EVALUATION OF POTENCY OF MUMPS VACCINE USED IN IRAN: COMPARISON OF WHO AND NIBSC METHOD IN CELL CULTURE

S. Tavajohi[1, 2], H. Rastegar[1], S. N. Ostad[2], S. M. Rezayat[3] and M. H. Ghahramani[*2]

1) Food and Drug Control Laboratory, Ministry of Health and Medical Education

2) Department of Toxicology and Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

3) Department of Pharmacology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract- Vaccination against mumps is included in world Health Organization (WHO) program of global immunization. Safety and efficacy of vaccines must be confirmed by control units in charge of public health. In Iran, the secondary control on potency of vaccine has not been set up yet. We decided to overcome this problem by developing WHO and NIBSC methods in Food and Drug Control Lab. Nine dilutions of vaccine from 10^{-1} to 10^{-5} in 0.5_{log10} steps were mixed with Vero cell suspension. In WHO method, the cell suspension was seeded in octaplicate (8 wells of each dilution) in 24-well plate and incubated at 36°C for 10 days, during which the cells were checked for cytopathic changes everyday and positive wells were recorded. We used 5% serum and 1×10^5 cells for the assays. The test was repeated with six different vaccines produced in one batch. The mean potency was $10^{4.475\pm0.134}$ $CCID_{50}$/vial for each ten-dose vial. For NIBSC method the dilutions of vaccine were added to hexaplicate (6 well per dilution) in 24-wells plate. After 3 hours, the medium was removed and overlay was added. Then the plates were incubated at 35°C for 10 days. After incubation period, the plaques were stained with methyl violet and counted. This assay was repeated three times and the mean of results was 5.93 $\log_{10}$ PFU/dose. Results indicate that the potency of the vaccine is in acceptable range in either method. WHO method is simple and less time consuming compared to NIBSC method.

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INTRODUCTION

Vaccines are essential tools in the prevention of diseases. They protect the vaccinated individual from developing a potentially serious disease and help protect the community by reducing the spread of infectious agents. The success of immunization initiatives is evident from the impact they have had on the occurrence of diseases in various parts of the world. Smallpox has been eradicated worldwide, poliomyelitis is on the verge of eradication and there have been dramatic reductions in the incidence of measles, mumps and neonatal tetanus in many areas (1, 2).

In the years immediately before the introduction of mumps vaccine in the United States, mumps was most commonly reported among young school-aged children, with more than half the reported cases of mumps being among children 5 to 9 years of age. There is evidence that preschool-aged children may also have played an important role in the epidemiology of mumps (3). Live attenuated mumps vaccine can prevent this disease effectively (2, 4). A

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*** Corresponding Author:**
M. H. Ghahremani, Department of Toxicology and Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 66959102, Fax: +98 21 66461178
E-mail: mhghahremani@tums.ac.ir

valuable vaccine must create high immunity with minimum harm. In 1982, with the aim of providing guidance for the standardization of tests needed to ensure the safety and efficacy of vaccines, the World Health Organization (WHO) issued a document entitled "Manual of details of tests required on final vaccines used in the WHO Expanded Program on Immunization" (1, 2). The prime responsibility for the safety and efficacy of a biological product rests with the manufacturer. However, it is a country's national control authority that is responsible for establishing procedures for assuring that biological products intended for use in that country are of adequate safety and efficacy. The activities required to meet these responsibilities are incorporated in the concept of assurance of quality (1, 5, 6). In our country, the potency of vaccines has not been checked by the national control authority and the report from manufacturer has been used as an authorized report. As a health issue, we decided to set up the vaccine potency evaluation using WHO and National Institute for Biological Standards and Control (NIBSC) method as two valid and internationally accepted methods in Food and Drug Control Lab (FDCL). In this experiment, we have also compared the result obtained from these two methods and the assay procedure in order to determine the proper method in this regard.

MATERIALS AND METHODS

Vaccines were obtained from Razi Institute, Tehran, Iran. All the mediums and fetal bovine serum were purchased from Gibco, UK. The cell lines were obtained from Iran Pasture Institute, Tehran, Iran.

WHO Method

In conformity with WHO method (1, 2, 7), we prepared serial dilution of vaccines in 0.5_{log10} steps from 10^{-1}-10^{-5} (nine dilution) in medium 199 supplemented with 2% fetal bovine serum. The Vero cells (passage number 12) was mixed with vaccine dilution and seeded in octaplicate (eight wells per dilution) in 24 well plates. The plates were incubated in 5% CO_2 at 36°C for 10 days. According to result of previous set up for measles, the assays were performed at 5% serum and $1x10^5$ cell per well. During incubation period, the cells were checked for cytopathic changes and positive wells were recorded. The titer was calculated in $CCID_{50}$ per vial on the basis of the final reading using the Karber formula:

$$\text{Log CCID50} = L - d\,(S - 0.5)$$

Where L= log starting dilution, d= log dilution step, S= sum of the proportion of positive replicate.

The cytopathic effect (CPE) in each assay was checked under microscope by two different individuals and recorded. The assay was repeated six times and the potency was calculated.

NIBSC Method

According to NIBSC method (8, 9), in the first stage, we prepared the medium composed of DMEM 91%, Fetal calf serum (FCS) 4%, sodium bicarbonate 4.4%, Penicillin-Streptomycin 1% and the overlay medium contain of DMEM 67%, FCS 4%, sodium bicarbonate 4.4%, Penicillin-Streptomycin 1%, Carboxy methyl cellulose 25%. Vaccine dilution was prepared similar to WHO method. The cells were mixed with vaccine and plated in 24 well plates (six of each). After 3 hours, the medium was removed and the overlay was added to each well. The plates were incubated at 35°C with 5% CO_2 for 10 days.

After incubation period, the tissue culture fluid was discarded into 10% chlorous and cells were washed gently three times with PBS. Then plates were stained with 1 ml methyl violet (5% in IMS; Ethanol: Methanol 19:1) for 20 min. Then the dye was removed and wells were washed carefully with PBS and allowed to dry. The plaques were counted and based on NIBSC protocol counts above 80 and below 10 were regarded as inaccurate or statistically unreliable and were not included in calculation. To calculate log of plaque forming unit per dose (Log PFU/dose) we followed the NIBSC protocol. Briefly, the valid counts were multiplied by the dilution factor to obtain the number of plaques per well. The mean of this figure over all dilutions were taken and the log10 of the mean gave the potency per volume used to inoculate each well.

Since we have used a 10-dose vaccine reconstituted in 1 ml, in assays using 50 µl (half dose) (according to previous assay), the log PFU/dose was calculated by adding 0.3 ($log_{10}2$=0.3) to the log PFU obtained from the assay.

RESULTS

Mumps Vaccine potency evaluated by WHO method

In order to determine the potency of the mumps vaccine, we plated Vero cells at 1×10^5/well mixed with 10^{-1}-10^{-5} dilution of vaccine. Each dilution was plated in eight replica (eight well/ dilution). The CPE was observable at day three in high concentrations of vaccine (10^{-1} to 10^{-2}). However, as indicated in WHO method, the CPE reading was recorded at day 10 by two independent observers.

Figure l indicates that CPE was observed in all dilutions. This assay was repeated independently on 6 different vials of vaccine. The mean potency calculated for Measles vaccine was $10^{4.475\pm0.134}$ $CCID_{50}$/vial for each ten-dose vial (n=6).

Since in WHO method the potency of one dose should be at least 3 $\log_{10}CCID_{50}$ then a ten-dose vial should be at least 4 $\log_{10}CCID_{50}$/vial therefore the potency of vaccine under test was in acceptable range.

Mumps Vaccine potency evaluated by NIBSC method

In order to determine the potency of the mumps vaccine (according to previous paper), we plated Vero cells at 5×10^5/well mixed with 50 µl of 10^{-1}-10^{-5} dilution of vaccine. Each dilution was plated in six replicas (six well/dilution).

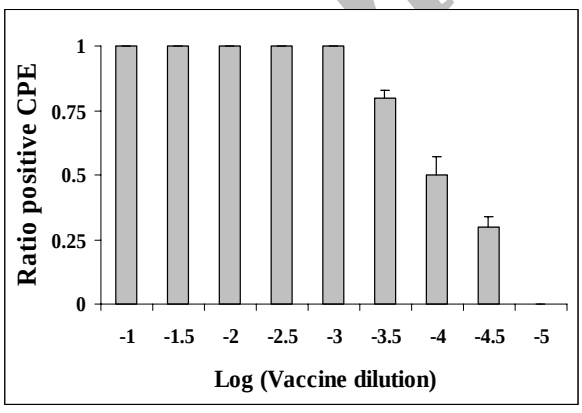

Fig. 1. The cytopathic effect of mumps vaccine on Vero cells. The cells were mixed at density of 1×10^5/ well with vaccine dilution and plated. At day 10, the positive cytopathic effect (CPE) in replica of each dilution was recorded and calculated as ratio= total positive CPE/total replica in each dilution. The results were plotted as mean ±SEM (n= 8).

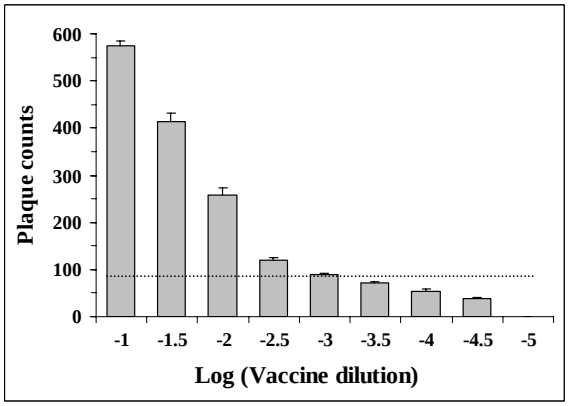

Fig. 2. The effect of plaque formation induced by mumps vaccine in Vero cells. The cells were mixed at density of 5×10^5/ well with 50µl of vaccine dilution and plated as described in methods. At day 10, the plaques in replica of each dilution was counted and mean ± SE of all dilutions is plotted (n= 6). The dotted line indicates plaque counts of 80, the count limit of NIBSC method.

Figure 2 indicates the result of potency evaluation in this assay. This assay was repeated independently on three different vials of Vaccine and the log PFU/dose of each assay was calculated. The mean log PFU/dose of this vaccine was 5.93 ± 0.03 (n=3).

DISSCUSSION

We have set up WHO and NIBSC methods in FDCL and compare these methods together. The WHO method is easy to set up and very economical with few steps for each to results. In this method, the results of potency are ready by end of incubation period.

However, NIBSC method has more steps and to obtain results one requires staining after the incubation time. Furthermore, the NIBSC assay procedure is very sensitive and related to the condition of the laboratory, which could harm the reproducibility and validity of the results. In this method, it is hard to determine if a large plaque belonging to one virus or formed from joining two near small plaques. Furthermore, in removing the overlay and staining the plaques, some cells have detached from the plate and created a shape similar to plaque. In addition counting of plaque is a very tiresome work and is time consuming. It is

noteworthy that the NIBSC method is more quantitative than the WHO method. Based on the NIBSC method, the method measures the total plaque forming unit (PFU) and directly counts the number of infecting unit. However, the WHO method measures the minimum limit of immunogenicity of the vaccine. Therefore, the NIBSC method is suitable for the quantitative analysis of the vaccine and reports the maximum number of infectivity of each sample. On the other hand, WHO method assesses the minimum viruses required for a suitable vaccine. The common method of potency evaluation, including the Razi Institute, is the titration of the vaccine in tube and reporting in $CCID_{50}$ unit. We have found that the WHO method in cell culture is sensitive, reproducible and valid for the vaccines we have checked. Therefore we suggest rechecking the vaccines produced in Iran by WHO method. Furthermore, based on the reasons mentioned, we recommend the WHO method to evaluate the vaccine in FDCL.

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