AN INNOVATIVE METHOD FOR THE DETECTION OF CONTAMINANT VIRAL GENOME IN CELL CULTURES

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INTRODUCTION

The use of cell cultures involves different fields of biology, from diagnosis to research. Moreover, technologies based on animal cells represent a useful tool to the development of biological products for the prophylaxis and therapy in humans and animals. Therefore, it is necessary to perform quality controls, including virological tests. Several tests performed in research laboratories are able to discriminate one or more viral species, but it is not possible to demonstrate the presence of contaminant viral genome with one non-specific method. The aim of this work consisted on the realization of a biomolecular method able to detect and to identify by sequencing extraneous viral genome in cell cultures of animal and human origin in the absence of any specific information about the virus.

METHODS

Positivization of test samples. Cell substrates were experimentally infected with known viruses (Table 1). Uninfected cells were used as negative controls.

Viral capsid extraction procedure to separate the viral genome from the cell genome (1). The separation was performed by RNase and DNase and by centrifugation, exploiting the different density of capsids. Several concentrations of nucleases (50U, 100U, 150U, 200U) were tested to determinate the appropriate one which is able to digest the cellular genome, leaving the viral capsids intact.

RNA and DNA extraction. RNA and DNA were extracted by High Pure RNA Isolation Kit (Roche) and DNeasy Blood & Tissue Kit (Qiagen) according to manufacturer protocols.

RNA retrotransciption. Retrotrascription was performed by SuperScript VILO cDNA Synthesis Kit (Invitrogen).

Cell Culture	Virus	Virus Characteristics
MDCK (Madin Darby Canine Kidney)	Canine Herpes Virus	DNA virus with envelope
MDCK (Madin Darby Canine Kidney)	Canine Adenovirus	DNA virus without envelope
FRhK-4 (Fetal Rhesus monkey Kidney)	Hepatitis A Virus (HAV)	RNA virus with envelope
VERO ORWELL (Orwell African green monkey kidney)	Canine Distemper Virus	RNA virus without envelope

Table 1. Cells and viruses used for experimental infection.

Degenerate Oligonucleotide Primed PCR. DOP-PCR was set up to amplify the viral genome in a single reaction using a single degenerated primer (5'-CCGACTCGAGNNNNNNATGTGG-3'). DOP-PCR is divided in two phases with different cycles:

- phase 1 (low stringency) - 5 cycles at low annealing temperature.
- phase 2 (high stringency) - 35 cycles at high annealing temperature that increases the specificity of primer during amplification of the sequence. Amplification products were resolved on agarose gel, excised from the gel and recovered using a gel extraction kit (2, 3)**.**

Cloning and sequencing. Amplification products were ligated to the pJET/blunt cloning vector. The ligated products were then transformed into XL1-Blue Competent Cells. DNA extracted from bacterial clones containing DOP-PCR-amplified products was sequenced. Sequences were compared with those submitted previously to Gene Bank.

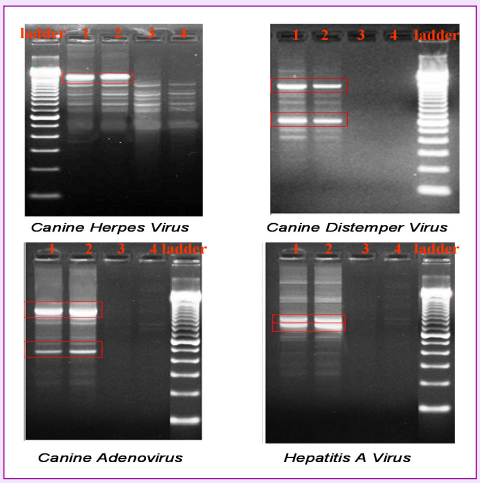


Figure 1. Lanes 1 and 2 are related to the genome amplified from pellet and supernatant samples experimentally infected with the virus. Lanes 3 and 4 are related to pellet and supernatant samples of control. The bands related to the virus are those in the red boxes.

RESULTS

The detection of the amplification products by electrophoresis showed that the optimal concentrations of nucleases used during viral capsid extraction were 50U of RNase and 150U of DNase.

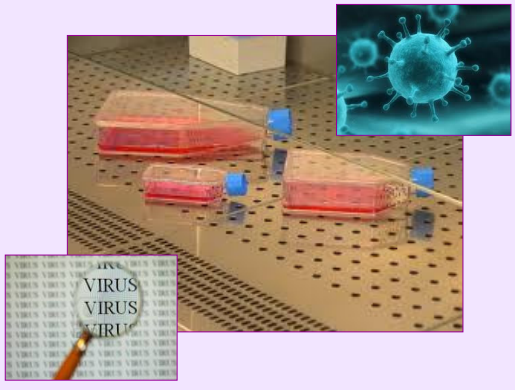
The confirmation that the viral capsids extraction protocol was developed successfully and that the method did not affect the viability of the viruses was obtained by inoculating the samples on new sensitive cell monolayers. All samples shown cytopathogenic effect demonstrating that the vitality of the virus used was not compromised by the steps of extraction.

DOP-PCR generated a smear of DNA fragments (200-1600 bp) visible on agarose gel (Figure 1). The bands present in lanes related to infected samples were compared with those present in lanes of samples used as negative control. The bands present in the lanes of the infected samples and absent in those of control samples were considered to identificate the extraneous genome. These bands were the most evident. The other bands represented the amplification product of cell genome residues. Specific PCR for the viruses used, cloning and sequencing confirmed the identity of the viral genome in the bands selected.

CONCLUSIONS

The sequence-independent amplification and subsequent sequencing of nucleic acids has shown to be a method capable to identify viruses of different nature, DNA or RNA viruses with or without envelope.

Therefore, it is conceivable the application of this method to the quality controls of cell lines used in diagnostics and research by optimizing and validating the procedures and expanding experimentation to retroviruses and circovirus. Moreover, this method may be particularly useful to ensure the virologic biosafety of mesenchymal stem cells that are used for *in vivo* implantation and cryopreserved.



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