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Detection of human pathogenic viruses in a human cell line

Dear Dr. Lam,

Thank you very much for your service order from 5 March 2014 to detect the human pathogenic viruses

- Hepatitis B virus (HBV)
- Hepatitis C virus (HCV)
- Human Immunodeficiency virus type 1 (HIV-1)
- Human Immunodeficiency virus type 2 (HIV-2)

in the cell line iNGN (P42). The analyses were carried out applying polymerase chain reaction (PCR).

On 20 March 2014 we received a Matrigel-coated 25 cm^2 cell culture vessel with partially adhered iNGN cells. We cultured the cells for four more days and harvested thereafter two cell pellets each consisting of ca. 3.3 x 10^6 viable cells for DNA and RNA preparation (viability of 94%) and 5 ml cell culture supernatant for the detection of HBV. Please find the detailed report of the assays on the following pages.

In summary, none of the human pathogenic viruses HIV-1, HIV-2, HCV, or HBV were detected in the iNGN (P42) cell line.

If you have any further questions, please contact me again.

Sincerely yours,

Dr. Cord Uphoff
Department of Human
and Animal Cell Lines

Report on the Detection of Human Pathogenic Viruses in the iNGN Cell Line

1. PCR Assay for the Detection of Hepatitis B Virus (HBV)

The testing for active hepatitis B virus (HBV) contamination of the iNGN cell culture was performed using cell culture supernatant. Five ml of the cell culture were centrifuged to remove cells and cell debris. Smaller particles (incl. viruses) were then collected by ultracentrifugation of the supernatant. The pellet was resuspended in lysis buffer containing proteinase K. We then separated and purified the DNA of the sample applying the Wizard DNA Clean-Up system (Promega). A hot start PCR was carried out using the isolated DNA and primers coding for a conserved sequence of the X gene region of the various sub-types of HBV. In case of a positive reaction, a 568 bp fragment is amplified and visualized in an ethidiumbromide stained agarose gel. In a parallel reaction, an internal control was coamplified resulting in a 911 bp DNA fragment. The internal control DNA was added in a limiting dilution to document a successful PCR amplification, a high sensitivity, and the absence of PCR inhibitors.

The DNA extractions performed from the cell culture supernatants showed no HBV specific bands after PCR amplification (Figure 1). The control reactions (sample plus internal control DNA, positive and negative controls) were as expected. According to these results, the cell culture is not infected with HBV.

Figure 1: Detection of HBV in the human iNGN (P42) cell culture

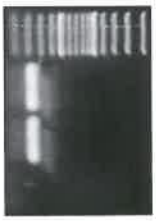
100 bp ladder
iNGN (P42)
iNGN (P42) + internal control
Internal control
Positive control
Positive control + internal control
Water control

2. RT-PCR Assay for the Detection of Hepatitis C Viruses (HCV)

For the detection of active HCV in the cell culture, total RNA from the cell pellet was extracted employing Trizol® reagent (Invitrogen). Five µg of the RNA of the cell culture were then transcribed to cDNA using reverse transcriptase (DyNAmo cDNA Synthesis Kit, Finnzymes) and random hexamer primers. This reaction was verified by the PCR amplification of a 220 bp fragment using specific primers for the human *abl* gene. The forward and reverse primers bind to sequences located in exons 2 and 3 of the *abl* gene, respectively. Consequently, the primers allow the descrimination of the cDNA products from those of the genomic DNA products, because the primers span an intron of the gene (Figure 2).

One µg of the reverse transcribed RNA were applied for the HCV-specific PCR reaction. The primers bind to HCV sequences of the conserved 5´-UTR and produce a 258 bp DNA fragment in case of a positive reaction. In a parallel reaction an internal control DNA was coamplified which produces a 452 bp fragment to document the successful PCR amplification and the absence of PCR inhibitors (Figure 3).

Figure 2: Amplification of the *abl* gene from cDNA



100 bp ladder
iNGN (P42) (cDNA)
Positive control (cDNA)
Water control

Figure 3: Detection of HCV in the Human iNGN cell line

100 bp ladder
iNGN (P42)
iNGN (P42) + internal control
Internal control
Positive control
Positive control + internal control
Water control

Applying the described method, no HCV sequences were amplified applying cDNA of the iNGN (P42) cell culture. The results of the control reactions were as expected.

3. PCR Assay for the Detection of Human Immunodeficiency Virus Type 1 (HIV-1)

For the detection of HIV-1 sequences (including proviruses) in the human iNGN cell line, genomic DNA of the cell culture was extracted employing the High Pure PCR Template Preparation Kit (Roche). The integrity of the DNA was verified by a PCR reaction for the demonstration of the human *abl* gene (Figure 4). The forward and reverse primers bind to sequences located in the exons 2 and 3 of the *abl* gene, respectively. The size of the PCR product is 778 bp.

Figure 4: Amplification of the *abl* gene from genomic DNA

100 bp ladder
iNGN (P42) (genomic DNA)
Positive control (genomic DNA)
Water control

Specific primers of the conserved region of the *gag* gene (SK 38 and SK 39) were employed to amplify a 115 bp fragment of the HIV-1 genome. Due to the high variability of the HIV genome, the PCR was run at temperatures slightly below the optimal annealing temperature to allow mismatches at variable positions. The amplified products were identified by agarose gel electrophoresis and visualized by ethidium bromide intercalation.

No HIV sequences were amplified in the PCR reaction with the genomic DNA of the above mentioned cell culture. The control reactions (negative control and positive control with plasmid DNA containing the *gag-pol* region of the HIV genome in a limiting dilution) were as expected. In parallel reactions a 394 bp internal control DNA fragment was amplified in the presence of the sample DNA and demonstrated the successful PCR amplification (Figure 5). The internal control DNA is amplified with the same primers as the HIV-1 wild type fragment.

Figure 5: Detection of HIV-1 in the iNGN (P42) cell culture

100 bp ladder
iNGN (P42)
iNGN (P42) + internal control
Internal control
Positive control
Positive control + internal control
Water control

4. PCR Assay for the Detection of Human Immunodeficiency Virus Type 2 (HIV-2)

For the detection of any HIV-2 sequences (including proviruses) in the iNGN cell line genomic DNA was extracted employing the High Pure PCR Template Preparation Kit (Roche). The integrity of the DNA was verified by performing a PCR reaction for the demonstration of the *abl* gene (Figure 4).

Sequence specific mixtures of forward and reverse primers (F-HIV-2 and R-HIV-2) were employed to amplify a 385 bp fragment from the HIV-2 genomic DNA. The amplified products were identified by agarose gel electrophoresis and visualized by ethidium bromide intercalation. The method was adopted from a protocol applied at the German Refence Centre for Retroviruses in Erlangen, which was updated according to the recently published HIV-2 sequences in the public sequence data bases.

Employing genomic DNA of the iNGN (P42) cell culture, no HIV-2 sequences were amplified (Figure 6) demonstrating the absence of HIV-2.

Figure 6: Detection of HIV-2 in the iNGN (P42) cell culture

100 bp ladder
iNGN (P42)
Positive control
Water control

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