difference between the two. Fractions are diluted 1 in 20 by comparison with the original serum therefore the column recovery is obtained by adding together the reciprocal titres of all the fractions and multiplying by 20.

Comment

The method is as inexpensive as possible granted that an automatic fraction collector is used. The total capital cost, assuming that one is already performing HAI tests is £50. The technique has been made as simple as possible by removing all strictly unnecessary steps. Accordingly interpretation of results has been based on an appreciation of the overall pattern of HAI activity within the IgM and IgG fractions so that no undue emphasis is placed on any one titration. Of course better separation can be achieved by using longer columns of gel but this inevitably increases the cost and technical difficulties.

Just as variations in any of the parameters of rubella HAI tests have been shown to alter the results obtained (Schmidt and Lennette, 1970), so do any changes in the method of fractionation of sera. It is emphasized that each of these variables has been investigated and that the detailed procedures described have been selected so that the method remains simple but gives accurate results without difficulties of interpretation. Although the use of the method has been illustrated only for rubella serology, it could be applied to any serological test which will accept an initial serum dilution of 1 in 20 and is not affected by the presence of Nairn PBS plus 0.02% sodium azide.

References

- Beest, J. M., Banatvala, J. E., and Watson, D. (1969). Serum IgM and IgG responses in postnatally acquired rubella. *Lancet*, 2, 65-68.
- Clarke, D. H., and Casals, J. (1958). Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *Amer. J. Trop. Med. Hyg.*, 7, 561-573.
- Dold, H. J., and Northrop, R. L. (1968). The non-specific inhibitors of rubella-virus hemagglutination. *Proc. Soc. exp. Biol. (N.Y.)*, 128, 577-581.
- Gupta, J. D., Peterson, V., Stout, M., and Murphy, A. M. (1971). Single-sample diagnosis of recent rubella by fractionation of antibody on Sephadex G-200 column. *J. clin. Path.*, 24, 547-550.
- Haire, M., and Hadden, D. S. M. (1972). Rapid diagnosis of rubella by demonstrating rubella specific IgM antibodies in the serum by indirect immunofluorescence. *J. med. Microbiol.*, 5, 237-242.
- Nairn, R. C. (1964). *Fluorescent Protein Tracing*, 2nd ed. Livingstone, Edinburgh and London.
- Schmidt, N. J., and Lennette, E. H. (1970). Variables of the rubella-hemagglutination inhibition test system and their effect on antigen and antibody titres. *Appl. Microbiol.*, 19, 491-504.
- Stewart, G. L., Parkman, P. D., Hopps, H. E., Douglas, R. D., Hamilton, J. P., and Meyer, H. M., Jr. (1967). Rubella-virus hemagglutination inhibition test. *New Engl. J. Med.*, 276, 554-557.

Wet ashing using a closed system

GEORGE P. LEWIS AND LINDA L. COUGHLIN *From the Section of Clinical Pharmacology, Boston Veterans Administration Hospital and Tufts University School of Medicine, Boston, Massachusetts*

Wet ashing procedures are being used more extensively because of the increased interest in the biological role of trace metals. The standard procedures, using fuming nitric acid, or mixtures of concentrated acids are normally carried out within a laboratory hood, the acid fumes being vented through the hood's exhaust duct. To obtain adequate digestion, such techniques require the addition of several aliquots of acid. When large numbers of samples are being prepared, a considerable quantity of acid can be used in one day. These 'open' standard procedures lead to the emission of highly acid fumes into the ambient atmosphere, result in marked erosion of the hood's exhaust system, and can give rise to unacceptable levels of acid fume in the laboratory air. In order to overcome these objections, a 'closed' wet ashing system has been used in this laboratory during the past two years for the preparation of cadaver tissue samples for atomic absorption spectrophotometry (Lewis, Jusko, Coughlin, and Hartz, 1972a and b).

Method

Samples (kidneys 0.5 g, liver 2 g, lungs 4 g) are each placed in a 100 ml Erlenmeyer flask. Each flask is fitted to a Stark-Dean distilling trap of 10 ml capacity. Above the receiver is fitted a water-cooled condenser. All components have ground glass joints. The whole is then placed over a hot plate sited within a laboratory hood. Initially, the hot plate is set at 250°C, and the acid and sample are allowed to simmer gently for two hours with the hood exhaust fan on. The circulation of air around the Erlenmeyer flask prevents distillation of the acid into the trap. After this time, when the tissue has been completely solubilized and digested, the hood fan is turned off, and the hot plate is turned up to 350°C. The acid then refluxes into the trap over 20 to 30 minutes. When the volume of digest is reduced to 0.5 to 1.0 ml, heating is stopped by placing an asbestos pad between the hot plate and the Erlenmeyer flask. Following cooling, 15 ml of distilled water is added to the digest, and the pH adjusted by titration to 3.5 with concentrated ammonium

Received for publication 1 December 1972.

hydroxide. The final volume is then made up to 25 ml with distilled water. The acid in the trap is discarded into free-flowing water in a sink fitted with a glass diluting chamber. The figure shows how six 'closed' wet ashing systems can be arranged within the confines of a small hood.

Comment

The 'closed' wet ashing procedure allows for a very controlled digestion of tissues over any preselected time period. The system is simple and very effective in completely solubilizing tissues. It carries with it the advantages that its use is not attended by pollution of the atmosphere with acid fumes; complete digestion of tissue can be effected using a small volume of acid and corrosion of the laboratory hood and ducting system is eliminated.

References

- Lewis, G. P., Jusko, W. J., Coughlin, L. L., and Hartz, S. (1972). Contribution of cigarette smoking to cadmium accumulation in man. *Lancet*, **1**, 291-292.
- Lewis, G. P., Jusko, W. J., Coughlin, L. L., and Hartz, S. (1972). Cadmium accumulation in man: influence of smoking, occupation, alcoholic habit and disease. *J. chron. Dis.*, **25**, 717-726.

Letters to the Editor

Immunofluorescent Technique for Rapid Virus Diagnosis of Cells in Nasopharyngeal Secretions

We feel that Dr Urquhart and Dr Walker will expect us to comment on their article (*J. clin. Path.*, 1972, **25**, 843).

There will be opportunities to discuss our mutual experiences in detail but some observations are called for now.

We have now used an immunofluorescent technique for rapid virus diagnosis of cells in nasopharyngeal secretions for five years. During the period September 1969-March 1972 this provided positive evidence of RS virus infection in 419 children out of 423 from whom the virus was isolated.

In our experience immunofluorescence carried out on tissue culture at five to seven days was less sensitive than the examination of cells in nasopharyngeal aspirates^{1,2}. We can therefore only conclude that Dr Urquhart and Dr Walker

have used a different and less effective method—either in taking their specimens or in preparing their slides. Either of these explanations would account for the paucity of epithelial cells in the preparation, which is exceedingly rare in our experience. The 22 children on which their conclusions are based are a very small proportion of the children in Glasgow with lower respiratory tract infection due to RS virus. In an average winter month we receive 700 specimens for respiratory virus diagnosis, and a reasonable service for the country, especially the large conurbations, would therefore be impossible if laboratories were persuaded to return to culture techniques alone. We are supported in asking them to review their method by the knowledge that other laboratories are achieving comparable results to our own.

We were also disturbed by the statement that 'unless specific or selective therapy is available for cases of serious RS virus infection rapid diagnosis is not essential'. Dr Urquhart and Dr Walker do not define the clinical categories used in their paper but we find that the application of consistent clinical and radiological definitions to children with lower respiratory tract

infections, together with rapid virus diagnosis, has enabled us to withhold antibiotics selectively and safely, especially in bronchiolitis. It would in our view be a backward step in the understanding and management of respiratory infections if virus diagnosis was only available several or more days after admission, when for most children and for the doctors concerned, the severe phase of the illness has passed. Since specific therapy is likely to come sooner or later it is illogical to abandon the essential diagnostic technique in the interval.

There are other cogent reasons for rapid virus diagnosis besides the evolution of rational therapy. In most seasons more than one respiratory virus is epidemic in our community at the same time and rapid virus diagnosis will help to separate them. Indeed, this technique is now a major tool in exploring the epidemiology of respiratory infection.

The extent of virus cross-infection in children's wards is neither widely recognized nor the ways in which it occurs fully understood; in our experience when wards are filled with respiratory infections rapid virus diagnosis has made possible the nursing of like with like and the be-