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# Comparison between of the Attenuated BR-Oka and the Wild Type Strain of Varicella Zoster Virus (VZV) on the DNA level

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Oka strain VR-795 (Varicella Zoster Virus, VZV) of American Type Culture Collection (ATCC) has been used for chickenpox vaccine production. In order to use this strain for vaccine production, the strain must be identified and its stability must be confirmed.The identification of the Oka strain has been confirmed using Restriction Fragment Length Polymorphism (RFLP) and DNA sequence analysis of glycoprotein-II (gp-II). The amino acid sequences of Oka deduced from the DNA sequence of gp-II have changed at three amino acids against Ellen and at one amino acid against Webster. To prove the stability of the Oka strain during the passage, RFLP and DNA sequence analyses were also used with 11, 15 and 23 times of virus passage. We found that the Oka strain was stable at passages of up to 23 times, based on the RFLP and DNA sequence analyses. The confirmed Oka strain was renamed as BR-Oka for the purposes of chickenpox vaccine production.

**Key words:** VZV, Oka, Chicken pox, Vaccine

## INTRODUCTION

Varicella zoster virus (VZV) was isolated approximately 50 years ago (Weller, 1953). Chickenpox, commonly known as varicella, is a highly infectious disease that spreads rapidly in susceptible populations. Chickenpox virus causes two distinct clinical conditions, chickenpox and herpes zoster (Takahashi, 1983). The Oka strain of the VZV was isolated from a three-year-old boy who was clinically diagnosed with varicella. It was passed 11 times in human embryonic lung cells at 34°C and 12 times in guinea pig embryo cells at 37°C. The virus was passed in human diploid cells (WI-38, MRC-5) several times to prepare an experimental vaccine (Takahasi *et al.*, 1974; Takahasi *et al.*, 1975).

The Oka strain is an attenuated virus, and it differs from the wild type VZV on pathological, biological and molecular levels (Hayakawa *et al.*, 1984). Michiaki Takahashi and his colleagues donated the Oka strain (U.S. Pat. 3,985,615) to American Type Culture Collection (ATCC VR-795). This strain has been used to produce chickenpox vaccine in Japan, Korea and U.S.A. (Yoshizo *et al.*, 1985; White *et al.*, 1991).

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Before the Oka strain (ATCC VR-795) is used as a master seed virus for chickenpox vaccine production, it should be re-identified by the manufacturer of the vaccine. The manufacturer must show the stability of the Oka strain during passage in the host cells. We compared the Oka strain to the Ellen and Webster strains of the wild type VZV. First, we analyzed the restriction fragment length polymorphism (RFLP) of the PCR products at the gene 38 and 54 regions in the Oka strain and the wild type strains (LaRussa *et al.*, 1992). RFLP analysis is a convenient method, and its results provide clear evidence when the Oka strain is identified. Second, we analyzed the DNA sequence of the Oka strain and wild type strains in the GPII region. Most glycoproteins have specific epitopes of each virus strain. Finally, the stability of the Oka strain was tested by DNA sequence analysis in the GPII region of the virus passed 11 and 23 times in MRC-5 cells. The stability of the virus is a very important factor in the quality and safety of the vaccine.

Our results showed that the identified Oka strain was attenuated VZV, which can be used as the seed virus for chickenpox vaccine production.

## MATERIALS AND METHODS

### Viruses and cells

Three VZV strains, Oka (ATCC VR-795), Ellen (ATCC

VR-1367) and Webster (ATCC VR-916), were obtained from the ATCC. MRC-5 cells were obtained from European Collection of Cell Cultures (ECACC, U.K.). The cells were grown and maintained in Dulbeccos Modified Eagle Medium:F12 (DMEM:F12, Gibco BRL, U.S.A.) supplemented with 2% fetal bovine serum (Gemini Bio Products Inc, U.S.A.), 2.0 g/L of sodium bicarbonate, 100 mg/L of kanamycin sulfate, and 0.1 mM non-essential amino acid (Gibco BRL, U.S.A.). All of the viruses were grown in human lung diploid fibroblast cells, MRC-5 (ECACC CB2719, passage number 20).

For the passage of the virus, each strain of VZV was inoculated at the 0.1 m.o.i. onto the fresh confluent monolayer cells. The infected cells were maintained for three days at 35°C. When the CPE was expended to approximately 70% of the monolayer culture area, the virus was harvested. After propagation of the Oka strain, we renamed it the BR-Oka strain of VZV. The virus was passed through the MRC-5 cells several times for large-scale virus production. The BR-Oka strain was classified into BR-Oka-11, BR-Oka-15, and BR-Oka-23 according to the passage time.

### Extraction and preparation of VZV genomic DNA

Viral DNA was prepared using the methods proposed by Martin (Martin *et al.*, 1982) and Straus (Straus *et al.*, 1982) with slight modifications. When extensive CPE appeared, the infected cells were harvested by scraping with a rubber policeman and collected by centrifugation at 3,000 rpm (Allegro 6R, Beckman, U.S.A.) for 10 min. The collected cells were added to 0.5 ml of 10 mM Tris-HCl (pH 8.0) containing 0.5% (v/v) NP40 (BDH chemical, UK) and 10 mM EDTA-2Na, and lysised by holding for 30 minutes at room temperature. The lysised cells were centrifuged at 3,000 rpm for 20 min at 4°C, and the supernatant was collected. This process was repeated three times, and the supernatant was collected in same tube each time.

This supernatant was centrifuged on a SW27 rotor (Hitachi, Japan) at 27,000 rpm for one hour at 4°C. After discarding the supernatant solution, the pellets were suspended in 1 ml of TE solution (10 mM Tris-HCl pH 8.0, 1 mM EDTA-2Na). The suspension was mixed with 1 ml of solubilizing liquid (10 mM Tris-HCl pH 8.0, 1% SDS, 10 mM EDTA-2Na, 200 mg/ml proteinase K, and 100 mg/ml RNase), and incubated overnight at 37°C. The DNA was extracted twice with PCI solution (water-saturated phenol:chloroform:isoamyalchol; 25:24:1) and precipitated with ethanol. The DNA pellets were resuspended in TE buffer, and the concentration of DNA was determined in terms of absorbance $OD_{260}$.

### PCR and restriction enzyme analysis

The VZV DNA was extracted from the infected MRC-5 cells and amplified by PCR with four pairs of VZV primers, as shown in Table I. The PCR reaction mixture contained 1.25 units of Taq DNA polymerase (Promega, Medison, U.S.A.), 200 mM deoxynucleoside triphosphate, 10 mM Tris-HCl (pH 8.0 at 25°C), 50 mM KCl and 0.1% Triton X-100, 1.5 mM $MgCl_2$, 1 ml of each primer (10 pmole), and 1 mg of template DNA, at a final volume of 50 ml. Thirty cycles of amplification were performed (denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 2 min in a DNA thermocycler (Takara SP, Japan).

To investigate the restriction profiles of each virus in the gene 38 and gene 54 regions, the PCR products of each region were digested with *Pst* I and *Bgl* I, respectively. The restriction enzyme digestion was carried out overnight at 37°C in the appropriate buffer, and the digested PCR products were resolved by electrophoresis using 2% SYNERGEL (Diverfie Biotech, U.S.A.) according to the manufacturer's instructions.

### Cloning and DNA sequencing

For the sequence comparison in the gp-II region, the PCR products of each VZV were ligated with pGEM-Easy plasmid (Promega, Medison, U.S.A.). The ligated plasmids were transformed into *Escherichia coli* Top10 according to standard protocols (Sambrook *et al.*, 1989).

Electrophoresis was performed on 6% acrylamide gels, and the sequences were read using an LI-COR automatic sequencer (LI-COR Inc.,U.S.A.).

Multiple sequence alignment analysis was performed

**Table I.** The oligonucleotide primers used in this study

| Name | Sequence | Location at genome | Product size (bp) | Usage |
|---|---|---|---|---|
| Gene 38-up | 5'-ttgaacaatcacgaaccgtt-3' | 69250-69269 | 350 | RFLP by *Pst* I |
| Gene 38-down | 5'-cgggtgaaccgtattctgag-3' | 69570-69599 | | |
| Gene 54-up | 5'-ggaacccctgcaccattaaa-3' | 95109-95128 | 222 | RFLP by *Bgl* I |
| Gene 54-down | 5'-tcccttcatgcccgttacat-3' | 95261-95280 | | |
| GPII-1-up | 5'-cgtttcagagatcacggacacc-3' | 57415-57436 | 497 | GPII-1 region sequencing |
| GPII-1-down | 5'-gttttggctttcagttccaacc-3' | 57890-57911 | | |
| GPII-2-up | 5'-gaaatcgcagtccatgagttg-3' | 58826-58847 | 481 | GPII-2 region sequencing |
| GPII-2-down | 5'-ttaacccctggttgtgagtgg-3' | 59285-59306 | | |

using the DNA star program (DNA Star Inc., U.S.A.).

## RESULTS

### Restriction Fragment Length Polymorphism of the PCR product

One primer pair (gene38-up and gene38-down) was used to amplify 350 bp fragment in the Oka strain and in the wild type strains. The amplified product of the Oka strain did not contain a *Pst* I site. Therefore, it would not be cleaved by the digestion of *Pst* I. However, the amplified product of the wild-type strains would be cleaved with *Pst* I, resulting in two fragments of 250 and 100 bp. Another primer pair (gene54-up and gene54-down) was used to amplify a 222-bp fragment in the Oka strain and in the wild type strains. The *Bgl* I site was present in the Oka strain, but not in the wild-type strains (LaRussa *et al.*, 1992).

Ellen, Webster, BR-Oka-11, BR-Oka-15 and BR-Oka-23 strains were tested in this study. The genomic DNA of these viruses was amplified in gene 38 and gene 54, and the resulting products generated the exact band of 350 bp in gene38 and the exact band of 222 bp in gene54, as expected (Fig. 1). The wild-type VZV DNA fragment amplified from gene38 was cleaved by *Pst*into two fragments, 250 bp and 100 bp, but the fragment of the BR-Oka strain remained in the 350-bp band on agarose gel (Fig. 2A). Conversely, the 222-bp PCR products of the wild-type VZV that originated from gene 54 were not cleaved by *Bgl*, but the PCR product of the BR-Oka strain was cleaved by 137 bp and 85 bp fragments (Fig. 2B). Based on RFLP data, Ellen and Webster could be grouped as wild-type strains, whereas BR-Oka could be

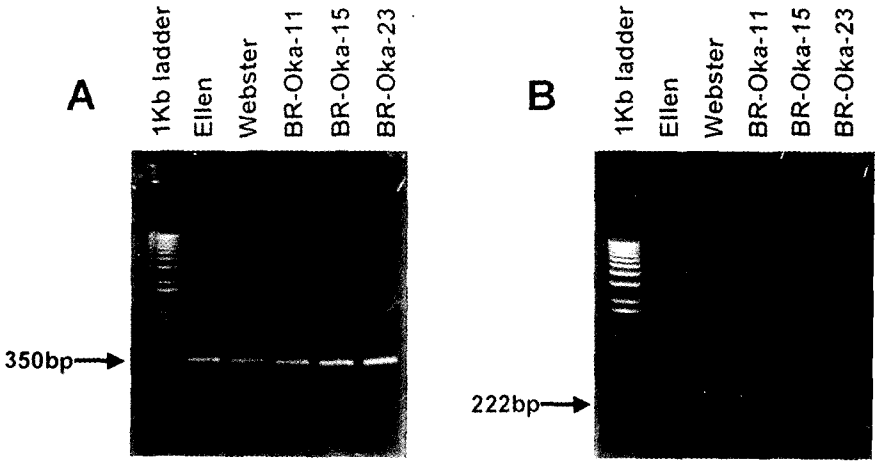


**Fig. 1.** PCR amplified VZV DNA of each strain for RFLP. A: Agarose gel electrophoresis of PCR products amplified from the gene38 of Ellen, Webster, BR-Oka-11,BR-Oka-15 and BR-Oka-23. B: Agarose gel electro-phoresis of PCR products amplified from the gene54 of Ellen, Webster, BR-Oka-11, BR-Oka-15 and BR-Oka-23

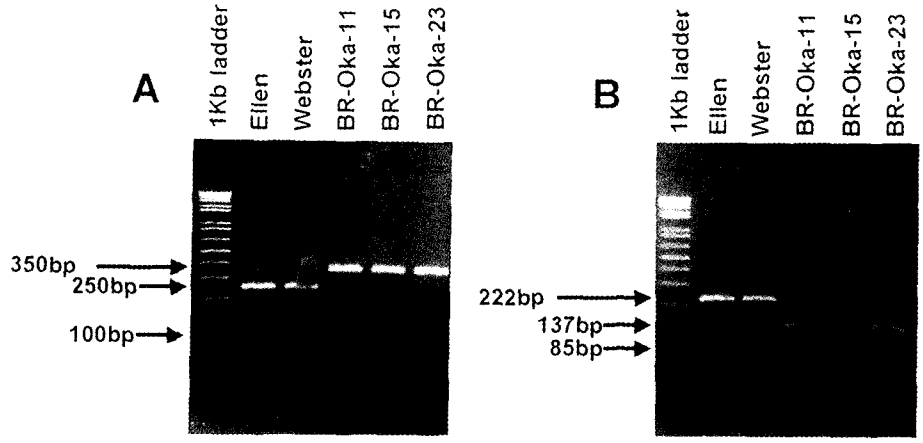


**Fig. 2.** RFLP of each strain of VZV. A: Agarose gel electrophoresis of PCR products of gene38 digested with *Pst* I in the strain Ellen, Webster, BR-Oka-11,BR-Oka-15 and BR-Oka-23. B: Agarose gel electrophoresis of PCR products of gene54 digested with *Bgl* I in the strain Ellen, Webster, BR-Oka-11, BR-Oka-15 and BR-Oka-23.

grouped as a vaccine-type strain (attenuated VZV).

The stability of the BR-Oka strain was also confirmed by these RFLP experiments. The viral genomic DNA of BR-Oka strain viruses was amplified at the same size at virus passages of 11, 15 and 23 times (Fig. 1). The PCR products of each number of passages retained the same digested fragment profiles after digestion with *Pst* I and *Bgl* I (Fig. 2A and Fig. 2B), demonstrating the stability of the BR-Oka strain for up to 23 passages in the MRC-5 cell.

### DNA sequence comparison between (the) wild and vaccine-type strain of VZV

To compare the DNA sequences between the wild and vaccine-type strains of VZV, two regions of gp-II in each virus were amplified and cloned in a pGEM-T-easy vector. The genomic localization of GPII-1 is 57415 to 57911, and the genomic localization of GPII-2 is 58826, according to the numbering system proposed by Davison *et al.* (Davison and Scott, 1986) (Fig. 3)

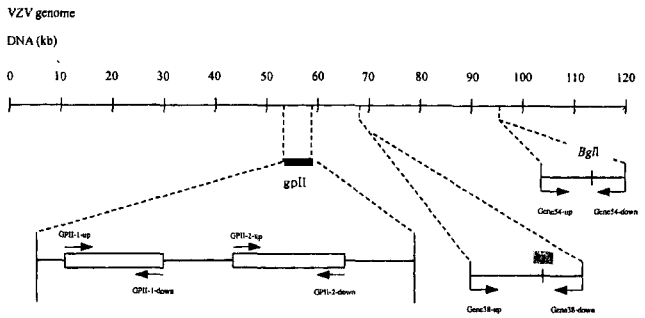


**Fig. 3.** Relative locations of the gpII-coding region, gene38 and gene54 fragment in the BR-Oka VZV genome. The Shaded *Pst* I site that is not present in BR-Oka strain but exists in wild-type strain marked for identification. Conversely the *Bgl I* site exist in BR-Oka strain but not in Wild type strains. The arrows indicate locations of primers.

The DNA sequences of BR-Oka, Ellen and Webster in the gpII region were newly determined and used as a new criterion for the characterization of VZV. The viral DNAs were amplified by PCR and produced a 497 bp fragment for gpII-1 and a 481 bp fragment for gpII-2 (Fig. 4). The DNAs used in this experiment had the same nucleotide sequences in the gpII-1 regions (data not shown). However, the gpII-2 region of the BR-Oka VZV strains differed from Ellen in three nucleotides and from Webster in one nucleotide. The amino acid sequences of BR-Oka deduced from these DNA sequences were also changed at three amino acids against Ellen and at one amino acid against Webster (Fig. 5). This result supports that BR-Oka strain is an attenuated virus, which differs from the wild type VZV.

In addition to these, the DNA sequence comparison between BR-Oka-11 and BR-Oka-23 in the gpII-1 and gpII-2 regions also gives evidence of stability. In this BR-Oka strain of VZV, there are no DNA sequence variations in the gpII-1 and gpII-2 regions until 23 passages in the MRC-5 cells (Fig. 5). This sequence analysis during virus passages indicates the stability of the BR-Oka strain.

## DISCUSSION

Based on the result of the RFLP and DNA sequence analysis of glycoprotein II, we believe that the BR-Oka strain has the characteristics of attenuated VZV, and that in our production process, its stability is maintained until it has been passed 23 times on the DNA level..

The RFLP experiment performed on a specific site is considered to be the simplest and most convenient method for the identification of a virus (LaRussa *et al.*,

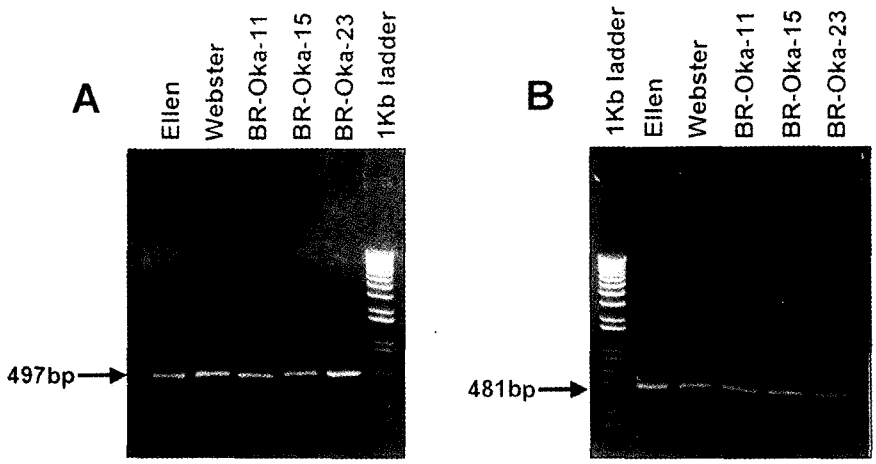


**Fig. 4.** PCR amplifed VZV DNA of each strain for the partial sequencing of GPII region. A: Agarose gel electrophoresis of PCR products amplified from the GPII-1 region in chromosomal DNA of the strain Ellen, Webster, BR-Oka-11, BR-Oka-15, and BR-Oka-23. *B: Agarose gel electrophoresis of PCR products amplified from the GPII-2 region in chromosomal DNA of* the strain Ellen, Webster, BR-Oka-11, BR-Oka-15, and BR-Oka-23

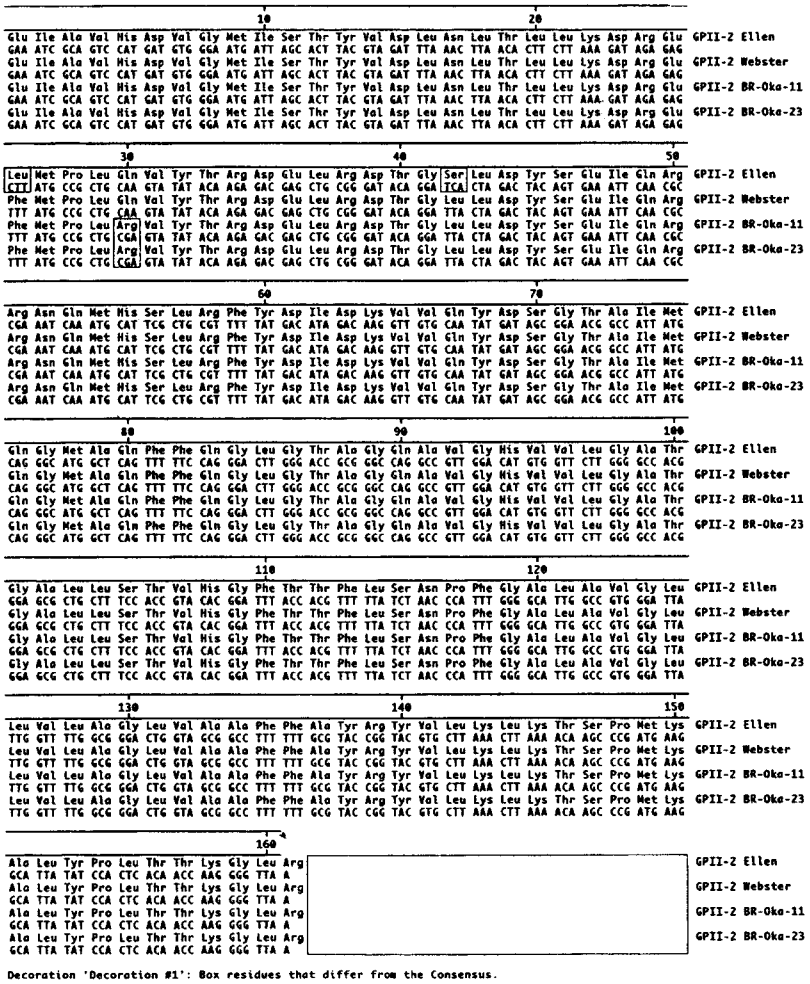


**Fig. 5.** Comparison of the sequences of GPII-2 region derived from strain Ellen, Webster, BR-Oka-11 and BR-Oka-23. A multiple sequence alignment was performed using the DNA star program. Box residues that differ from the consensus sequence.

1992). However, the results of RFLP provide limited information about DNA sequence variation. On the other hand, DNA sequence analysis of specific sites results in more precise information about the identification of viruses. The genomic DNA analysis of the Oka strain is difficult because of its large genome size and the lack of accurate information about the gene relating to its attenuation. In general, the antigenic determinants of virus-coded proteins are of great importance for virus classification. Viruses belonging to the same genus or major antigenic group share common determinants. These group-specific antigens are generally located in internal virus components. However, the most specific or individual viral antigenic determinants are usually located on the surface components of viral particles. An individual virus strain is identified by these specific antigens. The glycoproteins of the virus surface are frequently used as criteria for serotypic differentiation of viruses. The nucleotide sequence of glycoproteins displays more variations than other structure proteins (Wolfgang K. J., 1988). Therefore, the gpII region of the Oka strain was chosen for identification and stability confirmation at 11, 15 and 23 virus passages. The results of the sequence analysis of the GP-II region could prove to be molecular biological evidence for the safety and stability of this vaccine strain.

The live attenuated chickenpox vaccine recognized by the WHO Expert Committee exhibits the desirable property of low virulence, while also inducing adequate antibodies and protecting against disease (Hardy and Gershon, 1990). Because of its proved safety and efficacy, this vaccine is now licensed for general use in Japan, Korea and U.S.A., and for immunocompromised patients in several other countries (Yoshizo *et al.*, 1985; White *et al.*, 1991).

Vaccine-type viruses exhibit various biological and biophysical attributes that can be used to distinguish them from wild-type viruses (Hayakawa *et al.*, 1984). These characteristics include a difference in temperature sensitivity and levels of infection in guinea-pig embryonic fibroblast cells (GPEF) and human embryonic fibroblast cells (HuEF)

(Hayakawa *et al.*, 1986; Shiraki *et al.*, 1991; Martin *et al.*, 1982). Although the BR-Oka strain has been confirmed as attenuated VZV on DNA levels, its biological properties must also be identified. The study of the biological properties of the BR-Oka strain is currently underway.

## REFERENCES


Davison, A. J. and Scott. J. E., The complete DNA sequence of varicella-zoster virus. *J. Gen. Virol.*, 67, 1759-1816 (1986).

Hardy, I. and Gershon, A. A., Prospects for use of a varicella vaccine in adults. *Infect. Dis. Clin. North Am.*, 4, 160-173 (1990).

Hayakawa, Y., Torigoe, S., Shiraki, K., Yamanishi, K. and Takahashi, M., Biological and biophysical markers of a live varicella vaccine strain (Oka): Identification of clinical isolates from vaccine recipients. *J. Infect. Dis.*, 149, 956-963 (1984).

Hayakawa, Y., Yamamoto,T., Yamanishi, K. and Takahashi, M., Analysis of varicella-zoster virus DNAs of clinical isolates by endonuclease *Hpa*I. *J. Gen. Virol.*, 67, 1817-1829 (1986).

LaRussa, P., Lungo, O., Hardy, I., Gerrison, A. A., Steinberg, S. P. and Silverstein, S., Restriction fragment length polymorphism of polymerase chain reaction products from vaccine and wild-type varicella-zoster virus isolates. *J. Virol.*, 66, 1016-1020 (1992).

Martin, J. H., Dohner, D. E., Wellinghof, W. J. and Gelb, L. D., Restriction endonuclease analysis of varicella-zoster vaccine virus and wild-type DNAs. *J. Med. Virol.*, 9, 69-76 (1982).

Sambrook, J., Fritisch, E. F. and Maniàtis, T., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989.

Shiraki, K., Horiuchi, K., Asano, Y., Yamanishi, K. and Takahashi, M., Differentiation of Oka varicella vaccine strain from wild varicella-zoster virus strains isolated from vaccinees and household contact. *J. Med. Virol.*, 33, 128-132 (1991).

Straus, S. E., Owens, J., Ruyechan, W. T., Takiff, H. E., Casey, T. A., Vande Woude, G. F., and Hay, J., Molecular cloning and physical mapping of varicella-zoster virus DNA. *Proc. Natl. Acad. Sci. USA.*, 79, 993-997 (1982).

Takahashi, M., Otsuka, T., Okuno, Y., Asano, Y., Yazaki, T. and Isomura. S., Live vaccine used to prevent the spread of varicella in children in hospital. *Lancet* 2, 1288-1290 (1974).

Takahashi, M., Okuno, Y., Ostsuka, T., Osame, J., Takamizawa, A., Sasada, T. and Kubo, T., Development of a live attenuated varicella vaccine. *Biken J.* 18, 25-33 (1975).

Takahashi, M., *Advances in virus research*, American Press, Vol 26, 285p-355p (1983).

Yoshizo, A., Takao, N., Takao, M., Takehiko, Y., Shigemitsu, I., Koichi, Y. and Michiaki, T., Long term protective immunity of recipients of the Oka strain of live varicella vaccine. *PEDIATRICS*, 75(4), 667-671 (1985).

Weller, T. H., Serial propagation in vitro of agents producing inclusion bodies derived from varicella and herpes zoster. *Proc. Soc. Exp. Bio. Med.* 83, 340-346 (1953).

White, C., Barbara, J., K., Carol, S., H., Kathryn, L., I., Varicella vaccine, VARIVAX, in health children and adolescents: Result from clinical trials, 1987 to 1989. *PEDIATRICS* 87 (5), 604-610 (1991)

Wolfgang, K. J., *Virology*, 3rd ED., Prentice-Hall, Englewood Cliffs, New Jersey, (1988).