**ELISA for detection of HSV-2 specific IgM antibodies to glycoprotein G(gG-2)**

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**Abstract:**
Genital herpes is a sexually transmitted disease (STD) caused by the herpes simplex viruses type 1 (HSV-1) or type 2 (HSV-2). Most genital herpes is caused by HSV-2. In United States, 16.2% of people between 14 and 49 years of age have genital HSV-2 infection. HSV-2 is primarily transmitted sexually. Entry of HSV into the host cell involves interactions of several glycoproteins on the surface of the enveloped virus, with receptors on the surface of the host cell. The glycoprotein G(gG-2) purified from HSV-2 infected cells has been reported to be useful for determination of HSV-2 type-specific antibodies using conventional ELISA formats. The studies have also confirmed the specificity of the gG-2 and validated the feasibility of a specific IgM assay. This enhancement could be helpful in making ELISA kits that would distinguish between HSV-1 and HSV-2 on the basis of their unique glycoproteins. The idea is recalled from the paper “Indirect ELISA for the detection of HSV-2 specific IgG and IgM antibodies with glycoprotein G(gG-2)” published in *Journal of Virological Methods* (1992, 249-264) in 1991.

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**Method:**

Panel of serum that has been collected over the years for various purposes was used for all the testing. Serum samples in the panel chosen for “Standards” were positive for some kind of HSV. Serum samples in the panel chosen for “Negatives” were negative for some kind of HSV. Random assorted samples were chosen as “Normals” for testing on ELISA and IFA kits.

The combinations that showed a significant separation were tested again with more standard, negative and normal sera for determining a cut-off.

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**Conclusion:**

11 samples were positive on Indirect HSV 1&2 Combined IgM kit, which were then tested on individual HSV-1 IgM and it had 9 out of 11 samples as false positive, and on HSV-2 IgM, all 11 samples as false positive. It is possible that the samples were most likely not positive but in-house kit absorbed the background material that was not supposed to. In-house kits were more specific than Indirect but “Capture” kits are more reliable than the indirect ELISA due to their specificity because while capturing IgM, conjugate is more specific to a particular HSV-type.

So from ELISA and IFA results, it looks that individual “Capture” assay was more specific than Indirect HSV 1&2 combined. After confirming those results on IFA, samples showed non-specific binding in IFA while eliminated it on Capture assays. The samples from serum panel which were “Negative” showed false positive results on In-house indirect ELISA and were negative on Capture assays. For HSV-1 IgM, 14 out of 47 samples presented false negative results on HSV-1&2 Combined In-house kits. False negative results may also occur when the infecting virus is gG deficient.

A false negative value in IFA and ELISA may have occurred due to presence of virus-specific IgG which competes with the IgM for sites or presence of rheumatoid factor.

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**Future Work:**

Next step would be using Western Blot for characterization of HSV-1 and HSV-2 type-specific glycoproteins, according to their apparent molecular sizes. Further investigation can be done by getting confirmed samples along with the patient’s clinical evaluation and other diagnostic procedure.