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Preparation and Evaluation of Biological Activity of the Sublimated and Cryopreserved Forms of the Herpes Virus of Turkeys

L. B. Kutumbetov* and B. Sh. Myrzakhmetova

Kazakh Scientific Research Veterinary Institute, Almaty, Republic of Kazakhstan

Corresponding author.

Abstract

The article contains data on the technology of obtaining and biological activity of the freeze-dried and cryopreserved forms of the turkey herpes virus culture used for the manufacture of the vaccine against Marek's disease. Using the methods of stationary and roller cultivation, a culture biomass of the herpes virus of turkeys was obtained, which was subjected to sublimation and cryopreservation in a mixture of two different stabilizing components. The titration results of these two forms of the vaccine showed that the virus titer was maintained at a level sufficient to be used as a vaccine for the immunization of chickens against Marek's disease.

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Introduction

Poultry production of the Republic of Kazakhstan is maintained mainly through passive breeding, i.e., the method of periodic importation of birds from abroad at one-day age and / or in the form of hatching eggs with the subsequent production of chicks from them in local incubators. In future, each poultry farm will be required to hatch and produce chicks on site as the viral diseases are prone to lower the productivity (Syurin, 1998). According to this practice, immunization of chickens against Marek's disease (MD) is to be carried out in the territory of the Republic of Kazakhstan, where the breeding chickens are produced from hatching eggs at local poultry farms. However, MD vaccines are not produced in the country and there is no technology for its preparation, due to the lack of purposeful programs for vaccines development (Kulyashbekova, 1998). In connection with this situation, this work is devoted to testing the technology of obtaining the freeze-dried and cryopreserved forms of biomass of the herpes virus of turkey (HVT) vaccines against MD, and evaluating of its biological activity.

Materials and methods

The biomass of the HVT vaccine for the production of cryopreserved and sublimated samples was produced in chick embryo fibroblast (CEF) cultures in a stationary fashion in 1.5-liter mattresses and roller-based system in 0.5-0.7-liter circular bottles. Standard methods were adopted for cell culturing (Adams, 1983; Igudin et al., 1985).

For the production of the virus stocks, a monolayer culture of CEF was first prepared by seeding the primary-trypsized cells of 10-day chick embryos at a concentration of $3.5 \pm 0.3 \times 10^5$ cells / ml in a volume of 120 ml in mattresses and $6.5 \pm 0.3 \times 10^5$ cells / ml in a volume of 50-70 ml in circular bottles. Trypsinization was carried out on a magnetic stirrer with a 0.5% trypsin solution. CEF cultures were grown in nutrient medium according to the Eagle's formulation with the addition of 10% of blood serum of fetal bovine serum. The nutrient medium in the vessels was replaced every 2-3 days. The pH of the culture medium was adjusted with sodium bicarbonate solution. Primarily sown and virus-infected cells were cultured at a temperature of 37 ± 0.5°C. In a stationary method, the mattresses with the cell culture were left in the stationary state in the thermostat. When the roller was used, the circular bottles were placed in a special apparatus that continuously rotates the vessels around its axis at a speed of 20 rpm. CEF monolayer cultures were observed daily by microscopy.

After formation of a monolayer of cells in the vessels (mattresses and roller bottles), virus stocks were inoculated with a dose of 0,01-0,02 $FFU_{50/cell}$ and the CEF cultures were incubated under the same conditions for another 4 days, replacing the nutrient medium 24 hours after application virus. 96-120 hrs after the inoculation of the virus, the nutrient medium was removed, the monolayer cells were eluted using trypsin solution and suspended in nutrient medium with 5% bovine serum. The nutrient medium was introduced into the vessels in a volume that was four times less than the volume used in the culture.

The fibroblast suspension containing the cell-associated HVT cultivated in two separate methods was separately divided into two parts and one of them was mixed with a cryopreservation protective medium, the other with a similar sublimation medium.

For the cryopreservation, a protective medium, consisting of 8% peptone, 10% sucrose and 2% gelatin was used together with the suspension of infected cells in Eagle's nutrient medium containing 20% bovine serum and 10% dimethylsulfoxide in a concentration of 10%.

Each batch of the slurry was dispensed in 1.0 ml volume into insulin bottles. Two of these bottles, intended for cryopreservation, were placed in liquid nitrogen, after sealing the packing containers with rubber stoppers and rolling over with aluminum caps, and the remaining two batches were subjected to freeze-drying. Freeze-dried bottles were also sealed with rubber stoppers, rolled with aluminum caps and were stored at 4-6°C.

The biological activity of the virus in the freeze-dried and cryopreserved vaccines was examined by titration in CEF monolayers. For this, the freeze-dried vaccine in the vials was rehydrated in distilled water, and the cryopreserved vaccine was thawed in a water bath with a temperature of 39-40°C. Ten-fold dilutions of the vaccines were prepared separately from them in Hank's balanced salt solution and dilutions from 10^{-1} to 10^{-7} of each samples were used to infect CEF. Infected CEF cultures were incubated at 37 °C for 96 hours, medium removed and monolayer stained with 1% methylene blue solution and infected focus units were counted using microscope. The virus titer in FFU_{50} was calculated by Reed and Muench (1938).

Results and discussion

In total, CEF cultures in 8 mattresses and 8 roller flasks were prepared to produce the vaccine stocks. Of these, 4 mattresses and 4 roller flasks served as controls, the remaining ones were used for the production of the virus. The monolayer of cells in the mattresses was formed after 48 hrs, and in the roller bottles - 72 hrs later. At the indicated times, the cell culture in the mattresses and roller flasks was infected with HVT in doses of 0.01 $FFU_{50/cell}$, and in 0, 02 $FFU_{50/cell}$ respectively. This two-fold increase in the multiplicity of infection was based on the fact that the cells grown by the roller method were 24 hrs older than those grown in stationary cultures at the time of infection. Therefore, for comparatively active replication of the virus in culture with a different incubation interval for cells, an increased dose of the virus was used for the roller flasks.

The infected cell culture was incubated for 96 hrs at a temperature of 37±0.5°C, after which the nutrient medium was removed from the vessels, the cells were suspended in four times reduced volume of the nutrient medium. A total of 4 mattresses produced 120 ml of virus-containing cell suspension, while the roller bottle produced 60 ml. From each of the preparations, 40 ml of the suspension divided into 20 ml in separate two bottles to obtain two preparations each of the mattress and roller culture derived virus stocks.

One sample of the suspension of virus-containing cells

obtained by each of the two methods was mixed in equal volume proportions with a stabilizing medium intended for the preparation of freeze-dried samples, and the remaining two samples obtained by each of the two methods were stabilized by a protective cryopreservation. The stabilized suspension of virus-containing cells for sublimation was dispensed into 80 vials, 40 of which contained the suspension obtained from the stationary mattress cultures, the remaining 40-suspension obtained by the roller culturing methods. Samples for cryopreservation were also packaged in a similar fashion.

In order to determine the biological activity, the samples of cryopreserved and freeze-dried preparations from both stationary or roller flasks cultures were titrated on CEF monolayer cultures. The results of titration are given in Table 1.

Table 1. Titers of the herpes virus of turkeys in samples of culture biomass subjected to cryopreservation and sublimation.

№	Method of cultivation of CEF and virus	Method of preservation	Titer of virus, $FFU_{50/ml}$
1	Stationary	Sublimation	10^5, 13±0.41
		Cryopreservation	10^5, 84±0.07
2	Roller	Sublimation	10^5, 33±0.13
		Cryopreservation	10^6, 17±0.21

As can be seen from the data in Table 1, HVT vaccines, regardless of the cultivation method and subsequent processing by cryopreservation or freeze drying, have demonstrated sufficient biological activity suitable for the immunization of the birds against Marek's disease. The titers of the virus in the culture suspension obtained by different culturing methods did not differ appreciably from each other and varied from 10^5, 13±0.41 $FFU_{50/ml}$ to 10^5, 84±0.07 $FFU_{50/ml}$ with the stationary cultivation method and from 10^5, 33±0.13 $FFU_{50/ml}$ to 10^6, 17±0.21 $FFU_{50/ml}$ - with roller method. The titres of the virus stocks, preserved by deep freezing, were 0.71 – 0.84 lg $FFU_{50/ml}$ higher than the obtained by freeze drying. This relatively significant difference in titres is thought to be due to the fact that significant amount of virus particles undergo sublimation due to dehydration.

Conclusion

The results obtained from these studies indicate that HVT vaccines with sufficient titers can be produced in CEF grown by stationary and roller methods. The titres of the culture virus obtained by both methods are suitable for the manufacture of cryopreserved and freeze dried forms using the appropriate protective components. Cryopreserved samples of HVT showed that the residual biological activity was higher in the sublimated form of the vaccine.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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