Detection and Identification of Adenovirus from Ophthalmological Specimens by Virus Isolation and PCR

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Most epidemic kerato-conjunctivitis (EKC) are caused by human adenovirus infections. Adenovirus is classified into 6 subgenera and 49 serotypes (1). Adenovirus types 3, 4, 8, 11, 19 and 37 are responsible for most eye infections. In laboratory diagnosis of adenoviruses, virus isolation using tissue culture is routine, but requires more than 2 weeks for isolation and typing. More recently, diagnosis by combination of polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP) has been introduced for identification of adenoviruses in the conjunctiva swabs.

In this study, we used PCR and RFLP to detect and identify adenoviruses on swabs from conjunctiva of 73 patients who had visited an eye clinic in Chikushino City in Fukuoka Prefecture and been diagnosed with EKC (71 cases), pharyngo-conjunctivitis (1 case) or acute conjunctivitis (1 case). The swab specimens were dissolved in 3 ml Hanks' solution containing 0.5% gelatin and centrifuged at 10,000xg for 10 min. The supernatant was stock frozen at -80 C till the examination.

For virus isolation, Vero, FL, RD-18s and Hep-2 cells cultured in 96-well microplates were infected with the specimen in a volume of 25 μ l/well and cultured for 1 week. The cultures negative for CPE were subcultured further. Specimens that failed to induce CPE after 4 subcultures were considered negative. Isolated adenoviruses were typed by using antisera provided by the National Institute of Infectious Diseases, Japan and the DENKA SEIKEN Co., Ltd., Tokyo.

PCR was conducted according to the method of Saito and Imagawa, (3). 200 μ l of each specimen was centrifuged at 12,000xg for 30 min. The pellet was dissolved in 100 μ l of 0.31 mg/ml proteinase K, 0.5% Tween 20, 1 mM EDTA, and 10 mM Tris-HCl (pH 8.3), then incubated at 55 C for 1 hr. After the samples were heated at 95 C for 10 min, DNA was extracted. Nested PCR using the primers shown in Table 1 (external primer pairs were AdTU7 and AdTU4', and internal pairs were AdnU-S' and AdnU-A) was conducted. Both the first and second PCR amplifications consisted of 36 cycles of denaturation at 94 C for 1 min, annealing at 50 C for 1 min and elongation at 72 C for 2 min; after the second amplification the samples were incubated at 72 C for 7 min for completion of the elongation reaction. The amplified materials were digested with

*Eco*T14I, *Hae*III, or *Hin*fI and electrophoresed in 3% agarose gel (Fig. 1).

The results are summarized in Table 2. Virus isolation in culture and serotyping identified adenoviruses from 28 specimens: eight Ad3, one Ad8, one Ad11, fifteen Ad19, and three Ad37 specimens. PCR-RFLP identified adenoviruses from 54 specimens: nine Ad3, eleven Ad8, one Ad11, twentyfour Ad19, and nine Ad37 specimens. The data on all specimens agreed perfectly between the two methods performed. The PCR-RFLP method required 3 days for a complete analysis. The positivity was 74% (54/73) for PCR-RFLP, and 38.4% (28/73) for virus isolation and serotyping. For B subgenera Ad3 and Ad11, the sensitivities of the two methods were comparable, while for D subgenera Ad8, Ad19 and Ad37 (particularly for Ad8) the virus isolation in culture was very inefficient. In neutralization, Ad19 and Ad37 showed cross reactivity, but in PCR-RFLP the two viruses were clearly distinguished (Fig. 1). Our data thus demonstrated the usefulness of PCR-RFLP for the laboratory diagnosis of adenoviruses. However, we should also be aware of the limitations of the PCR method, e.g., PCR detects only a fragment of the virus genome and detects viruses only if they have a sequence suitable for the primers. Because virus isolation and PCR-RFLP provide complementary information, both should be used for the virus surveillance.

Fig. 1 PCR products digested by restriction enzymes

Table 1 Primers for nested PCR of Adenovirus

Primers	Nucleotide Position ^a	Sequence	PCR Product(bp)
AdTU7	20,734 - 20,753	5'-GCCACCTTCTTCCCATGGC-3'	1,004
AdTU4'	21,718 - 21,737	5'-GTAGCGTTGCCGCGCAGAA-3'	
AdnU-S'	20,743 - 20,762	5'-TTCCCATGGCNCACAACAC-3'	956
AdnU-A	21,679 - 21,698	5'-GCCTCGATGACGCCGCGTG-3'	

^aaccording to Ad2 nucleotide sequence(4)

Table 2 Detection of Adenoviruses

	Positive Specimens(%)	Ad3	Ad8	Ad11	Ad19	Ad37	negative
Virus Isolation	28 (38.4)	8	1	1	15	3	45
PCR-RELP	54 (74.0)	9	11	1	24	9	19

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REFERENCES

1. Schnurr, D. and Dondero, M.E. (1993): Two new candidate adenovirus serotypes. *Intervirology*, 36, 79-83.
2. Aoki, K., Isobe, K. and Ohono, S. (1994): Nationwide surveillance program of epidemic conjunctivitis in Japan. 309-316. *In* A.A. Bialasiewicz and K.P. Schaal (eds.), *Infectious diseases of the eye*. Butterworth Heinemann, Boston.
3. Saitoh-Inagawa, W., Oshima, A., Aoki, K., Itoh, N., Isobe, K., Uchino, E., Ohono, S., Nakazima, H., Hata, K. and Ishiko, H. (1996): Rapid diagnosis of adenoviral conjunctivitis by PCR and restriction fragment length polymorphism analysis. *J. Clin. Microbiol.*, 34, 2113-2116.
4. Chroboczek, K., Bieber, F. and Jackrot, B. (1992): The sequence of the genome of adenovirus type 5 and its comparison with the genome of adenovirus type 2. *Virology*, 186, 280-285.