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# New Molecular Method to Detect Infectious Adenoviruses and Its Applications

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## Background and Problem Statement

Adenoviruses are among the most important waterborne viruses and are on the US EPA CCL. The use of molecular methods targeting nucleic acids is increasingly popular for the detection of viruses and other waterborne microorganisms, including Adenoviruses, due to rapidity, high sensitivity and specificity. However, detection of Adenovirus by PCR indicates only the presence of DNA of Adenovirus and does not provide any information on infectivity, which is our primary interest for human health risk. The detection of AdV DNA is of great concern because Adenoviruses have been shown to have a high virus particle to infectious unit ratio after viral inactivation, due to the persistence of DNA. To address this problem we have developed a method to detect viable Adenoviruses.

## Research Plan and Approach

We have evaluated the use of mRNA detection in cell culture to detect viable Adenoviruses using Adenovirus types 2 (Ad2) and 41 (Ad41) as models. This method detects only infectious Adenoviruses because only infectious viruses can produce messenger RNA (mRNA) during replication in cell culture, and the resulting mRNA can be detected rapidly and at low copy number by RT-PCR. A549 and Graham 293 cell cultures were infected with Ad2 and Ad41, respectively. The mRNA of infected cells was collected from cell lysates using Oligo-dT at different time periods (6 hours - 10 days) after infection. The recovered mRNA was treated with RNase-free-DNase to remove residual DNA contaminants and then AdV mRNA was detected by RT-PCR.

## Results

We were able to detect mRNA of Ad2 as soon as 6 hours after infection at higher inocular levels. With as little as 1-2 pfu of inoculated Ad2, mRNA was detected but after longer incubation time (Figure 1).

We were also able to detect mRNA of Ad41 and the rapidity and sensitivity of this detection is characterized by both conventional and real time PCR. With as little as 5-50 IU of inoculated Adenovirus 41, mRNA was detected (Figure 2).

In order to confirm that our methods detect only infectious viruses, we exposed 1-ml volumes of $10^4$ pfu of Ad41 to different doses (0, 1, 30, 100 mg·min/L) of free chlorine. We detected no mRNA in cells inoculated with Adenoviruses treated with any dose of free chlorine (1, 30, 100 mg·min/L), however, as expected, mRNA of Adenovirus was detected in cells infected with untreated virus. (Table 1)

Significance. These results indicate that mRNA detection in inoculated cells is a very sensitive and specific method to detect infectious Adenoviruses in water and other environmental samples. This method has the potential for wide application in actual practice to detect Adenoviruses in air, water, and food.

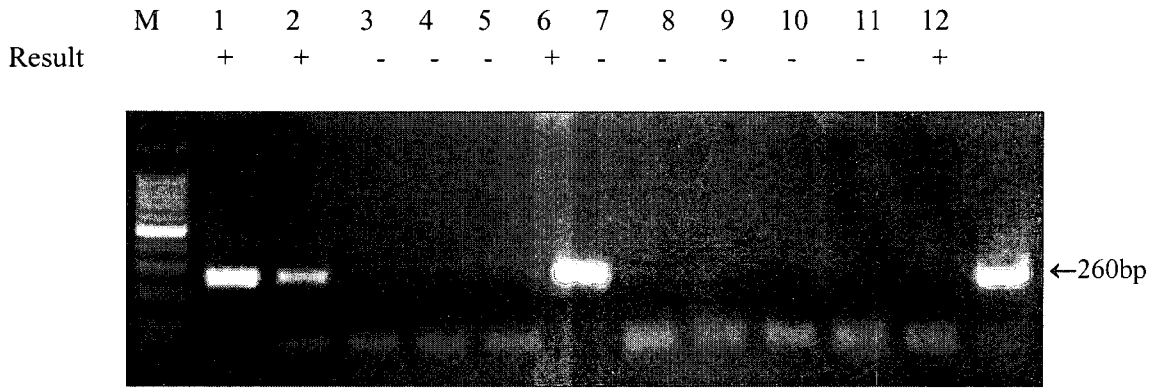


**Fig. 1. Sensitivity of detecting Ad2.** lanes 1 to 3, RT-PCR of Ad2 mRNA from A549 cells infected with 20, 2, 0.2 IU; lane 4, RT-PCR of mRNA from non-infected A549 cells; lane 5, negative control; lane 6, positive control; lane 7 to 9, PCR of Ad2 mRNA from cells infected with 20, 2, 0.2 IU; lane 10, PCR of mRNA in non-infected A549 cells; lane 11, negative control; lane 12, positive control.

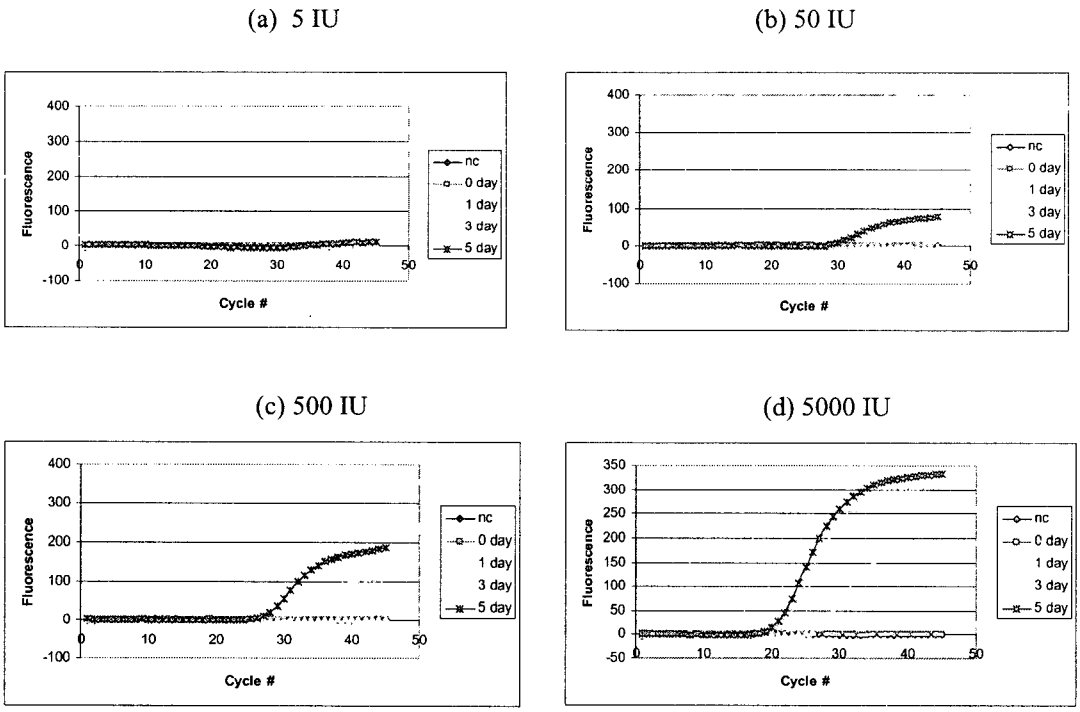


**Fig. 2. Sensitivity of detecting Ad41 mRNA by real time PCR.**

**Table 1. Results of culture, PCR, and mRNA RT-PCR of Ad41 disinfected by different Ct doses of free chlorine.**

| | Dose of free chlorine (Ct) (mg·min/L)[a] | | | |
|---|---|---|---|---|
| | 0 | 1 | 30 | 100 |
| CPE[b] | - | - | - | - |
| DNA detection (PCR) | + | + | + | - |
| Increased viral DNA during culture (PCR) | + | - | - | - |
| mRNA detection[c] (RT-PCR) | + | - | - | - |

[a] Ct Dose = concentration (mg/L)×contact time (minute) in phosphate buffer (pH = 7.2) at 4 °C.

[b] Cytopathogenic effect on Graham 293 cells after 7 days.

[c] mRNA was extracted from infected G293 cells at 7 days PI, and subjected to RT-PCR with Ad1/Ad2 primers.

[d] Approximately $10^2$ PFU of Ad41 was exposed to free chlorine (1 mg/L) for a predetermined time and then was inoculated into Graham 293 cells.