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Short Communication

Comparison of micro and macro titration for evaluation of potency of mumps vaccine

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ABSTRACT

Mass vaccination against viral infections such as mumps is a very noticeable and appropriate attempt in common health. Mumps infection has severe complications like deafness, infertility and meningitis. Attenuated live vaccine of mumps is produced and used to prevent the problems. Viral content in the monovalent vaccine is assayed with different methods. Nowadays international standards introduce microplate for titration of virus. In this study the different aspects were searched to assay vaccinal mumps strain, on microplate. Its results presented proper count of Vero cell and essential amount of viral inoculum to coculture, in certain concentration of CO_2, on 96-well plate. Therefore with these ideal conditions, potency test of mumps virus was performed exactly, rapidly and economic with micromethod instead of cultivation in tube .Furthermore evaluation and reproducibility of the method accompanied with decreasing titer of < 0.5 log on microplate. The results showed the method is an efficient procedure for potency test in comparison with previous method.

Keywords: Mumps potency test, Vaccine, Titration, Micro plate

INTRODUCTION

Mumps infection is a mild to severe illness during childhood. Therefore production of an immunogenic vaccine against mumps has been always noted. An efficient vaccine must be contained certain amount of virus particle to stimulate effectively humoral and cellular immune system, after vaccination (Carbon 2007). Several methods like plaque assay, hemagglutination test and titration on cell culture tube have been used for titration of mumps virus (Cann 2005, Mauldin 2005). However using micro plate (96 wells, flat-bottomed) has been known for potency test as an exact and rapid method to estimate viral content of in process and final products (Kenny *et al* 1970, World Health Organization 1997). The project was done for accession to the best conditions in potency micro method to report the nearest titer to macro method, about mumps virus. The attenuated mumps virus was Hoshino L-32 Japanese strain.

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MATERIALS AND METHODS

The research carried out in two major stages. At first stage, two certified lots of mumps vaccine were selected respectively as in-house standard and sample. Both of them had been tittered in cell culture tube (macro titration). Also a sample of a mumps single harvest was determined and known its titer. In-house standard vaccine was tittered nine other times with the same method, to calculate GMT[1] . Since the mumps is light sensitive, the test should be protected from direct light. In the procedure, tubes were cultured two days before with Vero cell line (Grist *et al* 1974). After formation of a confluent monolayer, the tubes were washed with phosphate buffer saline (PBS). Then serial dilution of vaccine from 10^{-1}-10^{-4} was prepared: in steps $10^{0.5}$. After PBS discarding from certain tubes, 0.1 ml of each dilution was added. Adsorption time for virus to cell surface was 1-1.5 hour, at room temperature. Then maintenance medium ($DMEM^{+}$ + 0.2% bovine albumin + 0.75% KN[2]) was added to the tubes. On 2nd or 3rd day, mumps cytopathic effect was visible and the primary results were written (Figure 1).Also medium change was done twice at 3 days intervals. Final observation was done on 10-12th days. Both in-house standard and sample vaccine, the titer in TCID50/ml was calculated using Karber formula. In second stage, to prepare cell suspension, a flask contained a confluent monolayer of Vero line was trypsinized and pipetted with growth medium: DMEM+5% serum + 0.25 µg/ml (World Health Organization 1997). In the next step 50λ of suspension was mixed with the same volume of trypan blue .It was counted on haemacytometer chamber and cell number was calculated. Then different cell suspensions were prepared including 1.1-2×10^5 cell/ml. Two micro plates were cultured with every concentration (100λ /well). One of them was incubated in common incubator and another one in CO_2 incubator, at 37 ^{0}C. After monitoring during 2-3 days, a confluent monolayer was formed with suspension 2×10^5 cell/ml in CO_2 5%. Afterwards viral dilutions of standard vaccine were prepared in DMEM +2% serum + 0.25 % KN. Then rang of inoculation volume of the dilutions, including 50λ, 75λ and 100λ per well, were cultured with the cell suspension above. Following CPE propagation, viral inoculation of 50λ was approved to acquire exact titer. Micro plate label (directly over all wells) were used to maintain pH and to prevent fungal and microbial contamination.

1- GMT: Geometric mean titer
2- KN: mixture of kanamycin and neomycin

RESULTS AND DISCUSSION

The titer of mumps standard vaccine was calculated after 10 times in tube method (Table 1). Also the titers of sample vaccine and in process product were 4.75 and 5.25 respectively, detected in the macro titration.

Table 1. The results of titration with macro method for mumps standard vaccine.

Test	Titer log
1	5.25
2	5.25
3	4.75
4	5
5	4.75
6	4.75
7	5
8	5
9	5.25
10	5
Mean	**5**

In the next step, the used range of cell concentration showed 2×10^5 cell/ml which provided a 2-3 –day confluent monolayer, of course in CO_2 incubator in term of proper pH (6.9±0.2). The other cell suspension needed to long time, more than 3 days, to be dense. Then viral growth and CPE observation was delayed. On the other hand, preparation of monolayer was impossible in low concentrations (1±10^5-1.3±10^5 cell/ml). The best volume for inoculation of virus dilution, was 50λ per well to

appear gradually and clearly mumps CPE. Thus in micro method to assay mumps virus was added: 100λ of cell suspension to all wells, 50λ of vaccine dilutions (10^{-2}-10^{-4}) to the appropriate wells and 50λ of maintenance medium to viral wells (100λ to cell control wells).

Table 2. Results of mumps virus titeration in micromethod.

Test	Titer log		
	Standard	Sample	In process Product
1	4.8	4.5	5
2	4.8	4.3	5
3	5	4.6	5
4	5	4.5	4.8
5	4.7	4.5	4.8
6	4.8	4.3	4.9
7	4.9	4.3	4.9
8	5	4.5	4.9
9	5	4.5	5
10	5	4.3	5
Mean	**4.9**	**4.4**	**4.9**

Table 3. Comparison of the results in the two methods.

Mumps Virus	Standard	Sample	In process Product
Titer log in tube	5±0.03	4.75	5.25
Titer log in plate	4.9±0.01	4.4±0.01	4.9±0.007
Log difference	0.1<0.5	0.35<0.5	0.25<0.5

The total volume in a well was 200λ which provided a complete and clear field of a well to observe. Incubation temperature was 37 ^{0}C in 5% of CO_2. The titration carried out for standard and sample vaccine, 10 times (Figure 2). Then micro method was used about single harvest sample of mumps, as intermediate product, 10 times .Thus GMTs of them were calculated (Table 2). According to the conclusions, difference titers between two procedures were calculated (Table 3). Mumps infection is a target in expanded program on immunization (EPI). The current standards recommend on using microplate to co culture virus and cell line (Numazaki *et al* 1987, Cusi *et al* 2000). This method is faster and more economic than other tests such as macro method with cell culture tubes.

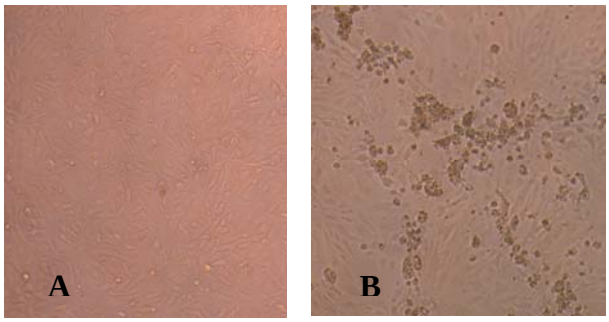

Figure 1. A- Control of Vero cell line (without virus); **B-** Mumps CPE includes cell with black margin and some internal vacuoles like dot (10 X).

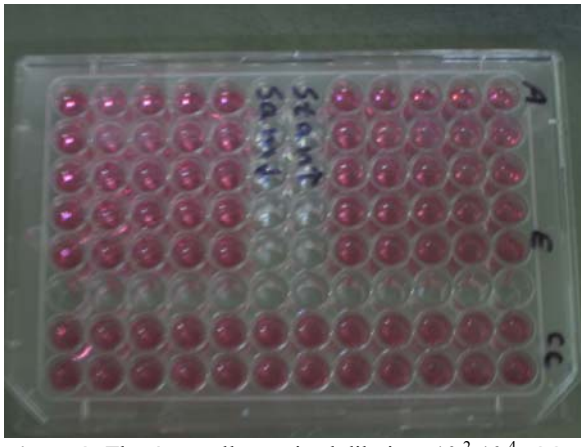

Figure 2. The *A-E* well contained dilutions 10^{-2}-10^{-4}. *CC*: cell control, *Stan*: standard and *Sam*: sample vaccines.

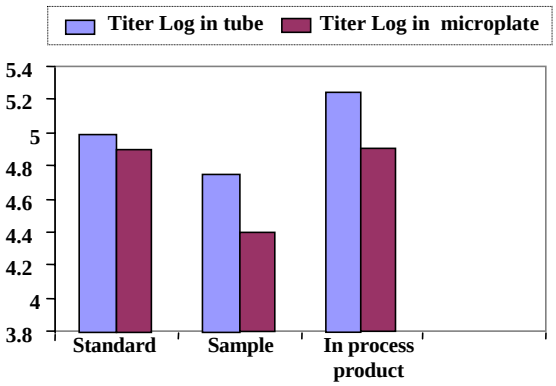

Figure3. The results comparison of titration of mumps virus between macro and micro plate methods.

In this study, examinations of different concentrations of cell and virus inoculation were

resulted to selecting the best data to achieve micro method for vaccinal mumps strain. Using of CO_2 - incubator was a critical factor for cell culture and virus growth on micro plate. After the titrations of standard and sample vaccines and also in process products, conclusions showed difference titer of <0.5log between micro and macro method (Figure 3). Otherwise, the new method did not need to medium change .Of course with this test will be saved expensive materials such as culture medium, serum, viral product and cell line. Its most important advantage is rapid report of titer sample to continue production processes and to use surely in vaccination. In this manner the micro method is a very proper replacement for macro method. Although, increase of virus concentration in product may be able to compensate the extenuation titers.

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