Recombinant VZV Glycoprotein B

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 Glycoprotein B (BA104R03)** and **VZV Envelope Glycoprotein E (BA104R02) 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. It is transmitted by droplets and aerosols or by contact with virus-containing vesicles or scabs. Polymorphic exanthemas with severe pruritus leading to papulation, vesicles and eschar are typical for this children's disease. In healthy children, chickenpox is usually a harmless and self-limiting

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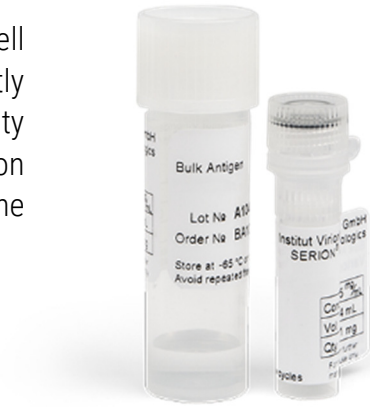
infection. However, commonly lifelong latency in cranial nerve and dorsal root ganglia is established and reactivation as herpes zoster (shingles) after decades may occur. Primary infection of adults, especially immunosuppressed patients, or newborns may be fatal and infection during gestation can lead to congenital varicella syndrome after transmission of the virus to the fetus [1]. 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, as immunocompromised patients are at increased risk of serious complications after infection or virus reactivation [2].

VZV glycoprotein B (gB) is highly expressed on VZV viral particles as well as on infected cells. **Virion\Serion's VZV gB (BA104R03)** 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 highly specific IgG, IgA and IgM detection with the new VZV gB (BA104R03).



Order Information and Related Products

Code	Description	Packaging
BA104R03 *New!*	**VZV Envelope Glycoprotein B** Envelope glycoprotein B ectodomain of Varicella zoster virus (VZV) Source: recombinant protein, expressed in insect cells Molecular weight: 81 kDa, Affinity tag: Strep-tag	1 mg
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
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

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Material and Methods

Antigen

Recombinant VZV gB (BA104R03) is produced in insect cells and highly purified by strep-tag affinity chromatography.

Immunoassay

Recombinant VZV gB antigen (BA104R03) was immobilized on magnetic beads with 5 µm in diameter, similar to those which are commonly used for CLIA. 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 detection of antibodies, antigen coupled magnetic particles were incubated with diluted human sera for 15 minutes at 37 °C and then washed. Bound VZV antibodies were detected after 15 minutes incubation at 37 °C with fluorescently labeled secondary antibody by using a Cytoflex S flow cytometer (Beckman Coulter). Reference ELISA 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 gB 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 gB 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.

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Results

Biochemical Analysis

Three independent lots of recombinant VZV gB 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 and an average hydrodynamic diameter of 16.00 d.nm.

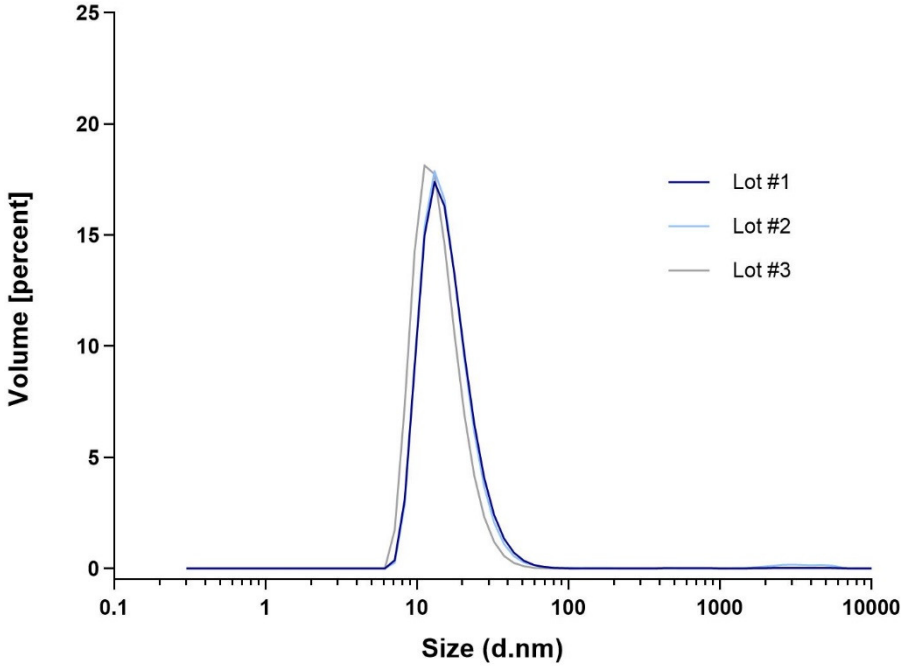
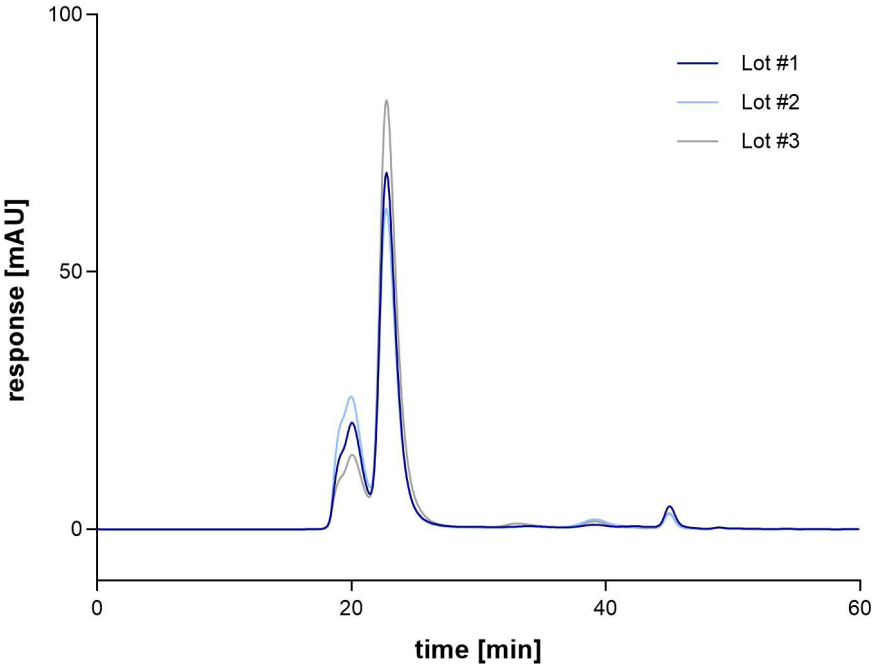


Figure 1: DLS analysis (volume distribution) of three independent lots of recombinant VZV gB. Peak overlay illustrates a high conformity. The hydrodynamic diameter of 16.00 d.nm indicates a globular protein structure.

To further confirm oligomerization, size exclusion-high-performance liquid chromatography (HPLC-SEC) was performed. As shown in Figure 2, two main populations are visible. The larger peak (right) shows the main population with an approximate molecular mass of 364 kDa. Considering the glycosylation (see glycosylation analysis below) and the calculated molecular mass (ProtParam, www.expasy.org) of the non-glycosylated monomer, these are probably trimers, since VZV gB is naturally present as a trimer [3]. The smaller peak on the left is at 683 kDa and may indicate hexamerization.



[3]

Figure 2: Analytic HPLC-SEC of three independent lots of recombinant VZV gB using a Superdex increase 200 3.2/300 SEC (Cytiva) column. Peak retention time (detection at 280 nm) shows two main populations with an average size of 683 kDa (left) and 364 kDa (right).

Glycosylation of recombinant VZV gB 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.

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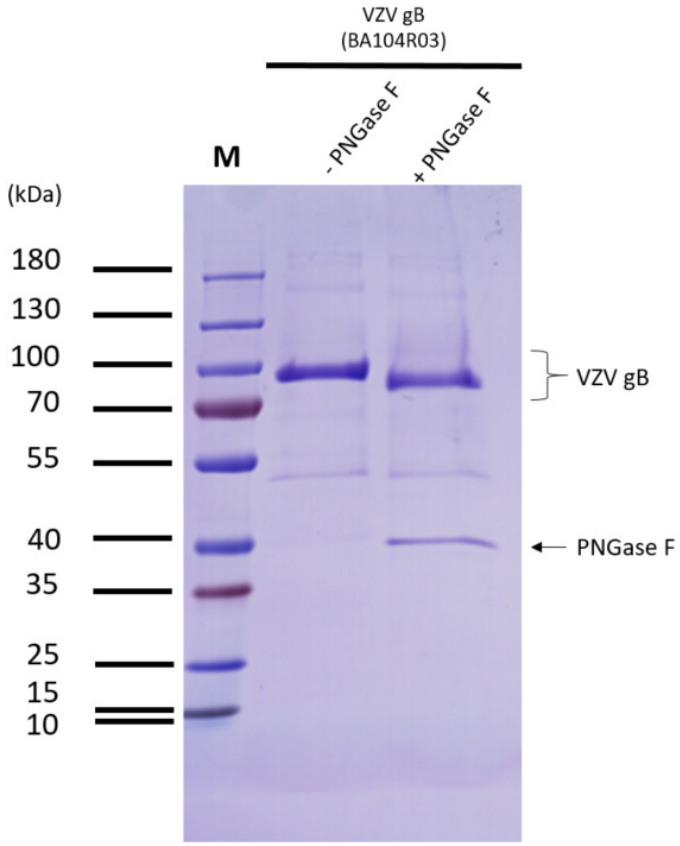

Figure 3: Analysis of glycosylation. VZV gB was incubated with 500.000 U/mL PNGase F according to manufacturer instructions, analyzed by SDS PAGE and stained with Coomassie solution. Band shift in PNGase F treated sample confirms glycosylation of VZV gB (exemplarily shown for Lot #1).

In addition three independent lots were compared by SDS-PAGE. The excellent lot to lot consistency is shown in Figure 4. In summary, these analytical methods demonstrate a high level of purity and homogeneity within the different lots.

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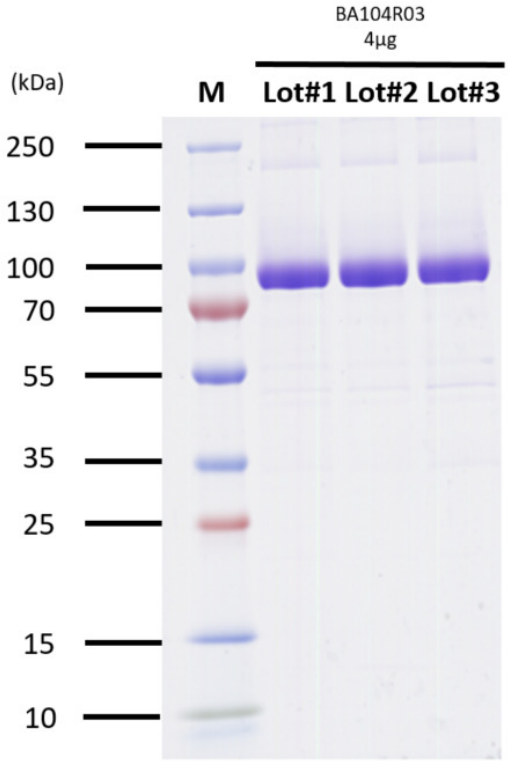

Figure 4: Comparison of three independent lots of VZV gB by SDS-Page, stained with Coomassie solution.

Lot to Lot Consistency

Binding of IgG and IgA antibodies by two independent lots of recombinant VZV gB was assessed by bead-based immunoassay with a fluorescence readout using a flow cytometer. Therefore 46 human serum samples for IgG analysis and 47 human serum samples for IgA analysis, covering a wide range from VZV IgG and IgA negative to highly positive, were evaluated.

Using Passing-Bablok regression analysis very high correlation coefficients of r = 0.99 for IgG and r = 1.00 for IgA could be achieved (Figure 5), underlining a high lot to lot correlation. The observed slopes of 1.03 for IgG and 1.05 for IgA indicate reproducible reactivity for all antigen lots (Figure 5). In summary, the high reproducibility proves excellent lot to lot consistency.

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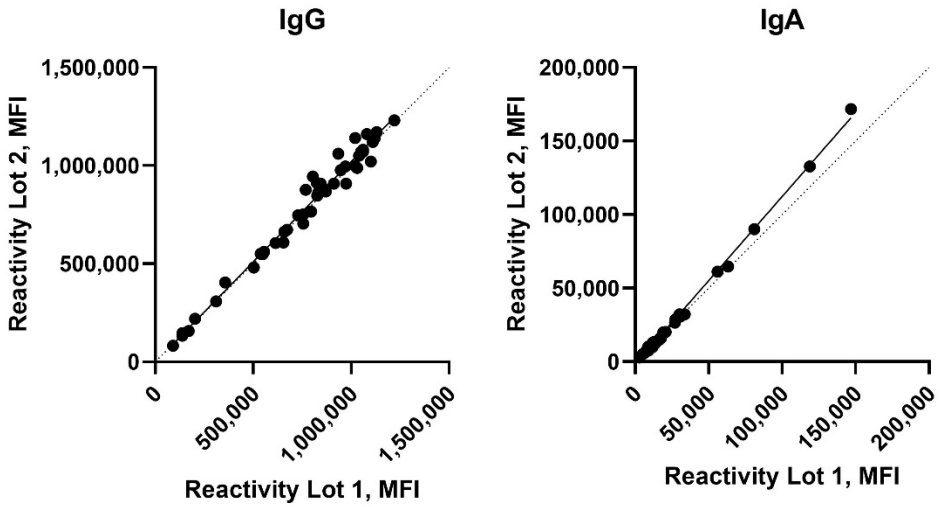

Figure 5: A comparison of two lots of recombinant VZV gB was done using an IgG and IgA bead-based immunoassay. As a fluorescent read-out was performed, the assay signals are displayed as median fluorescence intensity (MFI). The statistical analysis was performed using Passing-Bablok regression.

Reactivity and Measurement Range

In order to evaluate the reactivity of IgG, IgM and IgA antibodies towards the recombinant VZV gB antigen, human serum samples were tested using a bead-based immunoassay. A total of 153 human serum samples were subjected to IgG analysis for this purpose. IgM testing was conducted on 15 samples, while 47 samples were analyzed for IgA. Furthermore, other VZV antigens were included in the study, namely recombinant VZV Envelope Glycoprotein E (gE) (BA104R02) and native VZV Glycoprotein (gp) (BA104VSG).

The VZV gB antigen demonstrated excellent reactivity, comparable to that of VZV gE, indicating the potential superiority of recombinant antigens over native antigens. Antibodies of all immunoglobulin subclasses exhibited substantial reactivity with the recombinant VZV gB antigen (Figure 6). These findings underscore the advanced reactivity of the novel VZV gB antigen, which enables the development of immunoassays with an expanded measurement range.

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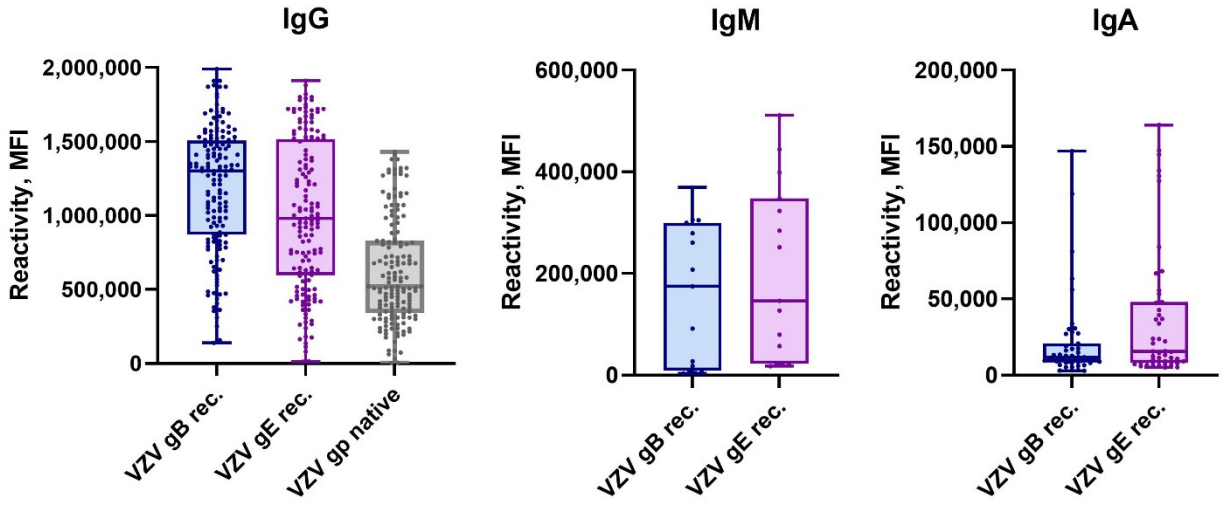

Figure 6: To demonstrate the reactivity of recombinant VZV gB in comparison to recombinant VZV gE and native VZV gp, bead-based immunoassays for detection of IgG, IgM and IgA antibodies were performed.

Sensitivity and Specificity

A method comparison was performed to assess the diagnostic efficacy of recombinant VZV gB for the detection of IgG antibodies. A bead-based immunoassay was performed on 151 human specimens, of which 149 were positive and 2 were negative. The positive and negative status of the samples was confirmed by SERION ELISA classic VZV IgG (ESR104G). The sensitivity and specificity of the method were found to be 100 % each.

Detection of IgG Levels in Vaccinated or Previously Infected Patients

A clinical study was performed to test the efficient detection of IgG by recombinant VZV gB in patient samples. For this, three different categories of patient samples, 98 from non-vaccinated individuals, 33 from vaccinated individuals and 11 from persons with confirmed herpes zoster, also known as shingles, in the past were included in the study. Furthermore, recombinant VZV gE (BA104R02) and native VZV gp (BA104VSG) were tested together with VZV gB in a multiplex bead-based immunoassay set-up.

As previously demonstrated, VZV gB displays robust IgG reactivity. In comparison to VZV gE and VZV gp, individuals with a confirmed past shingles exhibit particularly high IgG reactivity towards VZV gB. In contrast, vaccinated candidates demonstrate a markedly robust response to VZV gE. This observation is not unexpected, given that the VZV gE antigen is the immunogenic component of the vaccine used. The results for VZV gp are highly comparable to those for recombinant VZV gE, which is likely due to the fact that VZV gE is highly prominent in native VZV gp antigen (Figure 7).

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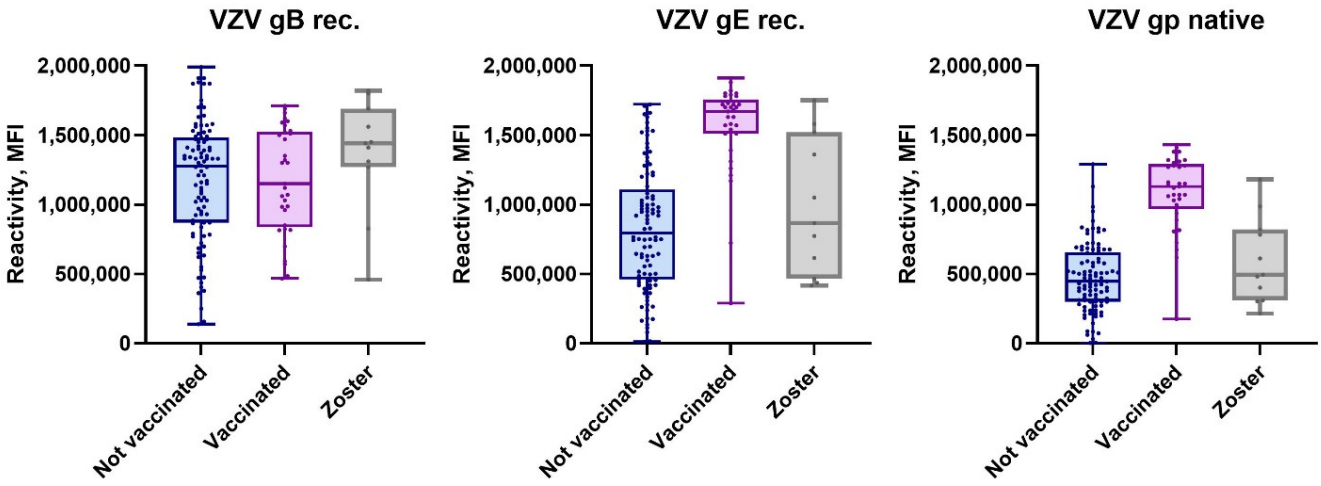

Figure 7: Multiplex serological immunoassay was performed with VZV gB, VZV gE and VZV gp for determination of IgG levels in three different categories of patient samples: non-vaccinated individuals, vaccinated individuals and persons with confirmed herpes zoster in the past.

The findings suggest that VZV gB may prove a valuable addition to VZV diagnostics, particularly for the detection of natural immunity to VZV after shingles. Therefore, the VZV data of individuals with a confirmed past herpes zoster was analyzed to see how long after the disease the antibodies remained. While IgG antibodies towards VZV gE and VZV gp decline over years, the VZV gB antibody remains (Figure 8). This shows even more that VZV gB is important for natural immunity to VZV.

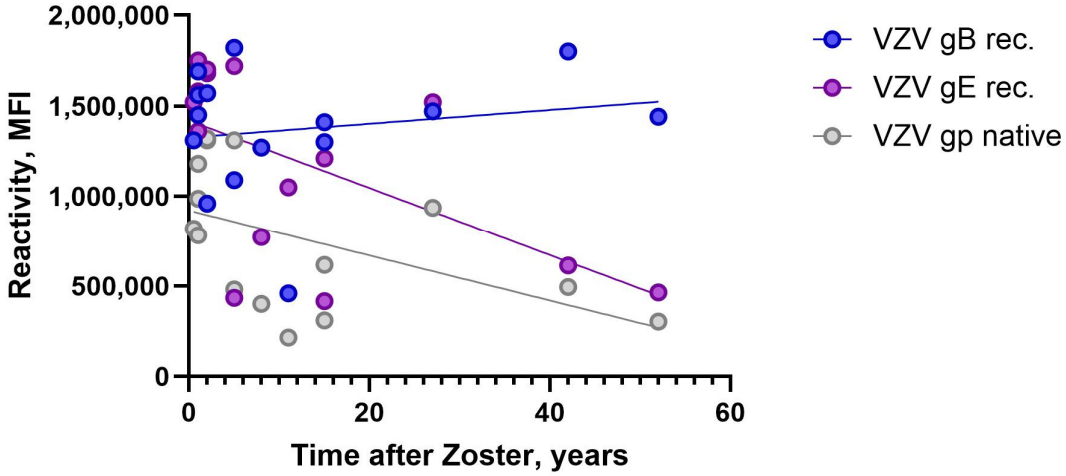

Figure 8: In individuals with a confirmed past shingles, the levels of VZV gB, VZV gE and VZV gp were analyzed with respect to time after acute herpes zoster disease.

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Summary

Recombinant VZV gB is a highly purified protein, which ensures the highest quality and availability. Furthermore, a high degree of consistency and reproducibility is achieved between lots. VZV gB enables serological assays for IgG, IgM and IgA with a wide measurement range. VZV gB appears to bring a new quality to VZV serology, in particular the ability to detect naturally acquired immunity to VZV after shingles.

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