

Procedures for the diagnosis of virus particles by electron microscopy

With reference to a recent research note on viral pathogens in piglet faeces samples⁴ and a similar report in children⁸, we would like to address the methods used by the authors in these surveys.

Electron microscopic viral diagnosis using the negative staining technique is well established³. While not as sensitive as some other methods, negatively stained specimens examined for the presence of virus particles remains a rapid alternative to conventional isolation and serology methods in virus diagnosis^{2,6}. However, the success of this diagnostic technique depends on several factors, for example: (a) the removal, as soon as possible after collection of a sample, of unwanted debris and bacterial components that may obscure virus particles; (b) the achievement of a homogeneously distributed monolayer of particles on the substrate of the grids; (c) adequate contrast of the specimen; (d) the use of a suitable magnification to scan samples (it is preferable to use a magnification of, for example, $\times 25\ 000$ rather than a higher magnification of $\times 40\ 000$ to $\times 45\ 000$, which is often used). Although a high magnification is ideal for obtaining suitable micrographs, it is of doubtful value when scanning grids for the presence of virus particles. A simple calculation shows that doubling any magnification will increase the area to be scanned 4-fold, thereby reducing the likelihood of detecting any virus particles 4-fold; (e) experience in identifying and recognising virus-like particles and an ability to distinguish them from structures resembling viruses and (f) achieving a threshold concentration of virus particles by e.g. the use of appropriate centrifugation procedures.

In our experience, very few samples can be successfully examined for the presence of virus particles without centrifugation. Moreover, the application of this technique in a systematic fashion provides a consistent and controlled method of sample preparation irrespective of the personnel involved. When doing a survey over a period of time, predetermined centrifugation times and speed (especially g-values) together with a constant dilution factor provide a reliable method for evaluating and comparing results.

The value of using a suitable centrifugation technique is reflected in the results

obtained in our laboratory during the past 6.5 years for the diagnosis of parvovirus (20–25 nm) in dogs. Initially, in the first 6 months, a success rate of about 50 % was achieved for samples using only low-speed centrifugation. In order to eliminate possible false negative results, the sample preparation method was modified to include 3 centrifugation steps¹. Samples were initially centrifuged at 3000 g for 15 min, 7000 g for 30 min and 27 500 g for 1 h. This method resulted in an increase in the success rate to about 70 %. In order to improve contrast quality and minimise unwanted debris and a background film of fine proteinaceous material, further changes were made to the technique. This involved reducing the centrifugation speed and number of centrifugations. To remove bacteria and debris the initial centrifugation was maintained at 3000 g for 30 min. This was followed by high-speed centrifugation (20 000 g for 1 h) to produce a virus-rich fraction suitable for electron microscopy. Using higher g-values to concentrate the virus particles is unnecessary and results in producing protein-like particles that resemble small virus particles, as well as depositing a fine proteinaceous background film that obscures particle detail on the grids. These refinements increased the positive identification of parvovirus to above 90 %, and the technique has been successfully employed to identify a range of virus particles in avian, bovine and equine samples as well.

A microlitre head was recently installed on our bench centrifuge, enabling us to decrease the centrifugation time for the 2 steps to 15 min and 30 min respectively, for about the same g-values. The sensitivity of the technique was increased 10-fold as well, as we now need only a 1.5 ml aliquot instead of 15 ml and a $\times 5$ dilution instead of $\times 20$ as used previously. In addition, the sensitivity of the technique can be further increased by any laboratory in possession of an airfuge ultracentrifuge³.

The disadvantage of not using a centrifugation step is reflected clearly, in our opinion, in the low percentage of positive results reported in some recent publications. An example is the 2.4 % incidence of small-sized virus particles and the 39.8 % incidence of rotavirus reported in piglets suffering from diarrhoea⁴. Apparently these authors⁴ also

experienced some difficulty in distinguishing entero- (28–30 nm), astro- (28–30 nm) and calicivirus (32–38 nm) -like particles from each other. This seems to apply to the recent survey in children as well⁸, in which the authors make unlikely claims about the presence of Breda-like virus particles. This may reflect a problem in recognising or identifying fringed membranous particles (considered to originate from fragmentation of epithelial cell linings^{5,7}) from true virus particles. Our centrifugation procedure provides sufficient contrast and concentration of material to allow us to make, with confidence, positive identifications and distinctions of the abovementioned small viruses, including parvovirus. The diagnoses were based on the appearance and size (irrespective of overlapping size ranges) of the particles, whether or not they occurred as a single or simultaneous infection, provided each virus concentration was above a threshold value necessary for identification. The high specificity and speed of electron microscopy in obtaining a positive virus diagnosis illustrate the value of this technique if the correct sample preparation methods are used. Taking into account the present situation where such electron microscopic diagnoses are no longer made free of charge but involve payment by the patient or customer, the need for a reliable procedure and diagnosis is essential.

We recommend that serious thought be given to alternative negative staining preparation methods for virus identification (whether for individual samples or surveys) rather than using a simple staining method that is prone to suffer from false negative results from the outset. Such situations can easily be avoided by following guidelines similar to those suggested here.

REFERENCES

1. De Beer M, Peenze I, Da Costa Mendes VM, Steele A D 1997 Comparison of electron microscopy, enzyme-linked immunosorbent assay and latex agglutination for the detection of bovine rotavirus in faeces. *Journal of the South African Veterinary Association* 68: 93–96
2. Dixon B R, Stone J L, Smith K, Mukherjee T M 1980 The role of electron microscopy in the identification of viruses in medical and veterinary specimens. *Micron* 11: 429–430
3. Doane F W, Anderson N 1987 *Electron microscopy in diagnostic virology*. Cambridge

- University Press, Cambridge
4. Geyer A, Steele A D, Peenze I, Lecatsas G 1994 Astrovirus-like particles, adenoviruses and rotaviruses associated with diarrhoea in piglets. *Journal of the South African Veterinary Association* 65: 164–166
 5. Goodwin M A, Brown J, Player E C, Steffens W L, Hermes D, Dekich M A 1995 Fringed membranous particles and viruses in faeces from healthy turkey poults and from poults with putative poult enteritis complex/spiking mortality. *Avian Pathology* 24: 497–505
 6. McFerron J B, McNulty M S, Curran W L 1978 Diagnosis of avian viral diseases by electron microscopy. *American Journal of Veterinary Research* 39: 505–508
 7. Schnagl R D, Brookes S, Medvedec S, Morey F 1987 Characteristics of Australian human enteric coronavirus-like particles: comparison with respiratory coronavirus 229E and duodenal brush border vesicles. *Archives of Virology* 97: 309–323
 8. Sebastian S, Bux S 1997 Viral pathogens found in children hospitalized for gastroenteritis in Kwa-Zulu Natal. *Proceedings of the Microscopy Society of Southern Africa* 27: 108

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Book review — Boekresensie

Developing a procedures manual for your veterinary clinic

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1996. *Veterinary Practice Publishing Company, Santa Barbara, California, 79 pp. Price not stated. ISBN 0 9603 534 8 8.*

Running a successful veterinary clinic usually implies rendering a sterling service to the community. Such service is usually the result of effective teamwork involving everybody working at the practice. Regardless of the number of people rendering the service, effective communication between them plays a pivotal role in rendering an excellent service. While it is not difficult for the owner or owners of a practice to explain the overall goal of the practice to staff members, it is much more taxing to explain every little detail that goes into consistently providing an excellent service. A clinic procedures manual could fulfil this role and provide all employees with guidelines on all aspects of their daily chores. Dr Fling's booklet provides guidelines for the compilation of a procedures manual, which, as he correctly states, is a living document that requires input from all employees, and should be revised, updated and changed as needed.

The following major points are covered in separate sections of the manual: clinic policies for all employees; clinic operational procedures; job descriptions; client relations and telephone protocol. Starting with a simple mission statement of the practice, the different sections then proceed to elaborate on guidelines regarded as attributes that, when demonstrated by an employee, should

result in a superior performance. The different sections are also supplemented with sample *pro forma* forms that detail various aspects of running a private practice such as staff disciplinary action; hospitalisation and surgery authorisation; permission for euthanasia; daily treatment sheet; veterinary assistant guide to cleaning kennel cages; separating a pet from the client; client take-home literature; telephone standards and first-aid procedures.

Dr Fling sees a procedures handbook as the Bible of a practice which should constantly be studied and consulted by all employees. He clearly states that there is absolutely no right or wrong approach in the preparation of such a handbook but that it is merely a process to get the information floating around in one's head or scribbled on scraps of paper all over the practice, collated into a single document.

This ring-bound soft cover manual is strongly recommended as essential reading for all practice managers as well as for staff employed in veterinary practices. It is bound to trigger a process that may improve the profits of the practice.

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