Letter to the Editor

Detection of Poliovirus Antigen by Enzyme Immunoassay

A paper with the above title appeared in the December issue of the *Journal of Clinical Microbiology* (P. Ukkonen, A. Huovilainen, and T. Hovi, J. Clin. Microbiol. 24:954–958, 1986). This paper described the attempt by a Finnish group to detect poliovirus antigen directly in stools. These stools were obtained from individuals infected with a new poliovirus type 3 strain, 3/Fin/K, isolated during an unexpected outbreak of poliomyelitis in Finland in 1984 and 1985. The purpose of the Finnish group was “to make an EIA [enzyme immunoassay] specific for one enterovirus type, poliovirus 3,” and, more specifically, “to apply the test to the detection of poliovirus antigen directly in fecal specimens.” The authors failed to detect unequivocally the viral antigen in most of the stools tested but still reported their findings.

In their introduction, the authors cited some reports on the detection of viruses directly in stools and insisted on the absence of tests for the detection of poliovirus. What they failed to acknowledge are the numerous reports on the detection of poliovirus antigens in cell culture supernatants, for both virus identification and serotyping, especially for inactivated poliovirus vaccine production and environmental virology. They failed to acknowledge several previous reports on the detection of poliovirus antigens by immunoassay and in particular those presented during an international symposium and published in *Developments in Biological Standardization* (1). They also failed to cite a very similar report on the rapid detection and serotyping of all three serotypes of poliovirus isolates (3), as well as other papers (2, 4).

Let us briefly review comparatively our assay and those described by Ukkonen et al. Our methods for the purification of poliovirus from infected Vero cells, as well as the preparation of antisera in rabbits and guinea pigs, were very similar to those of the Finnish group; we used similar doses of viral antigens and a similar immunization schedule. The major difference in our report is that we included all three serotypes of poliovirus in the development of our assay, while they prepared an assay specific for poliovirus type 3 only. Two enzyme immunoassays are described in our report: an indirect microplate (96-well) assay for the determination of specificity and serum titration and an indirect sandwich assay for the detection, identification, and serotyping of all three serotypes of poliovirus. The Finnish group described only an indirect sandwich immunoassay for poliovirus type 3.

The indirect enzyme immunoassay we have described uses similar amounts of globulins, incubation periods, and temperature and gave results very similar to those of the Finnish group. The major methodological differences are that we preferred a peroxidase conjugate, while they used an alkaline phosphatase conjugate, and they evaluated their results with a photometer (Multiskan), while we evaluated ours visually (a photometer was not available to us at the time).

Our results demonstrated that the assay could detect and differentiate the three serotypes of polioviruses in cell culture supernatants if they contained at least 10^5 50% tissue culture infective doses per ml. We challenged our assay with several other enteric viruses and did not find any specific reactions to these viruses; this was not done by the Finnish group. They were able to obtain specific reactions with purified poliovirus type 3, with a minimal detection limit of 4 ng. Their results with stools from infected individuals show, as we had reported in our conclusion, that the amount of poliovirus antigen in stools is relatively low and that its detection by enzyme immunoassay is difficult. Of 51 stools tested by the Finnish group, only 5 reacted barely above their cutoff value of 0.100, ranging from 0.104 to 0.172. We had been unable to detect poliovirus antigen in several dozen positive stools using our test. We thus concluded that amplification in an appropriate cell line was the best means of obtaining reactive samples with this type of immunoassay.

As we have shown, the paper by Ukkonen et al. is essentially a rewriting of procedures already described in great detail in the scientific literature and with similar results; it thus appears to be an example of failure to cite relevant papers and of partial duplication. They have only confirmed what had been demonstrated by other researchers, and they failed to properly acknowledge material already published in highly recognized journals.

LITERATURE CITED


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Author’s Reply

I agree with Drs. Payment and Trudel that their article (P. Payment, C. Tremblay, and M. Trudel, J. Virol. Methods 5:301–308, 1982) should have been cited in our paper, and I am sorry for this omission.

Although the two papers are related, our work is different from that of Payment and co-workers. Our study focused on the detection of poliovirus antigen directly in fecal specimens, and the assay was carefully optimized for that purpose, whereas the paper of Payment et al. dealt with cell culture supernatants. They did mention, however, that they failed to detect poliovirus antigen in stool specimens. But, for example, the number of tested specimens and the assay conditions were not given. The test of Payment et al. was described less accurately than was ours: the enzyme im-
munoassay results were scored by visual reading (instead of a photometer), which is not adequate for the definition of the type specificity of the test. We determined the sensitivity of the enzyme immunoassay in terms of nanograms of purified poliovirus per milliliter, which allowed us to estimate the concentration of poliovirus protein in stool. Furthermore, our test was done with poliovirus 3/Fin, which caused an unexpected outbreak of poliomyelitis in Finland in 1984 to 1985 and which proved antigenically different from previous type 3 strains; this antigenic difference could also be shown by the described enzyme immunoassay method.

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