JOURNAL OF MODERN BIOTECHNOLOGY, VOL. 1, NO. 1, pp.45–49, September, 2012

ISSN 2319−6645
JOURNAL OF MODERN BIOTECHNOLOGY
www.thebiotech.org

*Research Article*

# DEVELOPMENT OF BIOTINYLATED PROBE BASED METHOD TO DETECT ADENOVIRUSES FROM ENVIRONMENTAL WATER SAMPLES AND STOOL SAMPLES

Devakirubakaran Jayakar Santhosh, Senthilkumar Kamatchiammal* and Veerappan Saroja

*Virology Department, National Environmental Engineering Research Institute, Chennai Zonal Lab, Chennai 600116, Tamil Nadu, India*

Corresponding Author e-mail: kamatchi1956@gmail.com

Received: 28 July 2012; Revised: 18 August 2012; Accepted: 29 August 2012

***Abstract***

Water-related diseases are associated with exposure to water environments in many ways. Enteric viruses poses major health problem to humans and it is difficult to detect the entire enteric viruses from the environmental water samples. Adenoviruses, one of the members of enteric viruses whose prevalence is higher than other members may be considered as index organism for viruses. A PCR based detection method followed by dot blot hybridization using a specific probe was adapted to detect the presence of adenoviruses from environmental water samples. Sewage (treated and untreated), drinking water, seawater and stool (below 5 years) samples were collected from Chennai city and processed for the presence of Adenovirus. Sewage samples showed 100% positive for adenovirus, 3out of 10 drinking water samples and 2 out of 3 sea water samples were found to be contaminated with adenovirus. Among the 12 stool samples collected from children below 5 years 4 were infected with adenoviruses. The probe developed for the 140 bp PCR amplicon replicated the same results in dot hybridization technique. The method promises to be confirmative step and increases the sensitivity and specificity for Adenovirus detection from Environmental water samples.

***Keywords***: Adenovirus, biotin labeled probe, PCR, Drinking water, Sea water, Sewage, Stool

---

**INTRODUCTION:**

Adenoviruses are dsDNA viruses classified up to 52 serotypes causing several diseases such as conjunctivitis, gastroenteritis, acute respiratory diseases, meningoencephalitis, etc. Next to Rotaviruses Human adenoviruses (HAdVs) are the second most important viral candidate causing infantile gastroenteritis (Basu *et al.,* 2003; Logan *et al.,* 2006; Meqdam *et al.,* 2007; Shimizua *et al.,* 2007). The gastroenteritis causing adenovirus strains were frequently reported in aquatic environments and they are introduced through the human activities such as leakages of sewage, septic systems, urban runoff, agricultural runoff, and in the case of estuarine and marine waters, sewage outfall and vessel wastewater discharge. The important modes of adenovirus transmission are by fecal-oral route and inhalation of aerosols. The ubiquitous presence of HAdVs in the water environment made the researchers to consider them as preferred candidate for viral indicator organism (Griffin *et al.,* 2001; Jiang *et al.,* 2005; Pina *et al.,* 1998).

The virological analysis of water requires the need to concentrate and recover the low number of viruses from large volumes of sample. The concentration method should be rapid, simple, providing a smaller volume of concentrate with high virus recovery and has to be inexpensive. Positively

charged filters (Sobsey and Glass, 1980), glass wool (Vilagines *et al.,* 1993) and GAC UAPB (Jothikumar *et al.,* 1995) based methods are still among the best possibilities. Sampling large volumes requires a two-step concentration procedure, with polyethylene glycol precipitation (Lewis and Metcalf, 1988) and ultrafiltration (Rutjes *et al.,* 2006) as preferred procedures for re-concentration of the primary eluent.

Precise and reliable detection method was required to identify the HAdVs from the environmental samples. PCR based genome amplification methods are used for the rapid and sensitive identification of adenoviruses. Many primers were used previously targeting the hexon region of the viral genome (Allard *et al.,* 2001; Avelon *et al.,* 2001; Castignolles *et al.,* 1998; Echavarria *et al.,* 1998; Lu and Erdman, 2006; Puig *et al.,* 1994; Rohayem *et al.,* 2004) which has been considered as the highly conserved region for the adenoviruses. Genome based techniques have been applied either to detect all adenovirus types (Dalapathy *et al.,* 1998; Flomenberg *et al.,* 1997), or for the specific detection of some adenoviruses (Akerblom *et al.,* 1997; Tiemessen and Nel, 1996). The present work deals with the designing of a probe specific to the HAdVs serotypes and their implication to detect the Adenoviruses from environmental water samples and stool samples.

## MATERIALS AND METHODS:

### Concentration of viruses from environmental water samples

One hundred liter of drinking water samples from 10 different zones of Chennai city were concentrated for virus by ultra filtration method using hollow membrane fiber filter (Hill *et al.,* 2005). The hollow filter was directly connected to the tap and water allowed to flow through. Volume was calculated by the flow rate from the outlet tube. Treated and untreated sewage samples (1L) were collected from three sewage treatment centers of Chennai city (6 samples) and 10 L of seawater samples were collected from 3 different sites of Chennai coastal region and concentration procedure was carried out by GAC UAPB method (Jothikumar *et al.,* 1995). Twenty diarrheal stool samples of children below 5 years were collected from a clinical laboratory.

### Polymerase Chain Reaction

CTAB method was followed to isolate DNA from concentrated environmental samples and stool samples (Jothikumar *et al.,* 1995). Primer pairs ADRJC1 5′-GACATGACTTTCGAGGTCGATCCCATGGA-3′ ADRJC2 5′-CCGGCTGAGAAGGGTGTGCGCAGGTA-3′ (Cooper *et al.,* 1999) were used to amplify a 140 bp DNA segment. Reaction was carried out using 2X master mix (Bangalore Genei, Bangalore, India) having 5µL of DNA as template. Amplification was performed in MyGene series Gradient thermocycler by using the settings of 95°C for 5 min followed by 40 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min. The PCR products were analysed with 2% agarose gel.

### Dot blot hybridization

A 23 mer probe 5′-GAAGTCTTTGACGTGGTCCGTGT-3′ was designed in between the 140 bp sequence of PCR product. The probe was labeled with Biotin DecaLabel DNA Labeling Kit (Fermentas, USA). 15 µL of denatured PCR product were applied to Hybond $N^+$ Membrane (Amersham, USA) using a 96-well filtration manifold as previously described (Muscillo *et al.*,1992). Biotin Chromogenic detection kit (Fermentas, USA) was used to detect the purple colored precipitate formation.

## RESULT AND DISCUSSION

The importance given to enteric viruses has been increased as they are frequently observed in environmental sources which may result in waterborne viral outbreaks. The recent developments in the molecular detection substantially increases the knowledge about the contaminating viruses but practically it is difficult to detect all the viruses present in the water which results in holding onto a indicator for viruses. As the incidence of adenoviruses recorded are high when compared to the other enteric viruses makes their candidature better to suit the role.

### Concentration methods

Jothikumar *et al.* (1995) has compared the GAC UAPB method with the membrane filtration technique for the concentration of different viruses from the water samples and recorded the better recovery rate of viruses in GAC UAPB method. Although the method is very cost effective it is having its limitations while processing large volume of samples as it has the necessity of sample preprocessing. For larger volumes Hollow membrane ultra filtration is practically very

effective while using raw sample and it is very easy to sample and elute. Due to the pore size of the membrane it is difficult to process the high TDS samples and sewage samples. In this study we adapted the suitable concentration techniques for different types of environmental samples.

**Polymerase chain reaction results**

The sensitivity and the specificity of the primers were already demonstrated by Elnifro *et al.* (2000) and also showed the capability of the primer to amplify the different serotypes.

**Environmental water samples**

Samples from all the three sewage treatment plants regardless of treated or untreated showed positive for HAdVs which refers the treatment procedure fails to have their barrier on adenovirus. The study conducted by He and Jiang (2005) reveals the concentration of HAdv were at a level of 105 virus particles/litre in primary and secondary treated sewage. The prevalence and the frequency of adenovirus is more due to the excretion up to $10^{11}$ viral particles per gram of stool from the infected persons (Pina *et al.*, 1998, Wadell *et al.*, 1987). The 140 bp signature band (Figure 1) indicating the presence of Adenovirus was observed in three drinking water samples which accounts for 30% positive (Figure 2) and the contamination may be due to the intrusion of sewage into the pipelines of the water supply. Amongst the three seawater samples two are contaminated with adenoviruses were likely to be originated from an on-site contamination event related to human activity.

**Stool samples**

Samples were collected from children below 5years showed 4 out of 12 children whose stool samples analyzed were infected with adenoviruses. Normally adenovirus infections are acute but it might also cause persistent infections characterized by prolonged and intermittent fecal excretion. Allard *et al.* (1992) observed shedding of Adenoviruses from individuals affected with diarrhoea and also in healthy individuals.

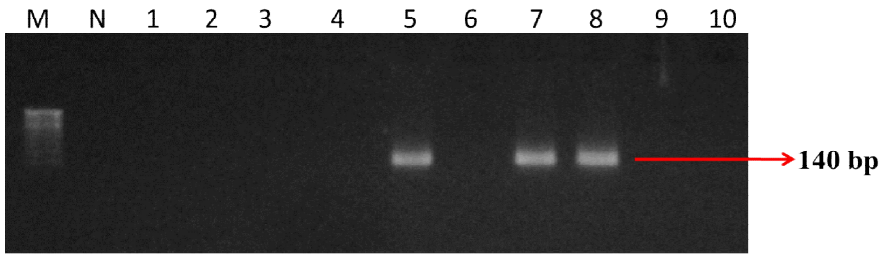


Figure 1: Gel photograph of PCR product from Drinking water samples

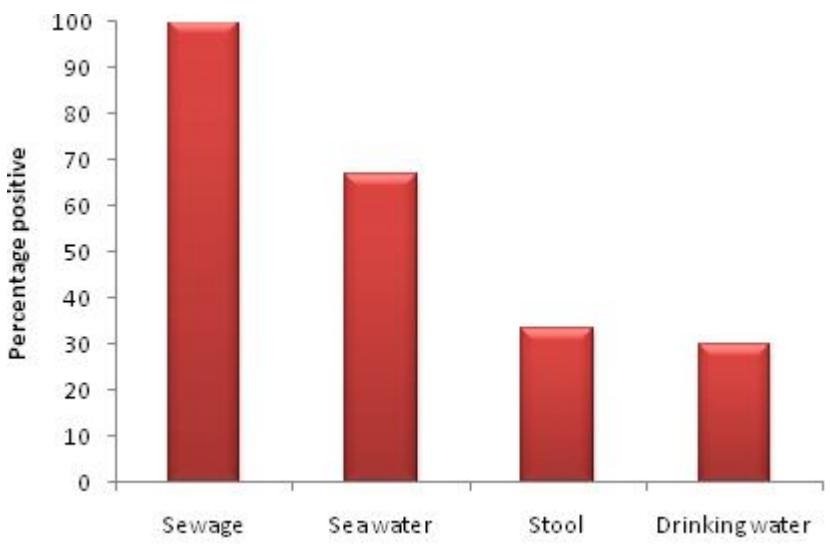


Figure 2: Percentage of adenovirus positive samples

**Dot blot analysis**

The newly developed probe is highly specific for 140 bp amplicon and it is also designed to hybridize with different serotypes. It gives further confirmation about the presence of adenovirus. The PCR positive samples replicated the same in the hybridization assay which increases the sensitivity, stability and reliability of the viral detection (Figure 3).

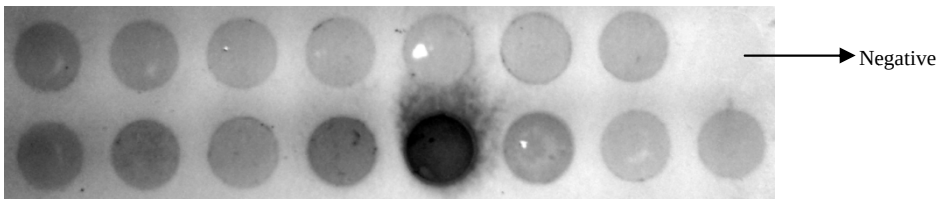


Figure 3: Blot Hybridization photo

**CONCLUSION**

Emerging opportunities for total virus and virus nucleic acid concentration and purification include improvements to adsorption/elution and filtration methods. The biotinylated probe based confirmation method demonstrated in the present study increases the reliability and sensitivity of the PCR method. Also this study supports the idea of considering adenoviruses as index organism for viruses in water analysis.

**REFERENCE:**

Akerblom P, Adrian T and Kostler T. 1997. PCR-based detection and typing of human adenoviruses in clinical samples. *Research in Virology* 148:225–231.

Allard A, Albinsson B and Wadell G. 2001. Rapid typing of human adenoviruses by a general PCR combined with restriction endonuclease analysis. *Journal of Clinical Microbiology* 39:498–505.

Allard A, Albinsson B and Wadell G. 1992. Detection of adenoviruses in stools from healthy persons and patients with diarrhea by two-step polymerase chain reaction. *Journal of Medical Virology* 37:149–157.

Avellon A, Perez P, Aguilar JC, ortiz de Lejarazu R, and Echevarria JE. 2001. Rapid and sensitive diagnosis of human adenovirus infections by generic polymerase chain reaction. *Journal of Virological Methods* 92:113–120.

Basu G, Rossouw J, Sebunya TK, Gashe BA, De Beer M, Dewar JB and Steele AD. 2003. Prevalence of rotavirus, adenovirus and astrovirus infection in young children with gastroenteritis in Gaborone, Botswana. *East African Medical Journal* 80:652–655.

Castignolles N, Petit F, Mendel I, Simon L, Cattolico L and Buffet-Janvresse C. 1998. Detection of adenovirus in the waters of the Seine River estuary by nested-PCR. *Molecular Cell Probes* 12:175–180.

Cooper RJ, Yeo AC, Bailey AS and Tullo AB. 1999. Adenovirus polymerase chain reaction assay for rapid diagnosis of conjunctivitis. *Investigative Ophthalmology & Visual Science* 40:90–95.

Dalapathy S, Lily TK, Roy S and Madhavan HN. 1998. Development and use of nested polymerase chain reaction (PCR) for the detection of adenovirus from conjunctivitis specimens. *Journal of Clinical Virology* 11:77–84.

Echavarria M, Forman M, Ticehurst J, Dumler JS and Charache P. 1998. PCR method for detection of adenovirus in urine of healthy and human immunodeficiency virus-infected individuals. *Journal of Clinical Microbiology* 36:3323–3326.

Elnifro EM, Cooper RT, Klapper PE and Bailey AS. 2000. PCR and restriction endonuclease analysis for rapid identification of human Adenovirus subgenera. *Journal of Clinical Microbiology* 38:2055–2061.

Flomenberg P, Gutierrez E, Piaskowski V and Casper JT. 1997. Detection of adenovirus DNA in peripheral blood mononuclear cells by polymerase chain reaction assay. *Journal of Medical Virology* 51:182–188.

Griffin DW, Lipp EK, McLaughlin MR and Rose JB. 2001. Marine recreation and public health microbiology: quest for the ideal indicator. *Bioscience* 51:817–825.

He JW and Jiang S. 2005. Quantification of enterococci and human adenoviruses in environmental samples by real-time PCR. *Applied and Environmental Microbiology* 71:2250–2255.

Hill VR, Polaczyk AL, Hahn D, Narayanan J, Cromeans TL, Roberts JM and Amburgey JE. 2005. Development of a rapid method for simultaneous recovery of diverse microbes in drinking water by ultrafiltration with sodium polyphosphate and surfactants. *Applied and Environmental Microbiology* 71:6878–6884.

Jiang S, Dezfulian H and Chu W. 2005. Real-time quantitative PCR for enteric adenovirus serotype 40 in environmental waters. *Canadian Journal of Microbiology* 51:393–398.

Jothikumar N, Khanna P, Paulmurugan R, Kamatchiammal S and Padmanabhan P. 1995. A simple device for the concentration and detection of enterovirus, hepatitis E virus and rotavirus from water samples by reverse transcription-polymerase chain reaction. *Journal of Virological Methods* 55:401–415.

Lewis GD and Metcalf TG. 1988. Polyethylene glycol precipitation for recovery of pathogenic viruses, including hepatitis A virus and human rotavirus, from oyster, water, and sediment samples. *Applied and Environmental Microbiology* 54:1983–1988.

Logan C, O'Leary JJ and Sullivan NO. 2006. Real-time reverse transcription-PCR for detection of rotavirus and adenovirus as causative agents of acute viral gastroenteritis in children. *Journal of Clinical Microbiology* 44:3189–3195.

Lu X and Erdman DD. 2006. Molecular typing of human adenoviruses by PCR and sequencing of a partial region of the hexon gene. *Archives of Virology* 151:1587–1602.

Meqdam MM and Thwiny IR. 2007. Prevalence of group A rotavirus, enteric adenovirus, norovirus and astrovirus infections among children with acute gastroenteritis in Al-Qassim, Saudi Arabia. *Pakistan journal of medical science*s 23:551–555.

Muscillo M., Aulicino FA, Orsini P, Bellucci C, La Rosa G and Volterra L. 1992. Standardization of a "dot-blot" method for the molecular detection of enteroviruses in environmental samples by cDNA probes. *Annali di igiene, medicina preventiva e di comunità* 4:255–261.

Pina S, Puig M, Lucena F, Jofre J and Girones R. 1998. Viral pollution in the environment and in shellfish: human adenovirus detection by PCR as an index of human viruses. *Applied and Environmental Microbiology* 64:3376–3382.

Puig M, Jofre J, Lucena F, Allard A, Wadell W and Girones R. 1994. Detection of adenoviruses and enteroviruses in polluted waters by nested PCR amplification. *Applied and Environmental Microbiology* 60:2963–2970.

Rohayem J, Berger S, Juretzek T, Herchenrŏder O, Mogel M, Poppe M, Henker J and Rethwilm A. 2004. A simple and rapid single-step multiplex RT-PCR to detect norovirus, astrovirus and adenovirus in clinical stool samples. *Journal of Virological Methods* 118:49–59.

Rutjes SA, van den Berg HHJL, Lodder WJ and de Roda Husman AM. 2006. Real-time detection of noroviruses in surface water by use of a broadly reactive nucleic acid sequence-based

amplification assay. *Applied and Environmental Microbiology* 72:5349–5358.

Shimizua H, Phana TG, Nishimuraa S, Okitsua S, Maneekarnb N and Ushijima H. 2007. An outbreak of adenovirus serotype 41 infection in infants and children with acute gastroenteritis in Maizuru City, *Japan. Infection, Genetics and Evolution* 7:279–284.

Sobsey MD and Glass JS. 1980. Poliovirus concentration from tap water with electropositive adsorbent filters. *Applied and Environmental Microbiology* 40:201–210.

Tiemessen CT and Nel MJ. 1996 Detection and typing of subgroup F adenoviruses using the polymerase chain reaction. *Journal of Virological Methods* 59:73–82.

Vilagines P, Sarrette B, Husson G and Vilagines R. 1993. Glass wool for virus concentration at ambient water pH level. *Water Science and Technology* 27:3–4.

Wadell GA, Allard A, Johansson M, Svensson L and Uhnoo I. 1987. *Enteric adenoviruses*, In: Enteric adenoviruses. John Wiley & Son, Inc.: Chichester; United Kingdom, pp73–91.