Preparation of a Dual-Purpose Cell Culture Mumps Virus Antigen for HI and CF Tests

A. Mohammadi, H. Mirshamsy, A. Shafyi, J. Razavi, M. Rahmani, A. Sassani, Z. Hamzehloo and S. Nasiri

Section of Biologics, Razi Vaccine and Serum Research Institute, PO Box 11365-1558, Tehran, Iran

Summary *A dual-purpose antigen (haemagglutinin and complement fixing antigen) was produced by a local mumps virus strain (S#12) in cell culture. The suspension was clarified by low speed centrifiugation.*

In most cases, the results of CF and HI tests using this virus antigen correlated with each other.

Introduction

In spite of recently developed methods for measurerment of mumps antibodies, complement fixation (CF) test is still one of the most specific and sensitive methods. The considerable quantities of complement fixing antigen in glandular suspension was shown to specifically react with sera of monkeys that had recovered from an attack of mumps(1).

Following the adaptation of mumps virus to the chick embryo by Habel(2) and others(1,3), the antigen was found in suspension of the allantoic(1) and amniotic membrane(1), yolk sac(2,3) as well as in the allantoic and amniotic fluid(1,2).

Pereira and Valentine(4), Wilcox and Ginsberg(5) and many others showed that cell-associated infective virus chould be concentrated by slow speed cell sedimentation prior to release from the virus bearing cells, others used this procedure to concentrate neo-antigens.

In this paper we have described the production of cell culture mumps

antigen which can be used in CF as well as in haemagglutination inhibition (HI) test.

Material and methods

Cell culture: A continous line of African green monkey kidney cells, designated Vero YY, (received from Dr. Makino, Kitasato Inst. Japan) was used. The growth medium consisted of Eagle's Basals Medium (EBM) supplemented with 5% newborn calf serum plus 100 mg of penicillin and 100 mg streptomycin/l. Cells were implanted in roller bottles (approx. surface 1500 cm^2) at a concentration of 2.5×10^8 cells per ml.

Monolayers were usually confluent on the 5th day, and the cultures were ready for use. Cultures were washed 3 times with phosphate buffered saline (PBS) in order to remove the residual calf serum before virus inoculation.

Virus: The virus strain used in this experiment was a local strain designated S#12. According to our experience, this strain in comparison to other strain (Sano, Mono, Hoshino) yields better antigenic titre. (Table 1)

Preparation of antigen: The virus was isolated in Vero cells and passaged 5 times in these cells for adaptation, and the material from the 5th passage was inoculated into roller bottles (MOI 0.01-0.001/cell).

Maintenance medium was MEM containing 0.2% bovine albumine (fraction V). The the cytopathogenic effect (CPE) appeared on the 3rd day whereupon the medium was removed and fresh medium was added; CPE was complete on day 5. The virus harvest, 3 days later, was carried out and in order to release all the viruses from the infected cells the material was frozen and thawed 3 times. The fluid was centrifuged for 30 minutes at low speed (3500) rpm, then the supernatant was collected and 0.2% bovine albumine plus 1% merthiolate (Merck) was added. We tried to concentrate this antigen by PEG without any success. This suspension was used as antigen in HI and CF tests.

Titration of HA: For titration of mumps HA, 1 drop of 50% suspension of guinea pig red blood cells (GPRBC) in PBS is added to serial twofold dilutions of HA in P.B.S. The plates are then shaken and allowed to stand at 37°C for 1 hour. The first well that showed "one-plus" pattern of haemagglutination was taken as the end point of titration and considered to contain 1 unit of HA.

HI Test: At least, 664 serum samples were HI tested to show the reprodability of test using the antigen (Table 2). Sera were treated by kaolin and GPRBC for removing inhibitors and non-specific agglutinins, respectively. This test was carried out in presence of:

a. positive serum control with known titre.

b. Negative serum control for showing the absorption of non-specific inhibitor.

c. Serum sample control at the end of each row.

d. GPRBC control as indicator for the test.

e. The control of 4 HA units of the antigen.

CF Test: This test was performed in the microtitre system "Cook Engineering" as described by Sever (6).

Results and Discussion

Stability: Experiments have shown that the antigen in cell culture for fixation of complement in the present of specific antibody is moderately persistant. The titre of antigen remained constant at least 3 year at the 4°C.

Storage at this condition not only have no effect on antigenic titre, but also it causes increasing in the titre. Storage of antigen in long time at 4°C didn't show anticomplementary effect.

Table 1 shows that HI titres were higher, when our local mumps virus strain (S#12) was used,. The results of HI test on 664 patients sera, using the local antigen, are shown in Table 2.

Table 3 illustrates the results of CF tests of 27 patients samples as compared with HI tests. The comparison of these results show that in, general, in each case of positive antiserum we had a positive reaction (Table 3-1). Experiments have shown that the amount of antigen needed in the 2 tests differed (Table 3-2). This was, perhaps, due to the difference that existed between the kind of antibodies involved in HI and CF test.

Table 4 shows comparison between a standard antigen (Behring alantoic antigen) and the local cell culture antigen used in CF test. Our local cell culture antigen showed better results in experiments, i.e., local antigen didn't show anticomplementary effect in very low dilution (less than $\frac{1}{2}$), while Behring allantoic antigen presented this effect in dilution of $\frac{1}{2}^2$.

Periera and Valentine(4) concentrated cell associated infective adenovirus by slow speed sedimentation and removal of a part of the

supernatanant fluid that did not containing cells. Schmidt et al.(5) and Sarma et al. (12), also used the same procedure for producing the antigen. Although many antigens have been produced with considerable success after much effort and costly manipulations these antigens, in some cases, have had anticomplementary effect.

Table 1. Comparison between antigenic titre of different strains on the 8th day after inoculation day Virus

Titre	Virus Sample
64	S#12
2	Hoshino (vaccine strain)
2	Mano
4	Sano
2	Hoshino Virulent
64	Mumpsirt (Rabbit)
2	R-
4UHA	Mumps HA

The procedure that we have described in this communication is the most simple whereby one can produce, in a short time, a large amount of high titred antigen without the anticomplementary effects. In addition, the antigen maintains its activity after a long term storage at 4°C. The little torbidity that occur after long term storage can be removed, simply, by low speed centrifugation. Most important of all, the dual antigen can be used both for HI and CF tests.

Table 2. HI antibody titre of serum samples

>	128	64	32	16	8	8		Total samples tested
2	2	18	73	201	119	13	244	664
0.30	0.30	2.71	10.99	30.27	17.92	1.96	36.75	100%

Table 3.1. Total result from comparison of two tests

CF		HI		Total case in test
(+)**	(-)**	(+)**	(-)*	27
0	27	0	27	
	100%		100%	%

Table 3.2. Similarity and dissimilarity of results in 2 tests

dissimlarity of titre in 2 tests		similarity of titre 2 tests	
CF>HI	HI>CF	HI=CF	Total case
(c)	(b)	(a)	in test
9	9	9	27
66.66		33.33	%

a. equality of titre in 2 tests

b. HI antibody titre > CF antibody titre

c. CF antibody titre > HI antibody titre

Table 4. Comparison between local cell culture antigen and Behring standard antigen

CF test by Behring standard antigen	CF test by local cell culture mumps virus antigen	Serum sample
1:64	1:64	1
1:2	1:2	2
1:64	1:128	3
1:64	1:64	4
1:2	1:2	5
1:64	1:64	6
1:32	1:32	7
1:128	1:128	R^+
-	-	R^-
OK	OK	G.P.RBC

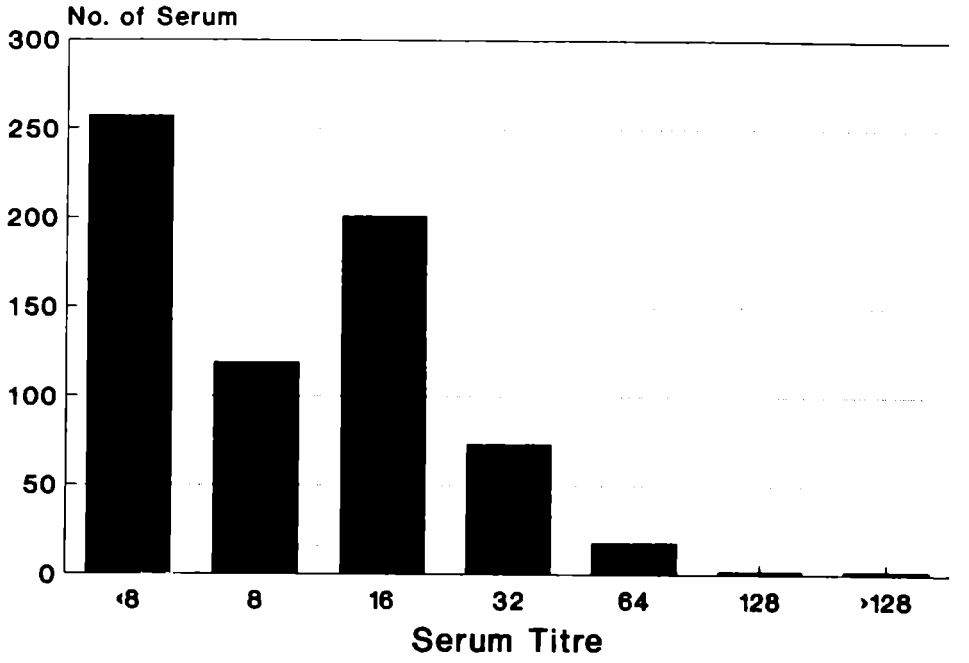

Fig 1. HI antibody titres of serum samples tested against the local antigen

References

1. *Enders, J. F., Kane, L.W., Cohen, S. and levens, J.H. (1945). Journal of Experimental Medicine. 81: 93.*
2. *Wolinsky, J. S. and Server, A. C. (1985). Mumps virus. Virology, Eds, B. N. Fields et al., Raveb Press, New York. Chapter five.*
3. *Henle, G. Henle, W. and Harris, S. (1946). The serolgical differentiation of mumps complenent fixation antigens. Journal of Experimental Medicine. 84: 290-295.*
4. *Pereia, H.G. and Valentine, R.C. (1958). Journal of Microbiology. 19: 178.*
5. *Schell, K.. Huebner, R. J. and Turner, H. C. (1966). Complenent fixing viral antigens. Journal of Experimental Medicine. 121: 41-46.*
10. *Sever, J.L. (1962). Application of microtechnique to virological investigation. Journal of Immunology. 88: 320. 320.*