

Recombinant VZV Glycoprotein E

Diagnostic CLIA Assay Development for VZV Antibody Detection

Introduction

For many years, enzyme-linked immunosorbent assay (ELISA) was routinely used for in-vitro diagnostics of infectious diseases. As advanced automation entered routine laboratories, and new needs for flexibility in testing arose, chemiluminescent immunoassays (CLIA) became more and more popular. CLIA technology is a very versatile and reliable method allowing fast and economic testing of patient samples. However, with the increasing complexity of CLIA assays, developers also face new technical challenges, e.g. demands for higher quality of raw materials or optimization of bead coupling.

Virion\Serion's Varicella zoster virus (VZV) antigens **VZV Lysate (BA104VS)** and **VZV Glycoprotein (BA104VSG)** were developed more than two decades ago for optimized ELISA performance. Though these antigens show excellent performance in CLIA assays too, we recently added the recombinant **VZV Envelope Glycoprotein E (BA104R02)** and **VZV Glycoprotein B (BA104R03) antigens** to our portfolio. The protein production in an ISO 13485 certified environment guarantees the expected high quality, traceability and lot-to-lot consistency paired with readily available bulk quantities.

Background

Varicella zoster virus, also known as Human Herpesvirus Type 3 (HHV-3), is a member of the *Herpesviridae* family. It is a highly contagious virus causing varicella (chickenpox) after primary infection. Transmission occurs via droplets and aerosols or contact with virus containing vesicles or scabs. Polymorphic exanthemas with a strong itch leading to papulation, vesicles and eschar are typical for this children's disease. In healthy children, chickenpox is usually a harmless and self-limiting

infection. However, commonly lifelong latency in cranial nerve and dorsal root ganglia is established and reactivation as herpes zoster (shingles) after decades may occur. Primary infection of adults, especially immunosuppressed patients, or newborns may be fatal and infection during gestation can lead to congenital varicella syndrome after transmission of the virus to the fetus. Thus, VZV serological tests are commonly used for confirmation of acute infection, control of vaccination effectiveness and in some countries they are even routinely added to the TORCH screening of pregnant women. Furthermore, VZV serology is an important diagnostic element in the context of transplantation, because immunocompromised patients have an increased risk for severe complications after infection or virus reactivation.

VZV envelope glycoprotein E (gE) is highly expressed on VZV viral particles as well as on infected cells. It is the immunogenic component in the licensed subunit vaccine Shingrix® (Glaxo Smith-Kline Biologicals), which elicits a strong immune response and protects against herpes zoster⁽¹⁾. Virion\Serion's VZV gE (BA104R02) is recombinantly produced in insect cells and highly purified by strep-tag affinity chromatography, which guarantees high quality and availability. Read on to learn more about efficient IgG and IgM detection with the new VZV gE (BA104R02).



Order Information and Related Products

Code	Description	Packaging
BA104R02	VZV Envelope Glycoprotein E Envelope glycoprotein E ectodomain of Varicella zoster virus (VZV) Source: recombinant protein, expressed in insect cells Molecular weight: 61 kDa, Affinity tag: Strep-tag	1 mg
BA104R03	VZV Envelope Glycoprotein B Envelope glycoprotein B of Varicella zoster virus (VZV) Source: recombinant protein, expressed in insect cells Molecular weight: 81 kDa, Affinity tag: Strep-tag	1 mg
BA104VSG	VZV Glycoprotein VZV Glycoproteins are extracted from infected cells by detergent treatment and further purified by Lectin affinity chromatography. Source: VZV strain Ellen, HEL 299 cell culture	1 mg
BA104VS	VZV Antigen Virus is extracted from infected cells by alkaline treatment and sonication, followed by purification through a sucrose cushion using ultracentrifugation. Source: VZV strain Ellen, HEL 299 cell culture	1 mg

Material and Methods

Antigen

Recombinant VZV gE (BA104R02) is produced in insect cells and highly purified by strep-tag affinity chromatography.

Immunoassay

Recombinant VZV gE antigen (BA104R02), native VZV antigen (BA104VS) or VZV glycoprotein (BA104VSG) were immobilized on magnetic beads with 3 µm in diameter, which are commonly used for CLIA assays. The coupling of the antigens as well as the subsequent immunoassays were performed in an automated workflow on a KingFisher™ Flex System (ThermoFisher Scientific™). For IgG or IgM detection, antigen coupled magnetic particles were incubated with diluted human sera for 20 minutes at 37°C and then washed. Bound anti-VZV IgG or IgM antibodies were detected after 20 minutes incubation at 37°C with fluorescently labeled secondary antibody by using a Cytoflex S flow cytometer (Beckman Coulter).

Reference ELISA and CLIA assays were performed according to manufacturer's instructions.

Dynamic light scattering and size exclusion chromatography

Dynamic light scattering (DLS) on a Zetasizer Ultra (Malvern Panalytical GmbH) was used for the measurement of size distribution and the detection of aggregates of the different VZV gE lots in solution. For evaluation, the volume distribution was used, which shows the total volume of particles in the different size bins.

Size exclusion-high-performance liquid chromatography (HPLC-SEC) is a high-throughput analytical method that allows to determine and quantify the level of aggregates and fragments of purified proteins. HPLC-SEC was performed using a Superdex increase 200 3.2/300 SEC column (Cytiva) on a 1260 Infinity II LC System (Agilent Technologies Sales & Services GmbH & Co.KG).

Glycosylation assay

VZV gE protein samples were treated with 500.000 Units (U)/mL PNGase F according to manufacturer instructions to analyze glycosylation. Denatured protein samples were separated by their molecular weight using SDS-PAGE and afterwards visualized by Coomassie staining.

Results

Biochemical Analysis

Three different lots of recombinant VZV gE were analyzed using dynamic light scattering (DLS) to check the homogeneity. Figure 1 shows the comparison of the three peaks highlighting a strong consistency and homogeneity with < 1% aggregates detected by DLS in all lots. The hydrodynamic diameter of 9.2 d.nm indicates dimerization of the protein. This is in line with a publication from Olson et al. ⁽²⁾, showing that VZV gE purified from insect cells dimerizes due to phosphorylation by a tyrosine kinase.

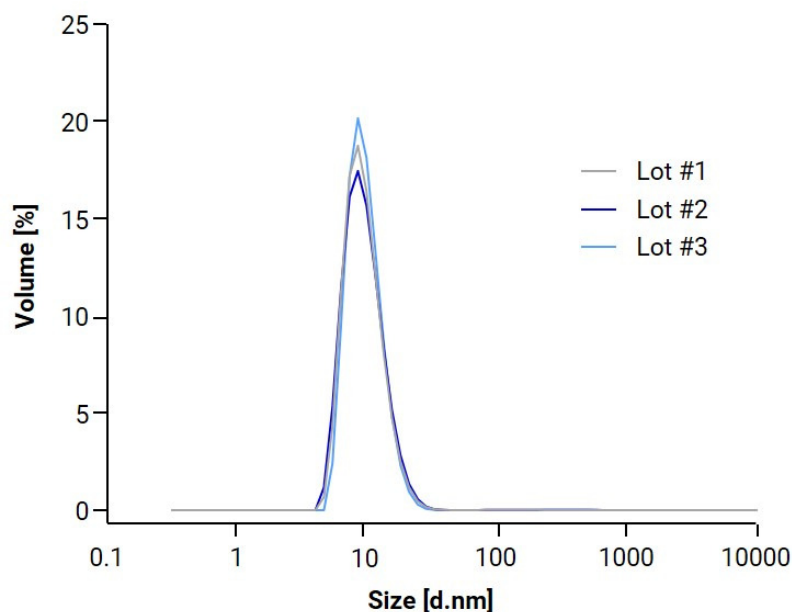


Figure 1: DLS analysis (volume distribution) of three independent lots of recombinant VZV gE. Peak overlay illustrates a high conformity. The hydrodynamic diameter of 9.2 d.nm indicates dimerization.

To further confirm oligomerization, size exclusion-high-performance liquid chromatography (HPLC-SEC) was performed. As shown in Figure 2, the VZV gE lots display a mean molecular weight of 139.4 kDa with < 4% aggregates or higher oligomers as determined by peak retention time analysis at 280 nm detection wavelength. This is 2.2 times the calculated molecular weight (ProtParam, www.expasy.org) of the unglycosylated monomer. Considering glycosylation (see glycosylation analysis below), this weight fits perfectly to a dimer. A study by Nordén et al.⁽¹⁾ showed VZV gE to be heavily glycosylated when it was produced in human fibroblasts.

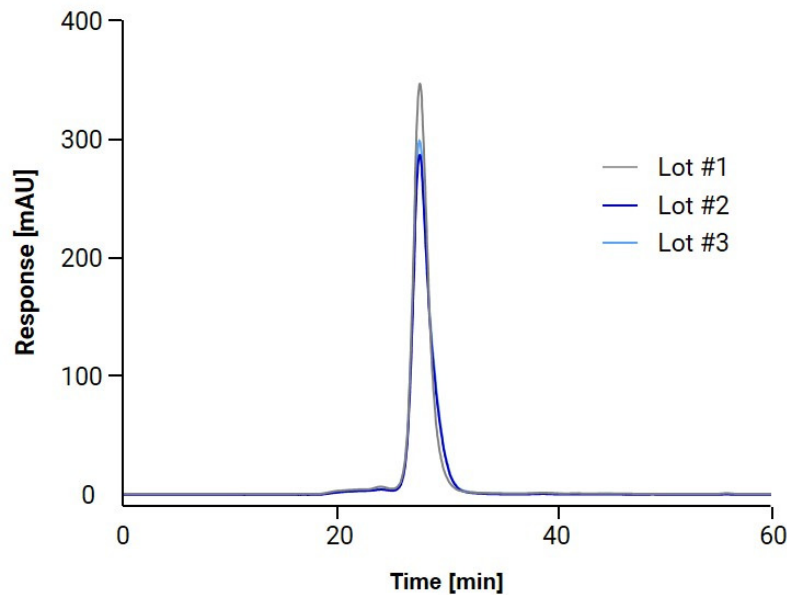


Figure 2: Analytic HPLC-SEC of three independent lots of recombinant VZV gE using a Superdex increase 200 3.2/300 SEC (Cytiva) column. Peak retention time (detection at 280 nm) indicates a size of 139.42 kDa pointing to dimerization and glycosylation of the protein.

Glycosylation of recombinant VZV gE was further assessed by PNGase F treatment. The enzyme PNGase cleaves glycoside bonds leading to glycan-free proteins. As shown in Figure 3, SDS-PAGE analysis revealed a band shift in the PNGase F treated sample confirming glycosylation of the untreated protein.

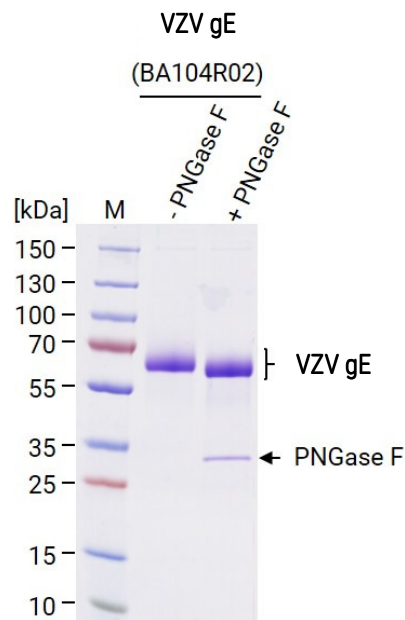


Figure 3: Analysis of glycosylation. VZV gE was incubated with 500.000 U/mL PNGase F according to manufacturer instructions, analyzed by SDS PAGE and stained with Coomassie solution. Band shift in PNGase F treated sample confirms glycosylation of VZV gE (exemplarily shown for Lot #1).

Titration Studies

To test the optimal coupling conditions, recombinant VZV gE antigen was serially diluted and coupled to magnetic beads. Subsequently, binding to IgG or IgM antibodies was assessed by immunoassay with a fluorescence readout in a flow cytometer.

Figure 4 shows the results of the titration experiment indicating a high titration performance of recombinant VZV gE. This data underlines that VZV gE is a well suited candidate for IgG and IgM detection in IVD assays. A concentration of 50 µg/mL was used for the subsequent experiments.

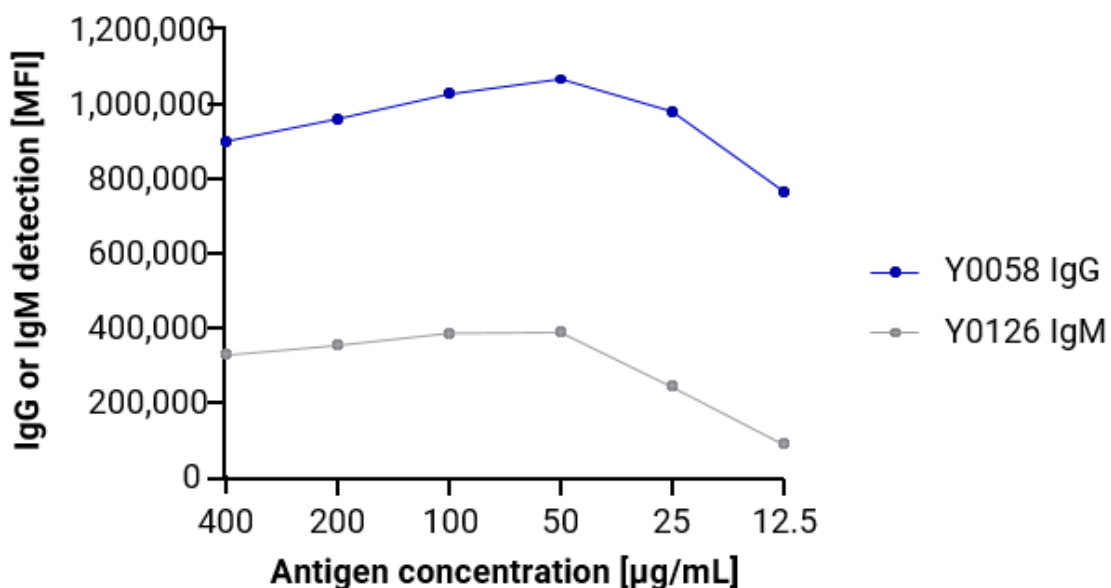


Figure 4: Recombinant VZV gE coupled to magnetic beads was titrated in 6 steps from 400 µg/mL to 12.5 µg/mL and binding of anti-VZV IgG or IgM antibodies was measured using VZV IgG (Y0058) and VZV IgM (Y0126) high positive human serum, respectively.

Lot to Lot Consistency

Binding of IgG and IgM antibodies by two independent lots of VZV gE was assessed by immunoassay with a fluorescence readout using a flow cytometer. 96 human serum samples, covering a broad range from VZV IgG or IgM negative to highly positive, were evaluated.

As shown in Figure 5, very high correlation coefficients of $r > 0.99$ could be achieved, underlining a high lot to lot consistency.

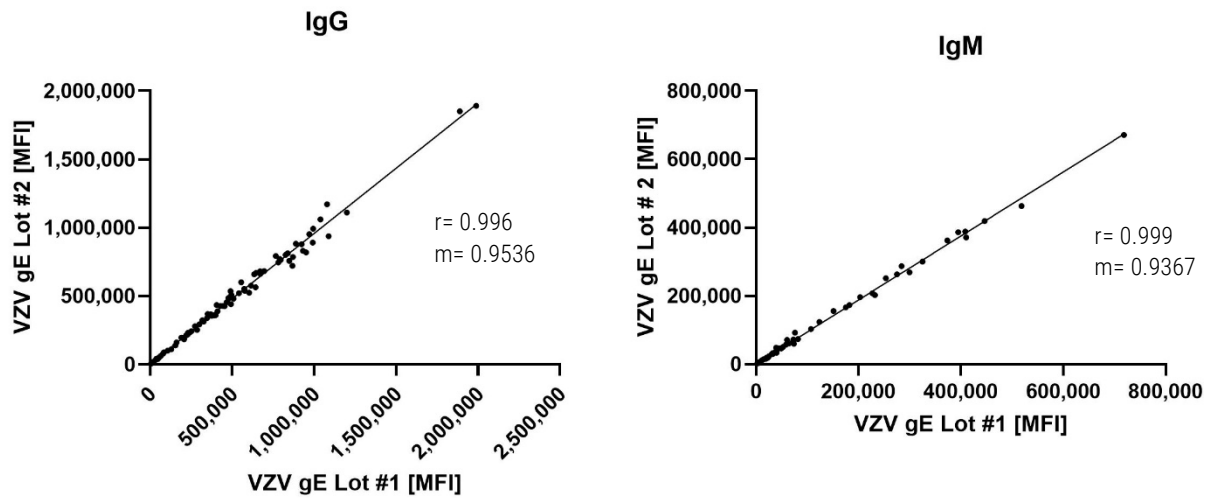


Figure 5: Comparison of different lots of recombinant VZV gE. Statistical analysis was performed using the Passing-Bablok fit. Pearson correlation coefficient $r = 0.996$ (IgG) and $r = 0.999$ (IgM) as well as slope $m = 0.9536$ (IgG) and $m = 0.9367$ (IgM).

Measurement Range

Recombinant VZV gE was coupled to magnetic particles and in a bead immunoassay setup, binding of IgG and IgM antibodies was compared to VZV glycoprotein (BA104VSG) or VZV lysate (BA104VS) (Figure 6). These results highlight an advanced reactivity of the new VZV gE antigen, which allows the development of an immunoassay with an enlarged measurement range.

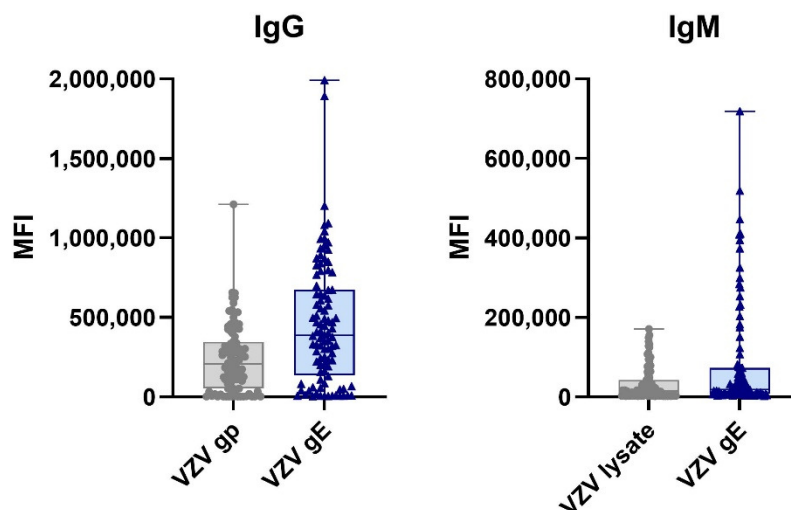


Figure 6: Comparison of VZV IgG (left) and IgM (right) antibody binding by recombinant VZV gE antigen in comparison to either VZV glycoprotein (left) or VZV lysate (right). 96 human serum samples covering a broad range from VZV IgG or IgM negative to highly positive were analyzed with the indicated immunoassay setups.

Sensitivity and Specificity of Recombinant VZV gE

Binding of VZV IgG or IgM antibodies by recombinant VZV gE coupled to magnetic particles was analyzed in the bead immunoassay in comparison to two commercially available diagnostic assay setups, a manual ELISA and a fully-automated CLIA system. Both reference assay setups are based on native viral antigens.

For VZV IgG binding analysis, 90 samples were measured with both reference assay systems. In consensus, 76 samples were determined positive. In comparison, in the bead immunoassay with VZV gE coupled to magnetic particles, one sample was defined false-positive. Additionally, 56 samples were studied for VZV IgM binding. 20 of those samples were determined positive with both reference assays. With the VZV gE assay setup, one sample was determined false-negative and three samples were false-positive in comparison to the consensus results of the reference assays. Calculated sensitivity and specificity rates for VZV IgG and IgM detection using VZV gE are summarized in the table below. The high sensitivities confirm highly efficient VZV IgG and IgM detection when the recombinant VZV gE is used instead of native antigens. False-positive rates are most likely diminishable by further adjustments of the assay.

VZV gE compared to consensus results	Sensitivity	Specificity
IgG	100 %	92.3 %
IgM	94.4 %	86.7 %

Detection of IgG Levels in Vaccinated or Previously Infected Patients by VZV gE

A clinical study was performed to test the efficient detection of IgG by VZV gE in patient samples. For this, 3 different categories of patient samples (not vaccinated, vaccinated, known zoster infection in the past) were used and binding of VZV IgG antibodies was compared between VZV gE coupled beads and Virion/Serion's VZV IgG SERION ELISA *classic* (ESR104G) as shown in Figure 7.

This data demonstrates that VZV gE efficiently recognizes IgG antibodies in vaccinated individuals or Zoster patients. The high variance in the group of not vaccinated individuals can be explained by high VZV IgG serum titers in most people/patients, as a great percentage of the population was infected with chickenpox in the childhood. Compared to the external reference assay, quantitative resolution of highly positive samples was better using VZV gE.

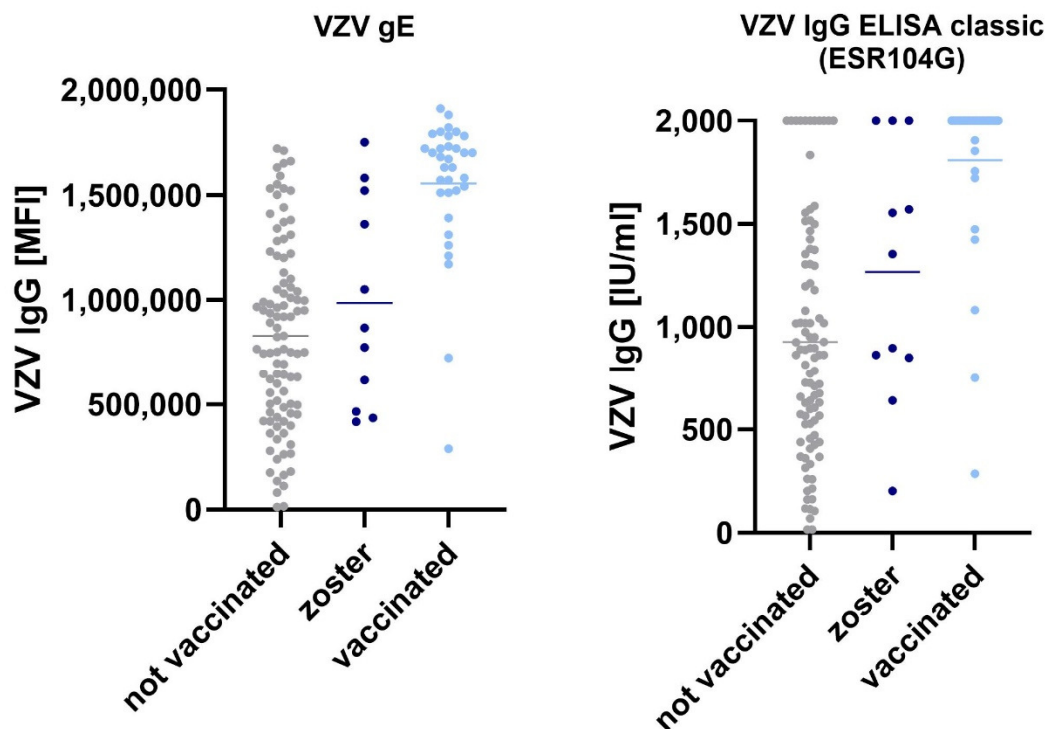


Figure 7: Recombinant VZV gE (BA104R02, left) effectively recognizes IgG antibody levels in vaccinated individuals and zoster patients compared to Virion/Serion's VZV IgG ELISA classic (ESR104G, right).

Summary

Recombinant VZV gE is a highly purified protein, which guarantees a high quality and availability. Moreover, a great lot to lot consistency and high specificity and sensitivity is achieved. It is well suited as reliable candidate for efficient IgG detection in serological IVD assays. To optimize IgM serology, VZV gE might be combined with further selected antigens. Especially the high performance on beads makes VZV gE antigen a promising candidate for diagnostic serology tools.

References

- (1) Nordén R, Nilsson J, Samuelsson E, Risinger C, Sihlbom C, Blixt O, Larson G, Olofsson S, Bergström T. Recombinant Glycoprotein E of Varicella Zoster Virus Contains Glycan-Peptide Motifs That Modulate B Cell Epitopes into Discrete Immunological Signatures. *Int J Mol Sci.* 2019 Feb 22;20(4):954. doi: 10.3390/ijms20040954. PMID: 30813247; PMCID: PMC6412795.
- (2) Olson JK, Bishop GA, Grose C. Varicella-zoster virus Fc receptor gE glycoprotein: serine/threonine and tyrosine phosphorylation of monomeric and dimeric forms. *J Virol.* 1997 Jan;71(1):110-9. doi: 10.1128/JVI.71.1.110-119.1997. PMID: 8985329; PMCID: PMC191030.