with 5% chromic acid and heat over low flame until it steams for 20 to 30 seconds.
- 3 Rinse in tap water gently for five seconds.
- 4 Immerse in 1% sodium bisulphite for 20 seconds.
- 5 Rinse in tap water for five seconds.
- 6 Immerse in distilled water for five seconds.
- 7 Immerse in a solution of working methenamine silver nitrate: DMSO (2 : 1) and heat gently over medium flame until cloudiness of solution fades and a swirling black silver precipitate appears, usually about one minute.
- 8 Remove slide and immerse in distilled water for 20 seconds, agitating to remove excess silver.
- 9 Counterstain in working light green solution for 30 to 45 seconds.
- 10 Dehydrate rapidly with two changes in 95% alcohol.
- 11 Place in absolute alcohol for 10 seconds.
- 12 Immerse in xylene and dip up and down completely in and out of the solution 10 times.
- 13 Immerse in a second change of xylene for 10 seconds.
- 14 Mount in a permanent mounting medium and examine microscopically.

Results

The staining characteristics and morphology of *P. carinii*, using the rapid method, are similar to preparations made by the Gomori method (Fig. 1). The cysts have a thin black capsule enclosing a pair of structures which resemble parentheses. The cytoplasmoid area of the pneumocyst frequently assumes a slightly golden cast and the organism stands out with clarity against a pale green background.

The rapid method was compared to the standard Gomori method in specimens from 10 patients with *P. carinii* pneumonitis. Seven of these specimens were lung aspirates and three were imprints made from lungs at necropsy. Preparations by the Gomori method were interpreted by one reader and the rapid method by another reader. After interpretations were completed comparisons were made between results of the two methods and were found to be in complete agreement.

Comment

The mechanism by which DMSO increases cellular uptake of various substances is presumed to be

A **B**

Fig. 1 *Pneumocystis carinii* in pulmonary aspirate stained by the standard method of Gomori (A) and by the rapid method described here (B). $\times 1\ 200$.

alteration in membrane permeability (Jacob, Bischel, and Herschler, 1964). Reversible alteration in membrane protein structure and/or the removal of fatty acids from the cell membrane may occur. These findings imply facilitation of movement of some substances by changes in membrane porosity.

As with most staining techniques, it is important that solutions be changed after frequent use. It is also helpful to the interpreter to have a control specimen with known *P. carinii* stained simultaneously with the test specimen. We found that necropsy lung sections from *P. carinii* pneumonitis can be maintained in refrigeration several months for this purpose.

The procedure has been carried out with excellent results by technicians after brief orientation.

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A simple method for studying nuclear division in free-living soil amoebae

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The study of mitotic figures is an essential investigation for the classification of free-living amoebae. Singh (1950) described a method requiring the growth of strains on a thin agar film attached to a glass slide. After fixation, cells which had penetrated the agar layer were found attached to the surface of the slide. Alternatively, trophozoites may be removed in a small volume of distilled water deposited on culture plates and transferred to slides for fixation and staining. In our experience, the latter technique fails to produce sufficient cells for easy study and those that are visible appear shrunken and hyperchromatic. The method of Singh, as the author states, requires considerable practice and patience.

Materials and Methods

CULTURE OF AMOEBAE

A variety of free-living amoebae, including pathogenic isolates of *Naegleria fowleri* (Carter, 1970), were grown on plates of 1.25% agar (Difco Laboratories) in distilled water. After solidification, the agar surface was spread with a culture of *Escherichia coli* ATCC 11229 to within approximately 3 cm of the periphery. After drying, the plates were inoculated in the centre using a micropipette charged with a distilled water suspension of amoebae. Cultures were incubated at 37°C for 24 to 48 hours and then examined under a stereoscopic zoom microscope (Olympus, Tokyo).

PREPARATION OF AGAR BLOCKS

Blocks are cut from selected areas of heavy growth under stereomicroscopic control using a sterile blade (Swann Morton no. 11) mounted on a suitable handle. Blocks are lifted out on the point of the scalpel blade and placed, surface upwards, on a glass slide. At this stage, the presence of abundant amoebae can again be checked by microscopy.

PREPARATION OF SLIDES

Clean glass slides are pressed gently onto the surface

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