F315 Rapid and Direct Diagnosis of Enteroviruses from Stool Samples

Jeong Koo Park*[,1], Ki Soon Kim[1,2], Yoon Seok Chung[1,2], Yoon Sung Lee[2], Ji Eun Kim[1], Young Mee Jee[1], Kwang Ho Lee[2], Chul Yong Song[2] and Jae Duek Yoon [1]

Laboratory of Enteroviruses, Department of Virology, NIH[1]
Department of Life Science, College of Natural Science, Chung-Ang University[2]

Aseptic meningitis, an acute inflammation of the meninges, is a common illness during childhood. Virus is the most important cause of aseptic meningitis. Especially enteroviruses cause approximately above 85% of all cases of aseptic meningitis. Current diagnosis of enteroviruses is based on cell culture-neutralization test or RT-PCR methods. However, the neutralization test takes too laborious time, and the use of conventional RT-PCR methods is inhibited by many contaminants derived from the stool. In this study, we focused the development of RNA extraction methods by co-amplification with standard RNA in the RT-PCR. We compared four RNA extraction methods including Tri-reagent, Catrimox, GTG-Silica and boiling methods from stool suspension for the detection of enteroviral RNAs. Consequently, we found that the Tri-reagent method is the most appropriate for the extraction of viral RNA prior to RT-PCR from stool suspension. We cloned the 5' non-coding regions (NCRs) of enterovirus and used the *in vitro* mutagenized version of the clone for identification of enteroviruses. The RNA extraction and RT-PCR amplification procedures could be checked it stepwise manner by adding the in vitro transcripts of the insertional mutant as a standard RNA into the stool suspension. Less than 5 ng of the standard RNA, when added directly to the first stool suspension, was enough to amplify through the RT-PCR method. Our results suggest that the RT-PCR method combined with the extraction of the viral RNA by Tri-reagent and standard RNA is the most effective and reliable procedures for the detection of enteroviral genomes from the stool samples. [Supported by Grant from MOHW, No. HMP-96-D-6-1054]

F316 Cloning of total genome of coxsackievirus B3 (CVB3) isolated in Korea and characterization of the proteinase activities.

Yoon Seok Chung*[,1,2], Jeong Koo Park[2], Ki Soon Kim[1,2], Yoon Sung Lee[1], Byung Kuk Na[1], Moon Bo Kim[1], Young Mee Jee[2], Jae Duek Yoon[2], Kwang Ho Lee[1] and Chul Yong Song[1]

Department of Life Science, College of Natural Science, Chung-Ang University[1]
Laboratory of Enteroviruses, Department of Virology, NIH[2]

Coxsackievirus B3 (CVB3), a member of the picornavirus group is an important human pathogen in at least 50% of acute myocarditis cases and approximately 25% of dilated cardiomyopathy cases. In addition, this virus is one of the most frequent infectious materials of acute inflammatory disease of the central nervous system including aseptic meningitis in childhood. Nevertheless, there is no available genetic information of the Korean isolate of CVB3, which is essential for the development of diagnostic tools or prophylactic/therapeutic systems of the disease. So we tried to characterize the full length of the viral genome through the reverse transcription (RT) and long-distance PCR amplification method using oligonucleotide primers derived from the prototype CVB3 genome (Nancy strain) sequence of the 5' and 3'-ends. About 7.5kb of the CVB3 amplicon, which contains whole length of the genome, was amplified and directly cloned into the pCR2.1 vector. Subsequent cloning followed by Eco RI digestion of the appropriate insert was performed into the pCR2.2 (modified pCR2.1 vector). Nucleotide sequences at the insert of clone pCB3K-F1, -F2, -F3, -F4 and -F5 were determined ,and analyzed sequence between the isolate and prototype. Among these subgenomic clones, the pCB3K-F1, which has the largest insert spanning from VP2 to P2-B region, was cloned into the pET30b for expression of polyproteins. In addition, pCB3K-F2, which encodes the proteinase (3CD) region, was also cloned into the pET30a and cis-, trans- function of the viral proteinases during polyprotein preocessing will be presented.
[Supported by Grant from MOHW, No. HMP-96-D-6-1054]