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RESEARCH ARTICLE

Development of Rapid Flow through Immunoassay using Recombinant Herpes Simplex Virus - 2 Glycoprotein G (gG) Antigen

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ABSTRACT

Serum panel were divided in five sub groups, High reactive sample, medium reactive samples, low reactive samples, borderline samples and negative samples. Spotting of the antigen for different concentration starting from 5 nano gram (ng.) per spot to 500 ng per spot was carried out in D.H (Dehumidifier room).Then the HSV panel was tested and according to the results obtained, concentration (150 ng./ spot) was standardized as the test spot because the intensity of this spot was same as the higher concentrations. At 150 ng. per spot the spot intensity was +7 for high reactive sample as compared with the reference standardize spot provided by Span. The signal reagent was also standardizing starting from 3 O.D to 6 O.D. The 4 O.D colloidal gold-protein "A" was sufficient to give positive high intensity colored spot, and it was standardized as final concentration. The negative samples were completely negative till the antigen concentration of 500 ng. per spot on test device. 202 Normal Human Plasma samples were analyzed for HSV-2 Ig G antibody, KAPPA Statistical analysis show, when compared with ELISA test show Sensitivity 100 % and specificity 98.5%. Positive predictive value 70% and Negative predictive value 100%.The results obtained are excellent for the detection of seroprevalence of HSV-2(Genital herpes) infection. The main feature of this test is its rapidity to detect HSV specific antibody mainly Ig G, to recombinant antigen gG.

KEY WORDS: *HSV-2 Ig G antibody, Recombinant antigen gG, Colloidal gold-protein "A".*

INTRODUCTION

Herpes simplex virus is ubiquitous, infecting the majority of world's population, early in life and persisting in latent form from which reactivation with shedding of infectious virus occurs, this maintains the transmission chain [1]. Some of the membrane glycoprotein have common antigenic determinant shared by HSV-1 and HSV-2 whilst HSV-2 has specific determinant for one type only membrane glycoprotein g (gG) [1,2]. It is estimated that over 50 millions people in United States have genital herpes [3]. A 30 % increasing in the seroprevalence of HSV-2 infection in this country over the past two decades demonstrate that the epidemic continues unabated [4]. Type specific glycoprotein, gG serological assay include Western blotting [5,6] Immunological strips [7], and Enzyme linked immunosorbent assays (ELISAs) [8,9,10].Although The Western blot is considered to be the "gold standard," It is expensive and labor intensive and its interpretation is operator dependent [11]. The main objective of present study is to establish a rapid diagnostic test kit that essentially and rapidly detects the seroprevalence of HSV-2 (genital herpes infection) in India, that is a great need to prevent the epidemic of genital herpes and it is also one of the step to prevent the S.T.Ds that is very common among sex workers. It was of particular interest to explore the usefulness of type specific antibody testing of a recently described HSV-2 specific glycoprotein, gG-2 [12].

MATERIAL AND METHODS:

Antigen Used

Recombinant glycoprotein (gG) HSV-2 antigen

Preparation of Colloidal gold

Gold chloride(HAuCl4) was procured from Amresco, Ohio, U.S.A. Colloidal gold with an average diameter of 25-30 nm was prepared by controlled reduction of a boiling solution of 0.02% chloroauric

acid with 1% Sodium citrate according to method of Frens [13]. Briefly, 50 ml. of 0.02% chloroauric acid in doubled distilled water was boiled. As soon as it started boiling, 1.2 ml. of 1% sodium citrate solution was added with constant stirring. The colour changes from grey to blue to purple to violet to full red within 60 second. After the colour change, heat was turned off. The solution was stirred for 5-10 minutes and then allowed to cool at room temperature away from light. The gold sol obtained by this method consisted of gold particles with diameters between 25-40 nm, as verified by electron microscopy. The batch having maximum absorbance between 525-530 nm and $A^{1cm}527$ nm = 2.0±0.05 was used for preparing the conjugate with Protein A.

Conjugation of Protein A to colloidal gold: To 1 ml. of colloidal gold sol, 5-10 micro liter of freshly prepared 1% K_2CO_3 solution was added to adjust the pH of the sol to 6.5. To this 15-20 mg of Protein A were added under stirring. It was incubated for 5-10 mins at room temperature and was centrifuged for 5 mins. at 5,000 rpm. 950µl supernatant was discarded and the pellet was resuspended (without washing) in the remaining 50 µl of supernatant. 5 µl of conjugate was added in each vial and lyophilized in individual vial, which can be stored for six months at 4º C.

Rapid flow through Immuno-assay Test

The Rapid flow through Immuno assay test was developed in association with Span diagnostics, India, with whom a confidentiality agreement was done for commercialization.

1) Test device: The test kit is developed with the glycoprotein G (gG) antigen using the Immunofilteration test device. The flow through device comprises of the polystyrene top and bottom layers of the discs with an absorbent pad (2.5 mm thick) above which nitrocellulose membrane (9 mm diameter) was embedded. Dimension of test device Top (51.4 × 38.45 × 5) mm, Bottom (51.2 × 38.45 × 7.5) mm, Inlet hole diameter, (external 14.5 mm, internal 10.3 mm). The device was assembled using a plastic welding machine (Imeco-Ultrasonic). The different concentration of recombinant antigen (gG) was coated starting from 5 nanogram/0.3 µl spot to 500 nanogram/0.3 µl. spot. The coating was done in D.H (Dehumidifier room).The device was dried in a 37º C incubator for 48 hours. The test devices were than packed in individual pouches containing desiccant.

2) Wash buffer: 10 mM Phosphate buffer saline containing 2% BSA+ Tween 20+Protein stabilizer + sodium azide.

3) Capture reagent (Colloidal gold - Protein A Conjugate)

4) Serum Panels: Positive control panel (high reactive sample, medium reactive sample, low reactive sample, borderline reactive sample, negative sample). Normal human plasma (N.H.P) 202 samples collected from SRK Blood bank, Surat

Assay procedure

Test device was labeled with Sample identification code & keep the test device on to a horizontal surface. Add (100 µl) of wash buffer to the centre of the device and allow soaking in. Add (100 µl) of serum/plasma sample using the disposable plastic dropper and allow to soak in. Add (100 µl) of wash buffer again and allow to soak in. Add 2 drops (100 µl) of Capture reagent and allow to soak in. Add 150 µl of wash buffer again and allow soak in. Observe the spot intensity of antigen at their designated region. Read the result after 2 minutes for interpretation.

Interpretation of result

The control spot containing goat anti human IgG antibody serves as in built positive control gives positive reaction (red magenta colored spot at the control area) after completion of test. The test spot contains the recombinant antigen (gG) develops red magenta spot when there is detectable IgG antibody is present in serum. Thus two red magenta spot at both side show positive reaction and vice versa.

RESULTS

Standardization of antigen concentration to be coated on Test device: As shown in figure1, the recombinant antigen concentration spotted on Nitrocellulose membrane filter paper starting from 5 ng./spot to 500 ng./spot the antigen 150 ng./0.3 µl was standardized to coat on nitrocellulose membrane filter paper of the test device.

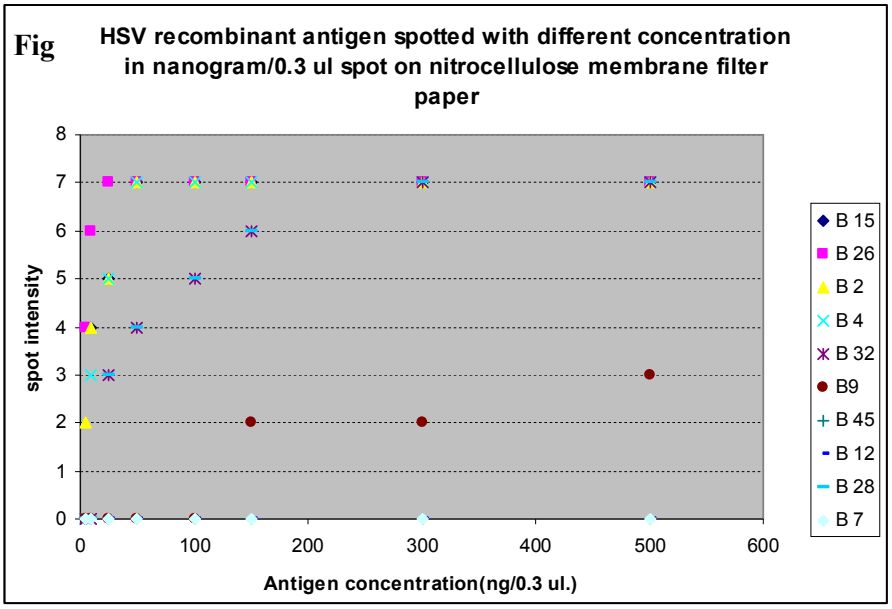
Standardization of Capture reagent (Colloidal gold-Protein A conjugate): As shown in figure 2, different O.D starting from 1 to 5 were used to observe the ideal spot finally 4 O.D is sufficient to capture antibody IgG for detection of colour spot.

Kappa and 95% C.I. Statistical analysis were done for Sensitivity, Specificity and Positive predictive value and negative predictive value after comparisons with Immunowell ™ ELISA test. The results are shown in table 1.

DISCUSSION

Sexually transmitted diseases are more prevalent all over the world. Herpes simplex virus is one of the TORCH panel organism and infection in pregnancy accounts for half of the morbidity and mortality among neonates. The seroprevalence of HSV-2 has increased by more than 30% over the past two decades it is seen that most seropositive individuals are unaware that they are infected to Herpes infection. Demographic and behavioral factors are associated with high risk of genital herpes infection. Approximately one in five Americans is infected with genital herpes and 600,000 new cases occurs annually in America [14,15]. Definitive diagnosis of genital herpes infection is fundamental to the management of patients and the development of strategies to prevent transmission to partners and neonates.

To establish a rapid diagnostic test kit that essentially and rapidly detect the seroprevalence of HSV-2 (genital herpes infection) in India, That is a great need to prevent the epidemic of genital herpes and it is also one of the steps to prevent the STDs that is very common in sex workers.



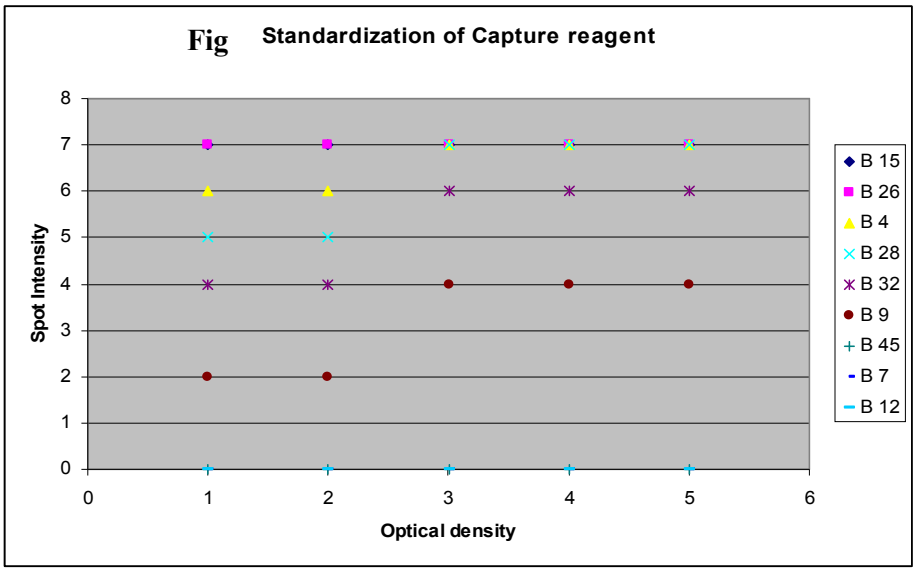


Table 1: KAPPA & 95% C.I. statistical analysis for Sensitivity & Specificity

Total No of sample (n)	**209**			
	ELISA			
Rapid Flow Through	Negative	Positive	Total	
Negative	199	0	199	
Positive	3	7	10	
Total	202	7	209	
Kappa statistic	0.82			
		95% C.I		
Sensitivity	100.0%	59.0%	to 100.0%	
Specificity	98.5%	95.7%	to 99.7%	
Positive predictive value	70.0%			
Negative predictive value	100.0%			

(Total 202 Normal human plasma were run and seven positive sample) n=209.

The Western Blot Test is the gold standard for researchers with accuracy rates of 99% but it is expansive and time consuming.

Immuno Flow through assay was selected as the ideal test. SPAN DIAGNOSTICS LTD. company has provided me the recombinant antigen, concentration 1 mg/ml., Colloidal gold - protein A conjugate and Assembled test device, the quality of nitrocellulose membrane and adsorbent pad is already standardize by Span Diagnostic company, and these all matter are confidential.

The recombinant antigens may be obtained from baculovirus HSV-1 gG (glycoprotein G) and E. coli, HSV-2 gG recombinant antigen. The antigens can also be obtained from the virus culture from the Simian kidney cells, partially purified protein can be obtained from HSV-1 (Macintyr strain) and HSV-2 (G strain). The test device consists of in-built positive control marked as “C” and test spot marked as “T”.

First of all I standardized the antigen concentration, for this the serum panel already checked by ELISA was taken and this serum panel was divided in five sub groups, High reactive sample, medium reactive samples, low reactive samples, borderline samples and negative samples. Spotting of the antigen for different concentration starting from 5 nanogram (ng.) per spot to 500 ng per spot was carried out in D.H(Dehumidifier room).Then the HSV panel was tested and according to the results obtained, concentration (100 ng./ spot) was standardized as the test spot because the intensity of this spot was same as the higher concentrations. at 100 ng. per spot the spot intensity was +7 for high reactive sample as compared with the reference standardize spot provided by Span. the signal reagent was also standardize starting from 3 O.D TO 6 O.D. The 4 O.D colloidal gold-protein “A” was sufficient to give positive high intensity colored spot, and it was standardized as final concentration. KAPPA & 95% C.I. statistical analysis for Sensitivity & Specificity show 100 % Sensitivity and 98.5 % sensitivity. 95 % C.I show 59.0 to 100 % sensitivity and 95.7 % to 99.7 % specificity.

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