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. Random samples from serum panel were chosen for testing at different antigen dilutions across different conjugate dilutions. IFA was run on some suspicious sample after ELISA. In-house kit assay were compared to "Capture" assay where there were many false positive results.

Introduction:

- The goal of the project is to make an ELISA kit for the detection of HSV-2 specific IgM antibodies with glycoprotein G(gG-2) by having a cut-off value below all the possible positive HSV-2 serums to avoid false negative results.
- The aim is to use purified recombinant gG-2 for antigenic coating of the ELISA plates which could be used for the early detection of HSV-2 specific IgM to gG-2 and, verify the sensitivity and specificity by Immunofluorescence assay (IFA).
- The locations of the type-specific glycoprotein G of HSV-2 carries mostly type-specific antigenic epitopes which could be very helpful in determining type-specific serologic tests.

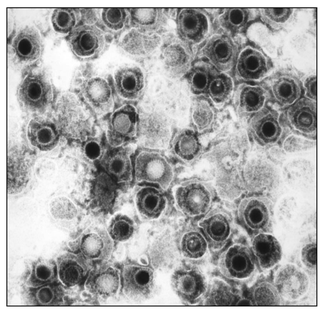


Figure 1: Negatively-stained transmission electron micrograph (TEM) of numerous herpes simplex virions. At the core of its icosahedral proteinaceous capsid, the HSV contains a double-stranded DNA linear genome. *http://phil.cdc.gov/phil/details.asp*

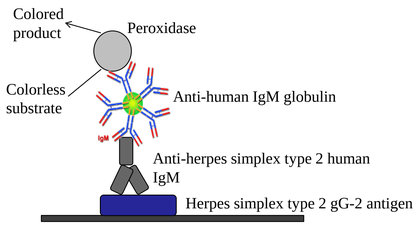


Figure 2: Principle of an indirect assay

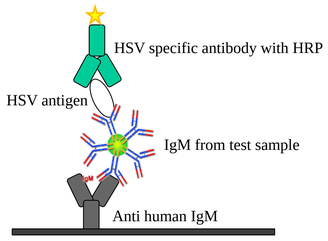


Figure 3: Principle of a "Capture" assay

- It is important to examine the enhancing effect of this specific glycoprotein G (gG-2) to detect IgM antibodies produced against HSV-2 for the diagnosis of HSV-2 in neonatal herpes and primary genital herpes, when cultures or rapid diagnostic techniques are unavailable.

Method:

Serum Diluent

Prepared Serum Diluent with Goat Anti-IgG Raw Absorbent (Serum Pretreatment) for diluting serum samples to be used in antigen coated plates.

Sample Investigation

Using Capture ELISA and an alternative indirect ELISA kits to confirm the specificity and sensitivity of the already manufactured HSV1 and HSV2 IgM kits.

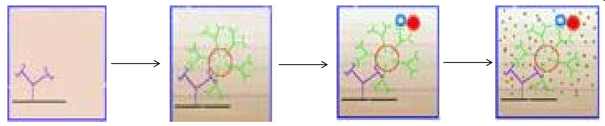
Hand coated 20 microtitre plates each with HSV type specific antigen, gG-1 and gG-2 (recombinant) at different dilutions.

gG-1
(2 plates each)
1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200, 1:6400, 1: 12800, 1:25600, 1:51200

gG-2
(2 plates each)
1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200, 1:6400, 1:12800, 1:25600, 1:51200

IgM Conjugate Dilution

Prepared different IgM conjugate (Peroxidase-conjugated AffiniPure Goat Anti-Human IgM) dilutions : 1:40, 1:160, 1:400, 1:800, 1:1280, 1: 1600, 1:2560, 1:3200, 1:5120, 1:6400 , 1:9000, 1:10240



Capture ELISA steps
(http://www.diaproph.com.ua/eng_product_test_system_5.htm)

ELISA

Tested confirm positive and negative serum samples with different antigen and conjugate dilution combinations to see which combination gave considerable separation of samples as they react with type specific antigen on the plate.

ELISA test for determining cut-off

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

IFA

Discordant samples which gave a positive Index value on the above ELISA were tested on Indirect Immunofluorescence Assay(IF) for qualitative and semi-quantitative detection of IgM antibody to HSV-1 and HSV-2.

ELISA Results

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.

Table 1: HSV-1&2 Combined IgM

In House \ Indirect	+	-
+	11	1
-	14	21

Result from testing 47 samples on In-house as well as "Capture" HSV-1&2 Combined IgM kits.

Table 2: HSV-1 IgM

In House \ Capture	+	-
+	2	9
-	0	0

Result from testing 11 samples on In-house as well as "Capture" HSV-1 IgM kits.

Table 3: HSV-2 IgM

In House \ Capture	+	-
+	0	11
-	0	0

Result from testing 11 samples on In-house as well as "Capture" HSV-2 IgM kits.

Relative Sensitivity and Specificity

Type	Relative Sensitivity	Relative Specificity
HSV Combined (Indirect)	91.67%	60%
HSV-1 (Capture)	81.82%	N/A
HSV-2 (Capture)	0%	N/A

IFA Results

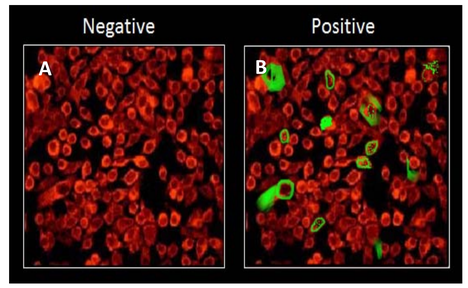


Figure 4: Procedure uses SCIMEDX 10-well HSV slides, serum or plasma samples, FITC-conjugated goat antihuman IgM(μ class) w/ Evans blue & Rhodamine counterstain(2nd antibody) and Mounting Media. Fig. (A) shows negative HSV sample; Fig. (B) shows positive HSV sample. Green staining cells indicate presence of HSV antigens. *http://www.abcam.com/ps/datasheet/Images/32/ab32037/ab32037IFA.jpg*

Table 4: HSV-1&2 Combined IgM

IFA \ Capture	+	-
+	3	0
-	13	0

Result from 16 samples on IFA as well as "Capture" HSV-1&2 Combined IgM kit.

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 it 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" assay.
- 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

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.
- Blocking and washing step will be refined.