Correspondence

Diagnosis of rotavirus infection

Sir,

We read with keen interest the paper entitled “Rapid ELISA for the diagnosis of rotavirus” by Kelkar et al and the commentary by TN Naik. We agree with Naik that rotavirus diagnosis in cases of acute diarrhoea/gastroenteritis is important in clinical settings and this will lead to judicious use of antibiotics. We congratulate Kelkar and her colleagues for making a diagnostic rotavirus ELISA kit available idigenously.

The National Institute of Virology (NIV) routine ELISA was developed at least a decade ago by Kelkar. Some modifications might have been incorporated since its publication in 1993 that the readers are unaware of. It would have been easier to understand the difference between the two assays if the routine ELISA procedure was either described in “Material & Methods” section or reiterated under “Discussion”. It is usually recommended that the capture and the detecting antibodies used in ELISA be from two different species of animals. Did the authors test such a combination for the rapid ELISA as they had done in the routine ELISA? Also, the authors mentioned that one sample was negative in the NIV routine ELISA and positive in the rapid ELISA. They used RNA-PAGE (polyacrylamide gel electrophoresis) method as a confirmatory test. This observation implies that RNA-PAGE is a more sensitive and reliable method than ELISA for detecting rotavirus in fecal samples. Kelkar et al have focused their attention on evaluating the newer method with the NIV routine ELISA and have not entered into discussing other methods of diagnosis of rotavirus infections. Naik also failed to point this out in the commentary.

Herring et al developed the method of detection of rotavirus double stranded RNA segments by polyacrylamide gel electrophoresis (RNA-PAGE, also called electropherotyping). It was found to be as sensitive as most ELISA systems. During the past two decades methods of RNA extraction have become more efficient and simple therefore RNA-PAGE is still considered as an extremely convenient method for large scale screening of stool samples for the presence of rotavirus. Being a generic procedure, electropherotyping is capable of detecting even those rotavirus that are antigenically diverse. Electropherotyping is possibly the most useful and practical frontline method available for screening large numbers of samples in a diagnostic setting for detecting rotaviruses belonging to non-A sero-groups. Indeed, one of the reasons for the failure to detect group B rotaviruses in different regions of the world may be the widespread use of commercial group A rotavirus-specific immunoassays for screening stool specimens in clinical settings.

In general, diagnosis of rotavirus infection is relatively easy as large numbers of virus particles are shed in feces (up to 10^11 particles per ml of feces) during the peak of the illness. For the past 3 years, we have successfully used the RNA-PAGE method to detect the presence to rotavirus in fecal samples collected from cases of gastroenteritis in Mumbai. We find that this method can be routinely performed in any small diagnostic laboratory. RNA-PAGE is done on stool samples. The method requires a microfuge for RNA extraction step, a constant current/voltage power supply (available in almost all laboratories) and a vertical PAGE chamber. One does not need any rotavirus specific reagents such as purified antibodies and antibody-enzyme conjugates. The characteristic separation of the 11 segments of dsRNA that make rotavirus genome identifies the presence of the virus in the sample. The results become available within 6 hours. The method is suitable in hospital settings where testing of small numbers of samples (one or two) is required on a day-to-day basis as well as large numbers of samples during outbreaks and surveys. Our intention is to draw the attention to RNA-PAGE method that is as sensitive and rapid as ELISA for detecting retavirus in stool samples of cases of diarrhoea.

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The authors reply:

We agree that procedure of routine ELISA should have been described in "Method's" section, but since detailed procedure was described in the published paper, we did not repeat it. We did try to use capture and detect antibodies used in ELISA from two different species of animals viz. guineapig and rabbit, but rabbit and rabbit combination worked better.

We have mentioned that one sample which was negative in the NIV routine ELISA was positive in the rapid ELISA and by RNA-PAGE. However, the result of this one sample does not prove that RNA-PAGE is a more sensitive and reliable method than ELISA. At the most, it may prove that the specimen had more of core particles, which could be detected by RNA-PAGE but not by routine ELISA. We did not enter into discussion on other methods of diagnosis of rotavirus infections because the aim of this publication was not to compare different methods of rotavirus diagnosis but to describe indigenous diagnostic kit for rotavirus and immunoassay is a method of choice for rotavirus diagnosis. The comparison of different methods for the diagnosis of rotavirus has been done long time back. There are many publications on this issue in the late 1980s. RNA-PAGE is not a new technique for rotavirologists. The limitations of RNA-PAGE include the need for extensive and expensive sample preparation, access to good electrophoresis apparatus, skilled laboratory worker and the relatively small number of samples that can be processed at one time. Further, it is somewhat less sensitive than optimal immunoassay procedure for the detection of rotavirus antigen. We do agree that it is very useful for detecting non group A rotavirus for which the immunoassays are not available.

We want to add that RNA-PAGE has been used by us for the detection of group B rotavirus, for the characterization of specimens showing multireactivity in MAb based ELISA, for detecting short/long electropherotype and also in the present publication to confirm rotavirus diagnosis. RNA-PAGE is very specific method for rotavirus diagnosis but not superior to confirmatory ELISA for large scale diagnostics.

Another advantage of ELISA is that single shelled empty particles of rotavirus also can be detected by ELISA, because of the presence of VP6, which is abundant in rotavirus particles. Moreover, we have developed combined tissue culture and ELISA based neutralization assay where rotavirus can be titrated and non neutralized rotavirus is detected by ELISA.

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**References**
