Eur. J. Clin. Microbiol. Infect. Dis., February 1988, p. 11–13
0722-2211/88/01 0011-03 $ 3.00/0

Laboratory Diagnosis of Viral Gastroenteritis

The importance of an accurate and rapid diagnosis of viral gastroenteritis cannot be overstated. Such infections cannot, at the moment, be prevented by vaccination or attenuated by chemotherapy; however, if the diagnosis is accurate and the patient well managed, they are rarely life-threatening. Management consists of replacing lost water and electrolytes either by mouth or intravenously for the duration of the diarrhoea and vomiting. The disease is usually self-limiting, rarely lasting more than a few days. In contrast, diarrhoea caused by bacteria and parasites such as *Vibrio cholera*, campylobacters, giardiae and amoebae often require treatment with an appropriate chemotherapeutic agent as well as fluid and electrolyte replacement therapy.

Traditionally the diagnosis of viral infections has relied upon (i) the identification of the virus in host tissue, (ii) the isolation and identification of the virus in a secondary host system such as cultured cells or fertile hen eggs, or (iii) the demonstration of a virus-specific antibody response. However, none of these strategies has been of much value in the diagnosis of viral gastroenteritis. This is because all viruses so far implicated in gastroenteritis have shown an almost exclusive tropism for the highly differentiated enterocytes of the small intestine. Diagnosis by examination of biopsy tissue is unnecessarily invasive, and although some viruses have been grown in cultured cells even this remains a difficult and unreliable method of diagnosis. Moreover it is likely to remain so until a cell line is developed that mimics more closely the morphology and biochemistry of the mature enterocyte. Serology is also of little use in rapid diagnosis because a specific antibody response occurs too late in most cases; however, retrospective epidemiological investigations can benefit from the method. This has given rise to an ironic situation whereby we have become dependent upon the traditional and time-honoured art of electron microscopy and the technical innovations of immunology and molecular biology. Furthermore, it can be argued that as a result of the study of gastroenteritis (and the equally fastidious hepatitis) viruses, many of the now familiar "third generation" tests have been adopted for both gastroenteritis and non-gastroenteritis viruses.

Electron microscopy remains the reference method for the diagnosis of viral gastroenteritis as it allows the detection of viruses other than those directly sought. Indeed all of the known viruses implicated in diarrhoea were initially discovered in this way. For some agents such as astrovirus, calicivirus and other small round viruses there is no other method that is widely available (Table 1). Although reliable, electron microscopy is not without its disadvantages. An electron microscope is an expensive piece of equipment. Given that the major impact of viral diarrhoea is in the developing countries this point should not be trivialised.

The ELISA method is becoming the mainstay test in diarrhoea virology. Its role is already well established in the detection of rotaviruses where it has proved invaluable in both developed and developing countries because of its sensitivity, specificity, safety and low price. The application of ELISA to the diagnosis of other gastroenteritis viruses has been hampered by the lack of specific antisera. These

Table 1: Diagnostic methods for diarrhoea viruses.

Virus	E.M.	ELISA	IF	PAGE	LATEX	N.A.HYB	RPHA	RIA
Rotavirus	+	+	+	+	+	+	+	+
Adenovirus	+	+	+	+				+
Astrovirus	+		+					
Calicivirus	+							+
Norwalk	+	+						+
SRV	+							
Coronavirus	+		+					
Torovirus	+	+						

EM = electron microscope, IF = immunofluorescence, N.A.HYB = nucleic acid hybridisation, LATEX = latex agglutination, RPHA = reverse passive haemagglutination, RIA = radio-immunoassay, SRV = small round featureless viruses, PAGE = polyacrylamide gel electrophoresis.

have proven difficult to produce, again because of the reluctance of these viruses to grow in cell culture, and (unlike rotaviruses) they are not present in stool samples in sufficient numbers for this to be used as a source of antigen.

Most existing ELISA procedures can be further refined. Enzyme/antibody conjugation methods, for example, often reduce the avidity of the antibody for the antigen and also the activity of the enzyme (1). Also many commercially available conjugates contain isoenzymes of lower activity which lower the titre and the sensitivity of the reagents. It has been estimated that irradiated polystyrene and polyvinylchloride solid phases bind less than 10 % of the protein (antigen or antibody) made available to them. Use of other materials such as nitrocellulose, which is known to have a far greater binding capacity, should significantly improve the sensitivity of the test system. The sensitivity of both direct and indirect assays has been increased by using avidin-enzyme conjugates and biotinylated antisera (1). Recently a method for amplifying the sensitivity of ELISA tests that utilize alkaline phosphatase has been described (2). This method is based on the dephosphorylation of nicotinamide adenine dinucleotide phosphate (NADP) by conjugated alkaline phosphatase. The NAD is then reduced and reoxidized in a cyclic enzyme-mediated reaction (Figure 1). This cyclic substrate system is 10–100 times more sensitive than conventional substrates of alkaline phosphatase. This method has been used to detect low levels of rotavirus serotype-specific antigens directly in stool samples and may prove to be of value in the development of assays for viruses present in faeces in only low numbers (3).

There are alternative tests which are non-serological. Polyacrylamide gel electrophoresis (PAGE) has been used successfully to diagnose rotavirus infections by

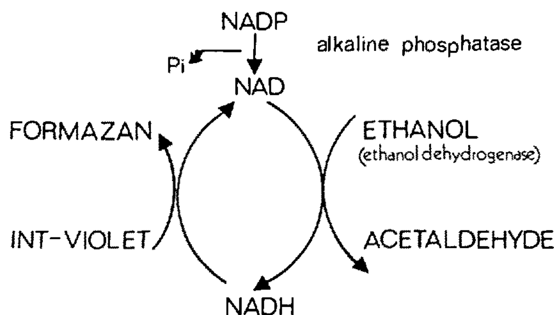

Figure 1: A secondary amplification system based on the reduction and oxidation of NAD. The primary system enzyme, alkaline phosphatase (conjugate), dephosphorylates NADP. The redox cycle is driven by alcohol dehydrogenase and diaphorase. Formazan dye is the deep red product.

demonstration of the characteristic profile of 11 RNA genome segments in silver-stained slab gels (4). Adenoviruses have also been detected in a similar way by using restriction endonucleases to segment their genomic DNA in vitro (5). Such methods do have advantages over serological ones. They often detect differences between strains which cannot be separated serologically (6). Such differences have proven useful in epidemiological studies. Furthermore serologically unique viruses can also be detected. Such strains are negative by ELISA. To date four previously unknown groups of rotaviruses have been discovered by PAGE of viral genomic RNA in faeces (7). There is no reason why other gastroenteritis viruses could not be diagnosed by this method, providing the appropriate restriction enzymes are used.

Future innovations will almost certainly include assays based on the hybridisation of suitable immobilized viral nucleic acid from clinical material with labeled, cloned, complementary nucleic acid probes. Such a "dot-hybridisation" assay has been described for rotaviruses based on the in situ hybridisation of radioactively labeled ssRNA transcripts to heat-denatured rotavirus RNA immobilized on nitrocellulose membranes (8). These probes do not need to be radioactive; they can be labeled with biotin and detected with an avidin-enzyme conjugate. Theoretically it should be possible to detect specific genes (i.e., those coding for serotype specificity) by such a method, thus avoiding time-consuming virus neutralisation tests. Enzyme amplification systems, such as the one based on NADP described above, might also be of value in this technique.

A number of epidemiological surveys have indicated that more gastroenteritis-associated viruses await discovery. Despite the *embarras de richesses* of candidate pathogens provided by electron microscopy in the 1970s, no new viruses have been detected by electron microscopy in recent years. The newly described toroviruses, which cause diarrhoea in cattle and might be human pathogens, were discovered by haemagglutination (9); they have proven difficult to detect by electron microscopy, particularly in older faecal samples (10). It is conceivable that the now well-established gastroenteritis viruses such as rotavirus and adenovirus were originally discovered and are now easily detected by electron microscopy because they look like we expect viruses to look. Other perhaps more pleomorphic viruses may go unnoticed or might be present in too few numbers to be detected by electron microscopy, which is a relatively insensitive technique. Dual infections, rotavirus and astrovirus for example, might also be common (Figure 2). "Secondary" viruses may go undetected. Could the familiar viruses associated with diarrhoea occasionaly get the "credit" for diseases aggravated by other agents?

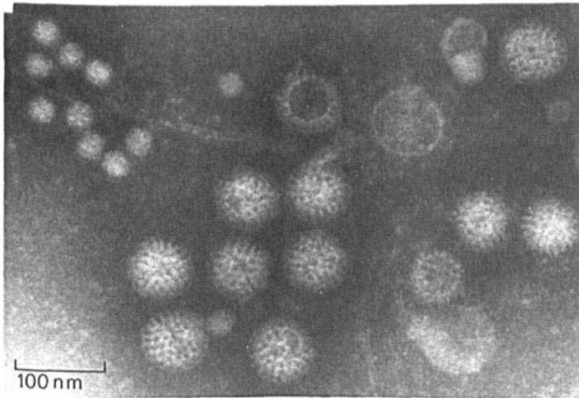

Figure 2: Transmission electron micrograph of a stool sample from a child with gastroenteritis. Rotavirus particles are clearly visible, but astroviruses (top left) and a "fringed" particle (right of centre) were also present in this specimen. PTA negative contrast. Bar = 100 nm.

Progress in the diagnosis of gastroenteritis viruses will depend equally upon the refinement of existing techniques and the development of novel diagnostic tests. The search for new agents might, however, become the domain of the immunologists and molecular biologists (11).

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References

1. **Kurstak, E.:** Progress in enzyme immunoassays. Bulletin of the World Health Organization 1985, 63: 793–811.
2. **Self, C. H.:** Enzyme amplification – A general method applied to provide an immunoassisted assay for alkaline phosphatase. Journal of Immunological Methods 1985, 70: 389–393.
3. **Beards, G. M.:** Serotyping of rotaviruses by NADP-enhanced enzyme immunoassay. Journal of Virological Methods 1987, 18: 77–85.
4. **Estes, M. K., Graham, D. Y., Dimitrov, D. H.:** The molecular epidemiology of rotavirus gastroenteritis. In: Melnick, J. L. (ed.): Progress in medical virology. Karger, Basel, 1984.
5. **Uhnoo, I., Wadell, G., Svensson, L., Johansson, M. E.:** Importance of enteric adenoviruses 40 and 41 in acute gastroenteritis in infants and young children. Journal of Clinical Microbiology 1984, 20: 365–372.
6. **Beards, G. M.:** Polymorphism of genomic RNAs within rotavirus serotypes and subgroups. Archives of Virology 1982, 74: 65–70.
7. **Pedley, S., Bridger, J. C., Brown, J. F., McCrae, M. A.:** Molecular characterisation of rotaviruses with distinct group antigens. Journal of General Virology 1983, 64: 2093–2101.
8. **Flores, J., Boeggeman, E., Purcell, R. H., Sereno, M., Perez, I., White, L., Wyatt, R. G., Chanock, R. M., Kapikian, A. Z.:** A dot hybridisation assay for detection of rotavirus. Lancet 1983, i: 555–559.
9. **Horzinek, M. C., Weiss, M.:** Toroviridae – a taxonomic proposal. Zentralblatt für Veterinärmedizin (B) 1984, 31: 649–659.
10. **Beards, G. M., Brown, D. W. G., Green, J., Flewett, T. H.:** Preliminary characterisation of Torovirus-like particles of humans. Journal of Medical Virology 1986, 20: 67–78.
11. **Rizzetto, M., Canese, M. G., Arico, S., Crivelli, O., Trepo, C., Bonino, F., Verme, G.:** Immunofluorescence detection of a new antigen-antibody system associated to hepatitis B virus in liver and in serum of HbsAg carriers. Gut 1977, 18: 997–1003.