Evaluation of three commercial enzyme-linked immunosorbent assays for determination of immune status to varicella-zoster virus

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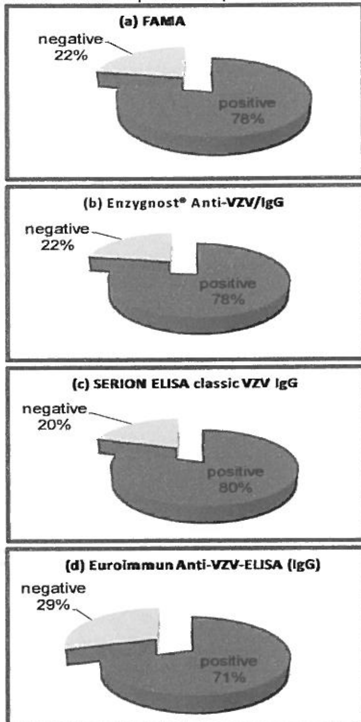
INTRODUCTION

Varicella-zoster virus (VZV) is one of the most common pathogens affecting humans. The primary infection leads to chickenpox. After endogenous viral reactivation, herpes zoster can be developed that is more prevalent in older adults and immunocompromised persons. In varicella, the incidence of reported complications is low (1), but their frequency and severity increase with age (2). In addition, varicella is a special risk for immunocompromised patients (3), and pregnant women are at risk of life-threatening maternal pneumonia or congenital diseases of the newborn (4). In individuals who are at risk of serious varicella or its complications, it is important to clarify the susceptibility to varicella and the protection against subsequent infection, both of which correlate with the presence of immunoglobulin (Ig) G class antibodies to VZV in serum (5). The fluorescent antibody to membrane antibody (FAMA) test, detecting VZV glycoprotein (gp)-specific antibodies, has been considered the "gold standard" and correlates best with susceptibility to varicella and protection against clinical varicella (6). However, FAMA is labor-intensive, time-consuming, not amenable to automation, and the inherent subjectivity of interpreting results requires extensive experience (7). Thus, there is a need of VZV commercial serologic assays allowing reliable and automated assessment of VZV-specific IgG antibodies for the determination of VZV immune status.

The objective of this study was to compare the VZV whole cell ELISA Enzygnost® Anti-VZV/IgG distributed by Siemens Healthcare Diagnostics (Marburg, Germany), the VZV protein-based ELISA Anti-VZV-ELISA (IgG) from Euroimmun (Lübeck, Germany) and the VZVgp-based SERION ELISA classic VZV IgG distributed by Virion\Serion (Würzburg, Germany). For this evaluation, FAMA was used as a reference method.

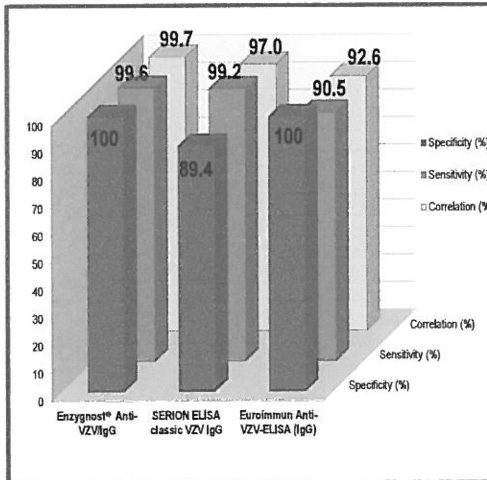
RESULTS

The **highest degree of concordance** was between the qualitative results of **Enzygnost® Anti-VZV/IgG** and the reference procedure of **FAMA** concerning the summary of 638 sera from panel 1 to panel 4.



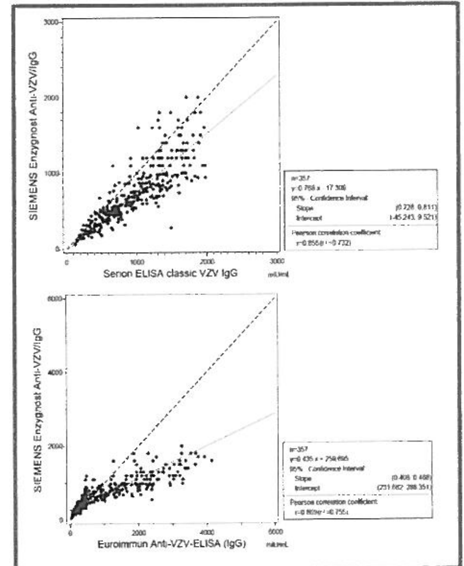
Summary of positive and negative sera as percentages tested by **(a)** FAMA, **(b)** Enzygnost® Anti-VZV/IgG, **(c)** SERION ELISA *classic* VZV IgG and **(d)** Euroimmun Anti-VZV-ELISA (IgG) under inclusion of 638 sera from panel 1-4.

The VZV whole cell ELISA **Enzygnost® Anti-VZV/IgG** and the VZV protein-based ELISA **Anti-VZV-ELISA (IgG) from Euroimmun** had **100% specificity** while 89.4% specificity was calculated for the VZVgp-based SERION ELISA *classic* VZV IgG. The **sensitivity** of the **ELISAs Enzygnost® Anti-VZV/IgG** was **99.6%** and for the **SERION ELISA *classic* VZV IgG 99.2%**. In comparison, the Euroimmun ELISA Anti-VZV-ELISA (IgG) had 90.5% sensitivity. There was **99.7% correlation** between the results of **Enzygnost® Anti-VZV/IgG** and the **FAMA** while the correlation of SERION ELISA *classic* VZV IgG was 97.0% and the Euroimmun ELISA Anti-VZV-ELISA (IgG) correlated with FAMA in 92.6% of all samples tested.



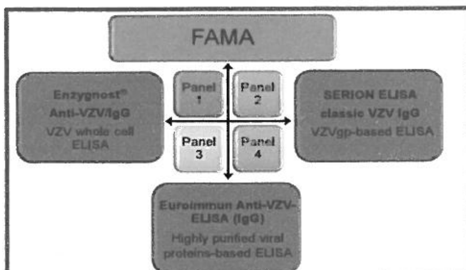
Sensitivity, specificity and correlation of Enzygnost® Anti-VZV/IgG, SERION ELISA *classic* VZV IgG and Euroimmun Anti-VZV-ELISA (IgG) on the basis of 638 sera from panel 1-4 in comparison with FAMA as reference procedure. The results were expressed as percentages.

The **VZV IgG concentrations** measured by **Enzygnost® Anti-VZV/IgG** and **SERION ELISA *classic* VZV IgG** had a **good correlation**. The Anti-VZV-ELISA from Euroimmun resulted in considerably higher values above 1000 mIU/ml. The **Pearson correlation coefficient** ranged between 0.855 for correlation between Enzygnost® Anti-VZV/IgG and SERION ELISA *classic* VZV IgG and 0.869 for correlation between Enzygnost® Anti-VZV/IgG and Euroimmun Anti-VZV-ELISA (IgG).



Comparison of quantitative results obtained by Enzygnost® Anti-VZV/IgG, SERION ELISA *classic* VZV IgG and Euroimmun Anti-VZV-ELISA (IgG) in positive sera from blood donors of the panel 2. 357 sera within the upper quantification limit of 2000 mIU/ml for the SERION ELISA *classic* VZV IgG were included. Correlation was analyzed statistically by Passing-Bablok regression.

MATERIALS AND METHODS



Study design to compare the performance of three commercial VZV IgG ELISAs

Panel 1	Panel 2	Panel 3	Panel 4
VZV-seronegative children, between 5 month and 3 years of age	Blood donors aged 19-65 years, 4-10 male and female individuals per year of age	Varicella vaccinees aged 2 to 35 years, sera were taken ≥4 to 6 weeks after one or two doses of Varilrix® or Varivax™	Sequential sera (2-4 per patient) showing seroconversions or seroreversion from 21 bone marrow transplant recipients aged 5-14 years
Number of sera: 109	Number of sera: 420	Number of sera: 57	Number of sera: 52
Total number of sera P1-P4: 638			

Panels of sera

Fluorescent antibody to membrane antibody (FAMA) test

The test was performed as in-house modification of the standard version (8) described recently (9, 10). Suspensions of human embryonic lung fibroblasts seeded into flat-bottomed micro-titer plates were infected with VZV Oka strain at a concentration of about 0.005 multiplicity of infection. The medium used was Minimum Essential Medium Eagle with 25 mM Hepes (Lonza, Cologne, Germany) supplemented with 2 mM L-glutamine (Lonza), 50 µg/ml ciprofloxacin (Fresenius Kabi, Bad Homburg, Germany), 1% non-essential amino acids 100x (Lonza), and 10% fetal calf serum (PAA Laboratories, Pasching, Austria). Cellular monolayers were fixed with 0.1% glutaraldehyde. VZV-infected cells were incubated in micro-plate cavities with the serially diluted sera. The cell preparations were covered with fluorescein isothiocyanate-labeled anti-human IgG from rabbit (Dako, Hamburg, Germany). Infected cells were evaluated using the inverse fluorescence microscope DIAPHOT-TMD (Nikon, Tokyo, Japan). Titers of ≥1:2 were considered positive.

Enzyme-linked immunosorbent assays (ELISA)

Three ELISAs were compared. The VZV whole cell ELISA Enzygnost® Anti-VZV/IgG distributed by Siemens Healthcare Diagnostics (Marburg, Germany), the VZV glycoprotein (gp)-based SERION ELISA *classic* VZV IgG distributed by Virion\Serion (Würzburg, Germany) and the Anti-VZV-ELISA (IgG), a highly purified viral proteins-based ELISA from Euroimmun (Lübeck, Germany). The test plates coated with the viral antigens were prepared with the diluted samples according to the manufacturer's protocols. All tests were performed and evaluated automatically using BEP® III system (Siemens Healthcare Diagnostics).

SUMMARY AND CONCLUSIONS

- Both the whole cell ELISA Enzygnost® Anti-VZV/IgG from Siemens and the VZV gpELISA SERION ELISA *classic* VZV IgG distributed by Virion\Serion had high sensitivity of approaching nearly 100%.
- Siemens ELISA showed an excellent specificity of 100%, but specificity of Serion ELISA was reduced to 89.4%.
- The Euroimmun ELISA had also an excellent specificity of 100%, but sensitivity was diminished clearly to 90.5%.
- The automated performance of the Enzygnost® Anti-VZV/IgG correlated best with the FAMA reference assay.
- This study does not show any superiority of commercial gp- or protein-based ELISAs compared to whole cell ELISA for detection of VZV-specific IgG.

REFERENCES

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