Serodiagnosis of Herpes Simplex Virus-1 Infections by a Prepared Antigen

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Summary

For diagnosis of Herpes simplex virus (HSV) primary infections by use of complement fixation test, the HSV-CF antigen was prepared. Virus was isolated from herpetic lesions on lips and confirmed by electron microscopy and specific antisera. The results confirmed correct HSV isolation. The isolated virus inoculated to susceptible cell line-Vero-with a high infections dose (MOI=0.1) and incubated at 33ºC. When complete CPE appeared, supernatant harvested as source of antigen. 93 serum samples were tested by complement fixation test (Kolmer method) using both prepared and imported antigens. The specificity and sensitivity of the test using prepared antigen in comparison of imported one were 100% and 84.7%, respectively. Because of we prepare the antigen using only one of two serotypes of the virus, in some cases that antibody directed against other serotype in patient's serum, false-negative results occur. This led to reduction the sensitivity of test by homemade antigen.

***Key words*:** Herpes simplex, complement fixation test, antigen

Introduction

Herpes simplex virus is an important member of Herpesviridae family. This virus can cause various infections from a mild fever blister to sever visceral infections and lethal encephalitis. One of the most important properties of this virus is the ability to establishment of latent infection in nerve cells and then, recurrent infection under

some stimulator conditions such as stress, UV, trauma, pregnancy, cold shock and hormonal changes. Virus is widespread in the world and transmission occurs by direct contacts such as kissing and sexual contact. Despite of most viral diseases, there are some effective drugs against herpes virus infections. Thus, early diagnosis and beginning to treatment may prevent the progression of infection to life-treating conditions such as Herpes simplex encephalitis[HSE] (Bernard 1996, Colee *et al* 1989). One of the routine methods for diagnosis of primary HSV infections is the measurement of HSV-specific antibody in sera by a complement fixation test (Carlton 1952, Grist 1974, Habel & Salzman 1969, Martines 1985).

Presently, the HSV-CF antigen is imported from foreign companies. In this research, we tried to prepare the antigen and compare with imported one in specificity and sensitivity aspects.

Materials and Methods

Cell culture preparation. Vero, HeLa and MRC-5 were prepared in Roux bottles. 100ml of cell suspension containing 10 cells per ml dropped in each Roux bottle. For preparation of MRC-5 cell culture 8% v/v calf serum and for cell cultures 5% v/v were added to BME and DMEM media, respectively. After formation of monolayer, the media removed and replaced with a serum-free medium.

Virus isolation. Vesicle fluid of fever blister lesions transferred to virus transport medium (VTM) and then inoculated to each of the cell culture. After 24-48h CPE appeared and a suspension of infected cells was prepared and evaluated by electron microscope and microneutralization test. The results confirmed the HSV strain MK#3 was isolated.

Preparation specific antisera in animal. MK#3 strain of HSV was cultivated in MRC-5 cell(MOI=0.1)and harvested when maximum CPE appeared. Cell debries also removed by centrifugation and 2ml of supernatant was injected into the rabbits subcutaneously three times with one-week interval. In the fourth week rabbits were bled. The potency of complement fixation test was evaluaterd by kolmer method. The

results suggested that the potency of complement fixing antibodies is 1:80, so this antisera used as positive reference throughout the experiments.

preparation of HSV-CF antigen. 5ml of suspension of infected cells with MOI=0.1 were inoculated to each of three cell cultures and incubated at 33ºC. When complete CPE appeared, antigen was harvested in four different manners: 1) Harvesting of culture media after shaking. 2) Trypsinization of infected cells after removing of spernatant, mixing them after washing of the cells, centrifugation in 1500 rpm for 5 min, resuspension of packed cells with PBS and finally sonication in 10-12 Amplitude Microns for 50 seconds. 3) Mixing of the antigen, which prepared in above manner with 5%v/v chloroform, keeping at 4ºC for 24h, centrifugation in 3000 rpm for 15min and then harvesting of supernatant. 4) Keeping of Roux bottles in -40ºC for one week, thawing and shaking them and finally harvesting of the cell-supernatant suspension.

All kinds of prepared antigens were used in CF test with prepared (reference) antisera. Here after many experiences, Vero cell line selected for production of antigen.

Reproducibility of prepared antigen. CF test was carried out against prepared antigen on 10 serum samples, which were selected randomly four times with the same batch of sRBC and complement.

Complement fixation test. 93 serum samples from healthy and HSV-infected persons were tested with prepared and imported (Orion company, Finland) CF antigen. Micro CF test was carried out with Kolmer method. 2 complete units of antigen and sensitized sheep RBC were used. 1:20 and lower titers and, 1:40 and higher of anti-HSV antibody considered as negative and positive, respectively.

Results and Discussion

The result of comparative study of different cell lines/strains for preparation of HSV-CF antigen summarized in table 1. It suggests that Vero and HeLa are better to use for preparation of antigen.

Table 1. *Evaluation of all 8 prepared antigens*

Method of Ag harvesting	**1**		**2**		**3**		**4**	
Cell culture type	Vero	HeLa	Vero	HeLa	Vero	HeLa	Vero	HeLa
Titer	1:64	1:16	1:64	1:32	1:8	1:4	1:64	1:32

Reproducibility test. The results suggest that the prepared antigen is reproducible (Table 2).

Table 2. *Reproducibility test on prepared antigen*

No.test	**CF- titer of 10 serum samples**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	1:80	1:40	1:80	1:20	1:80	1:80	1:20	1:80	1:40	1:40
2	1:80	1:80	1:40	1:20	1:40	1:80	1:20	1:80	1:40	1:40
3	1:80	1:40	1:40	1:20	1:40	1:80	<1:20	1:80	1:40	1:40
4	1:80	1:40	1:40	1:20	1:40	1:80	1:20	1:160	1:40	1:40

Complement fixation test. From 93 serum samples, which evaluated by CF test using prepared antigen 72 sera were positive and 13 were false negative. By imported antigen of those, 80 sera were positive and 8 were false negative. So, the specificity and sensitivity were calculated 100% and 84.7% for prepared and, 100% and 90.9% for the other antigen.

Based on specificity and sensitivity, the prepared antigen was acceptable in comparison of imported one. But there are some false-negative results that lead to reduction of sensitivity. The CF-antigen of two serotypes of HSV (HSV-1 & HSV-2) is similar but not identical, and in a few cases this differences are wider. On the other hand, our antigen prepared from only one of two serotypes of HSV. Thus, in such cases the antigen cannot detect the antibody that produced against other serotype and false-negative results occurred. We believe that if a new antigen makes using other serotype of HSV by the same manner, and mix with the recent antigen, false-negative results will reduce and sensitivity will increase.

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