Establishment and Application of an Indirect Immunofluorescence Assay for Surveillance of Prevalent Enteroviruses Strains in Taiwan

Abstract

Taiwan CDC set up a national contract laboratory-based surveillance system specifically for Enterovirus infection in 1999. It was shown that Coxsackieviruses A group (CA) were actively circulating during 1998-2006 in Taiwan area; theses were exemplified by CA2, A4, A6, A16 in 1998, CA10 in 1999, CA6, A16 in 2001, CA4, A5, A6, A16 in 2002, CA2, A5, A6, A16 in 2003 CA2, A5, A6, A16 in 2004,CA4,5,6,16 in 2005and CA2,4,5 in 2006. The trend of enterovirus epidemics has favored the recirculation of these CA serotypes.

Among the circulated CA serotypes, only the CA16 can be specifically identified by a commercially available monoclonal antibody; other CA serotypes can only be identified by genetic sequencing or neutralization test. In view of the situation, we have started to develop indirect fluorescent immunoassays for CA2, A4, A5, A6 and A10, with the CA4 and CA10 being completed for use in the contracted laboratories.

In the second year, we have generated rabbit polyclonal antibodies, and assessed their homotiter and heterotiter by means of checkerboard titration for optimal conditions for an indirect fluorescent immunoassay. We started with enterovirus prototypes and followed by clinical isolates, with a total of 268 strains for CA2 and 213 strains for CA5; these clinical isolates were collected during 1998-2006 and substantiated by genetic sequecing and neutralization test. The sensitivity and specificity of the assay for CA2 were 100.0% and 100.0%, respectively, while those for CA5 were 96.1% and 95.9%, respectively.

The data indicated that these newly developed assay is suitable for routine virus isolation, with the advantages of being more simple, rapid, sensitive and specific, and being less workload and expenses. The other advantages are that they would be useful for the untypable enteroviruses, and lead to develop and predict the trend of enterovirus epidemics. The immunoassays developed herein would be beneficial to taking measures for the next possible enterovirus epidmics.

Keyword: Prevalence strain、Indirect Immunofluorescence Assay、polyclonal antibodies、checkerboard titration、sensitivity、specificity