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Evaluation of commercial herpes simplex virus IgG and IgM enzyme immunoassays

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Serological methods are used widely for the determination of herpes simplex virus (HSV) IgG and IgM antibodies in virological laboratories. The present study evaluates the automated performance of the Virion\Serion (Würzburg, Germany) and Orgentec (Mainz, Germany) enzyme-linked immunosorbent assays (ELISA) for the determination of HSV type-common and type-specific IgG and IgM antibodies. Two hundred sixty-three sera from HSV-negative children, healthy blood donors as well as patients without and with acute HSV infections were included. The Serion ELISAs classic HSV 1 + 2, HSV 1 and HSV 2 IgG showed sensitivities between 89.1% and 98.0% and specificities from 82.8% to 100%. Sensitivities of the Orgentec ELISAs Anti-HSV-1 and Anti-HSV-2 IgG were calculated as 91.0–96.0% and 88.5–95.4% accompanied by specificities between 93.1% and 100%. The HSV type-common Serion IgM ELISA revealed also a high sensitivity and specificity. However, the single-type HSV-1 and HSV-2 IgM ELISAs from both companies did not detect reliably HSV-1- and HSV-2-specific IgM antibodies. In conclusion, the automated performance of Serion ELISAs classic HSV 1 + 2, HSV 1 and HSV 2 IgG as well Orgentec ELISAs Anti-HSV-1 and Anti-HSV-2 IgG provide highly dependable results for identifying HSV-1 and HSV-2 IgG-positive or -negative individuals. While HSV type-common IgM ELISAs can be useful to confirm acute newly acquired HSV infections, the use of single-type IgM ELISAs on the basis of whole-virus antigen is dispensable.

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1. Introduction

Herpes simplex virus (HSV) has been described as one of the most common pathogens affecting humans. Once acquired during primary infections mostly in infancy, the virus remains latent life-long in sensory local ganglia. Endogenous viral reactivations may occur frequently resulting in recurrent infections such as labial or genital herpes. Both, primary and recurrent HSV infections may lead to substantial physical and psychological morbidity. There are two types, HSV-1 and HSV-2, which show genetic differences and differ also in the way of transmission, the body site affected predominantly and the seroprevalence. The viruses are transmitted mainly by direct contact. The type 1 affects preferably the body above the waist, and HSV-2 infections occur primarily below the waist. While newly acquired HSV-1 infections occur mostly during infancy and childhood, HSV-2 infections and recurrent HSV-1 infections are observed mainly in adolescents and adults. In developed countries such as Germany, the seroprevalence of HSV-1 increases gradually in childhood and adolescence and reaches levels up to about 90% in adults (Wutzler et al., 2000; Sauerbrei et al., 2011). In contrast, a significant increase of HSV-2 IgG antibodies is observed usually with the start of sexual activity (Smith and Robinson, 2002; Sauerbrei et al., 2011). The seroprevalence of HSV-2 varies as a function of age, sex, number of life-time sexual partners and socio-economic status (Smith and Robinson, 2002) and ranges from 10% to 60% (Wutzler et al., 2000; Gupta et al., 2007). Although HSV-2 is the main cause of genital herpes, genital lesions may often harbor HSV-1 (Gupta et al., 2007).

During the symptomatic phase, the direct detection of the virus using polymerase chain reaction (PCR) and/or virus isolation in cell cultures has been considered as the gold standard for the laboratory diagnosis of HSV infections (Sauerbrei et al., 2000; Finnström et al., 2009). The domain of HSV serology is usually the determination of HSV type-specific IgG antibodies to identify virus carriers, in particular of HSV-2, transferring potentially the virus to susceptible persons (Sauerbrei and Wutzler, 2004; Bentley et al., 2012). Herpes simplex virus type 2-specific assays offer the opportunity to confirm the clinical diagnosis of previous HSV-2 infection

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without using relatively expensive and labor-intensive methods for viral detection (Bentley et al., 2012). In combination with the PCR technique, the type-specific HSV serology can also be useful to distinguish between the early phase of primary infections and recurrent HSV-2 infections (Brown, 2002). Thus, HSV type-specific serological assays may be of special value in view of the care of pregnant women and their newborns. In contrast, the significance of HSV-specific IgM antibodies for diagnosing acute HSV infections is overestimated frequently since HSV IgM can only be detected with a time delay of up to 10 days after exposure (Page et al., 2003) and can cross-react with other herpes viruses, especially with the varicella-zoster virus (VZV) (Sauerbrei, 2007). In addition, IgM antibodies may be reactive not only in newly acquired HSV infections but also in cases of asymptomatic HSV reactivation without clinical relevance (Sauerbrei et al., 2000). The latter may result in false conclusions for the further diagnostic and therapeutic approaches. Additionally, the determination of HSV type-specific IgM antibodies seems to make little sense because both virus types show a high cross-reactivity and the reliability of diagnostic IgM assays based on type-specific epitopes in a single viral protein suffers from limited sensitivity.

The objective of the present study was to evaluate the HSV type-common and type-specific IgG and IgM enzyme-linked immunosorbent assays (ELISA) provided by the manufacturers Institut Virion\Serion GmbH (Würzburg, Germany) and Orgentec Diagnostika (Mainz, Germany) for the diagnosis of acute and latent HSV infections. To this end, 263 sera from HSV-negative children, healthy blood donors as well as patients without and with acute HSV infections were included in this study.

2. Materials and methods

2.1. Serum panels

To compare the performance of different HSV IgG and IgM ELISAs, a total of 263 sera were included. All sera were taken randomly between 1997 and 2012 from healthy children between 5 months and 3 years of age, healthy voluntary blood donors aged 18 to 65 years or hospitalized patients between 14 and 70 years of age, living in towns and their rural surroundings of the federal state of Thuringia/Germany. Concerning the blood donors, informed consent for the use of their sera was obtained prior to processing samples. For children aged up to 18 years, consent was not sought. In accordance to recommendations of the Central Ethical Committee of Germany (Zentrale Ethikkommission bei der Bundesärztekammer, 2003), no patient consent is required for studies on anonymised residual diagnostic samples. In this study, the second use of sera was approved by the Ethical Committee of the Jena University Hospital, Germany (process numbers: 3233-08/11, 3670-01/13). Sera were tested utilizing reference procedures (see chapter 2.2.) for the determination of HSV-specific IgG and IgM antibodies and stored in aliquots at −20 °C without interruption. Only samples with concordant results in all reference assays were included.

According to the results of reference tests, all sera were classified into six panels which are summarized in Table 1. Serum panel 2 was used to exclude cross-reactions of HSV antigens to VZV-specific antibodies. Panel 6 consisted of 25 anti-HSV IgM-positive residual diagnostic samples from patients with symptomatic acute HSV infections such as herpes genitalis, herpetic gingivostomatitis or herpes simplex pneumonia. These sera were sent to the German Reference Laboratory of HSV and VZV for examining HSV-specific IgM and IgG antibodies between 2000 and 2012. In 11 patients, the clinical diagnosis was confirmed by detection of viral DNA using PCR or determination of viral antigens using indirect immunofluorescence antibody test (IFAT) supporting the positive IgM results. In further 8 sera, the Enzygnost® Anti-HSV IgM ELISA (Siemens Healthcare Diagnostics, Marburg, Germany) was positive, and in the remaining 6 patients with herpes genitalis, HSV-2-specific IgG was detectable. Whereas all sera (Table 1) with the exception of panel 5 were tested in the Serion ELISAs, sera of panels 1–3 were only tested partially in the Orgentec assays. The reason was that several sera were not available for both. All sera were allowed to attain room temperature immediately before ELISA testing. Antibody testing was carried out blindly in groups of up to 75 serum samples. Serum samples were tested in duplicate on different days.

2.2. Reference procedures

For the determination of HSV type-specific IgG class antibodies, the HerpeSelect 1 ELISA IgG and the HerpeSelect 2 ELISA IgG (Focus Diagnostics, Cypress, CA, United States) were used. These ELISAs licensed by the United Food and Drug Administration use recombinant glycoprotein gG-1 and gG-2 antigens, respectively. They were available as one of the first tests to determine HSV type-specific antibodies but distributed formerly by the companies Gull and Meridian. Because of robust sensitivity and specificity, these assays have been used as standard methods in many HSV seroprevalence studies (Ashley-Morrow et al., 2004; Delany-Moretlwe et al., 2010; Sauerbrei et al., 2011). The sensitivity and specificity of the HerpeSelect ELISAs for samples from sexually active adults have been shown to be 91.2% (HSV-1) to 96.1% (HSV-2) and 92.3% (HSV-1) to 97% (HSV-2) (Focus Diagnostics, 2011a,b). All tests were carried out manually according to the instruction for use (IFU). Samples were considered positive if the index value was greater than 1.1. Negative samples had an index value of less than 0.9 and those with index values between 0.9 and 1.1 were considered equivocal.

The immunoblot assay recomLine HSV-1 & HSV-2 IgG (Mikrogen, Neuried, Germany) was used for confirmatory examination of HSV type-specific IgG antibodies. This test is based on nitrocellulose membranes blotted with purified recombinant gG-1 and gG-2 as well as with proteins common to both types of HSV. The assay was performed manually taking into account the IFU. According to the manufacturer's publications (Mikrogen, 2011), the HSV-1 assay has been shown to have 99% (in routine diagnostic samples) to 100% (in samples from blood donors) sensitivity and 88.7% (routine diagnostic samples) to 94.6% (blood donors) specificity. For the HSV-2 immunoblot, the sensitivity was given as 75.0% (blood donors) to 93.8% (routine diagnostic samples) and the specificity as 92.5% (routine diagnostic samples) to 100% (blood donors).

For semi-quantitative detection of IgM class antibodies to HSV, a modification of the IFAT was used as described previously (Sauerbrei et al., 2000). In short, before measuring IgM, serum samples were pretreated with anti-human IgG (BAG Health Care, Lich, Germany). Sera diluted initially 1:20 were incubated with HSV-2 US strain-infected HEp-2 cells grown on microscopic glass slides. Non-infected HEp-2 cells were used as controls to exclude anti-cellular antibodies. Fluorescein-labelled anti-human IgG or IgM from rabbit (Dako, Hamburg, Germany) served as conjugates. Sera with titers of ≥1:20 were considered IgM positive. In addition, this modified IFAT was used for the determination of VZV-specific IgG antibodies. Varicella-zoster virus prototype Oka strain-infected A549 cells grown on glass microscope slides served as antigens (Sauerbrei et al., 1999), and titers of ≥1:5 were regarded as positive. This test has been recommended as alternative to the fluorescent antibody to membrane antigen assay for the determination of VZV immune status (Sauerbrei et al., 2004).

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