# ACTIVITY OF HERPES SIMPLEX VIRIONE VACCINE FOR MEN AFTER FORMALDEHYDE NEUTRALIZATION

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**Introduction:** Formaldehyde in viral vaccines coagulates proteins and prevents their decomposition. For inactivation of virione herpes simplex vaccine, sulfitization is necessary to neutralize formaldehyde. A small amount of 0,2% of formaldehyde is used from pure, 37% stock concentration.

Herpes simplex virone vaccine stimulatates cellular mechanisms of resistance in the human body against herpes simplex virus types I and II. It increases the activity of white blood cells and lymphocytes, thereby suppressing the herpes simplex virus in up to 87% of cases permanently. The vaccine against herpes simplex virus stimulates and increases after specific vaccination 30-60 days after specific vaccination, raising blood levels of specific antibodies which suppress viral activity. The virus enters a dormant state, resulting in reduced skin rashes, mucosal ulcers, ophthalmic herpes, and genital herpes. Frequently after vaccination, herpes simplex virus type 1 and 2 symptoms completely disappear. However, before starting vaccination, the patient must undergo a blood test to check immune status, including sensitivity of lymphocytes to herpes simplex virus. If percentage of inhibition is more than 8%, intradermal pure vaccine injection is contraindicated, as the patient's body Because body is sensitized to herpes simplex virus. In this case, the vaccine must be deluted three time and requires initial desensitization of the body.

**The aim.** To deternine the activity of herpes simplex vaccine after formaldehyde neutralization by sulfitization.

**Material and Methods.** For inactivation of herpes simplex virus, 37% formaldehyde was used. Formaldehyde it an aliphatic aldehyde of methanol and formic acid. Sodium metabisulfite $Na_2S_2O_5$ contains approximately 55% $SO_2$, therefore, for sulfitization of formaldehyde in herpes virion vaccine, sodium metabisulfite $Na_2S_2O_5$ in added in twice the amount.

Formaldehyde grade - A producing was produced by Inter-Sinthesis Company, Borislav, Ukraine.

In the inactivation vaccine 0.05 ml from stock concentrated formaldehyde (37%) was diluted in 100 ml purified water for injections.

The herpes simplex virus was grown using a Vero cell culture originally obtained from green African monkey (Cercopithecus aethiops). We received for research two cell lines, Vero and Vero-76: standard internationally used line kindly provided from Cell Bank of Vertebrates from Doctor of Biological Science Galina Poljanskaya of the Research Institute of Cytology.

The Vero cell culture strain of German origin was obtained from the Research Institute of Epidemioligical Diagnostics, Wustrhausen (Dosse), Germany. This cell culture strain was kindly provided for research by the Doctor of Biological Science Lev Dyakonov and Tatyana Galnbek, Laboratory of Biotechnology, Cell Bank of Vertebrates and Invertebrates at the Research Institute VIEV.

**Results and Discussion:**

**First stage of virus inactivation:** Carefully shake the formaldehyde and add 0.05 ml from the stock concentrated 37% formardehyde solution to the vaccine. Transfer the mixture to a sterile bottle containing 100 ml of virion vaccine, seal the bottle neck with a sterile rubber or silicone stopper, and place in a thermostat at 36±1ºC for cultivation for 72 hours. During cultivation, carefully shake the bottle with vaccine twice daily. After completing the first stage of virus inactivation, produced to the second stage.

**Second stage of virus inactivation:** Place the bottle containing the vaccine in a refrigerator at +4±1ºC for 7 days. As in the first stagecarefully shake the bottle twice daily during refrigerated cultivation.

After the second stage of virus inactivation, clarification or lightening is performed by centrifugation at 3,000 rpm for 20 minutes. The supernatant is used for vaccine production, while the sediment after centrifugation is autoclaved at a pressure of 1.0 atmosphere at 121ºC before disposal.

For formaldehyde neutralization, we used sulfitization with sodium metabisulfite ($Na_2S_2O_5$).

A 2% sodium metabisulfite solution is prepared in purified water and filter-sterilized. The solution should be stored in a refrigerator at 6±1ºC, for ut to 48 hours.

The required dose of 2.0% sodium metabisulfite is 1.0 ml per 100 ml of vaccine. The pH of sodium metabisulfite should be adjusted to pH=7,3±0,1. If the pH is lower, it must be adjusted to 7,3 using a 7.5% sterile solution of sodium bicarbonate – ($NaHCO_3$).

To distinguish antibody titres between the control group and the test group (test group II), 12 Chinchilla breed rabbits were by agreement in the vivarium. The rabbits were devided into two groups: group I injected herpes virion vaccine without formaldehyde neutralization (control group), and group II received the vaccine after formaldehyde neutralization.

Herpes simplex virus type I - UC (scin form) was obtained for research from the Culture Collection USRCB No. HSV - 0010.

Herpes simplex virus type II - BH (genital form) was obtained for research from the Culture Collection No. HSV - 0011.

The herpes virion vaccine was prepared from specific antigens of herpes simples virus type - 1 (skin form) and type - 2 (genital form). The virion vaccine was made from safe and carefully pre-selected viral immunogen strains. To prepare the virion vaccine, it was necessary to reproduce viruses by cultivation both virus types in Vero-76 cell culture. This cell culture is derived from the kidney of African green monkey (Chlorocebus sabaeus) and is a clone of the Vero cell line with epithelial-like characteristics. The Vero cell line was established on March 27, 1962, by Yasumura Y. and Kavakita Y. at the University of Chiba, Japan. Vero-76, a subline of Vero established in 1968, is one of the most widely used continuous cell lines in the world for viral vaccine production.

Cultivation of Vero and Vero-76 cells: Eagle's MEM with double concentrations of amino acids and vitamins is used, with ex tempore addition of 300 mg stelile L-glutamine per 500 ml of media and 10% bovine serum. A protective amount of

antibiotics must must be added to prevent mycoplasma infection:either gentamicin at 200 μg/ml or kanamycin at 100 IU/ml.

Cultivation of Vero cells of German origin: RPMI-1640 media is used, with ex tempore addition of 300 mg stelile L-glutamine per 500 ml of media and 3-5% embryonal bovine serum.

A protective amount of antibiotics must be added to prevent mycoplasma infection: either gentamicin 200 μg/ml or kanamycin - 100 IU/ml.

**Herpes siplex viris strains:**

**US (type I, skin form):** This strain belongs to herpes siplex virus I-antigenic type.

**Cultural and biochemical properties:** Reproduction in monolayer cell culture is accompanied by cytopathic effects (synplasts), characterized by areas of multinucleated syncytia (multinucleated giant cells with intranuclear inclusions and vacuolated protoplasm). The infectious titer in cell culture for the dry strain is: 3.5-4.5 lg/BOE/ml (Plaque-forming result). Total protein content is 5.0±1.5 mg/ml. Bovine serum serum protein is 4.5±1.5. pH is maintained 7.3±0.1.

**Immunogenic properties:** Triple immunization of rabbits includes the production of virus-neutralizing antibodies. The neutralization index is 3.0-4.0 logarithm (lg).

**Virulent and toxigenic properties:** Intracerebral infection of outbred laboratory mice causes herpetic encephalitis and death of animals. The negative logarithm of titer $LD_{50}$ (lethal dose 50%) is no less than 5.0 lg/ml.

**Storage condition:** Store at -20ºC.

**Cultivation condition:** The strain is cultivated in cell culture with a miltiplicity hour at 37ºC after which the inoculum with unadsorbed virus is removed. The plaque-forming result represents the number of viral particles per milliliter (BOE/ml). Eagle's MEM with 5-10% bovine serum is used at the supporting media.

**Condition for obtaining stock culture:** After 2-3 passages, the strain in dry conditions with infectious titer in cell culture 5.0-6.0 BOE/ml is bottled in ampoules and lyophilized.

**BH (type II, genital form):**

This strain belongs to herpes siplex virus II-antigenic type.

**Cultural and biochemical properties:** Reproduction in monolayer cell culture is accompanied by cytopathic effect (synplasts), characterized by areas of multinucleated syncytia (multinucleated giant cells with intranuclear inclusions and vacuolated protoplasm). The infectious titer in cell culture for the dry strain is: 2.5-3.5 lg/BOE/ml. Total protein content is 5.0±1.5 mg/ml. Bovine serum protein 4.5±1.5mg/ml pH is maintained 7.3±0.1.

**Immunogenic properties:** Triple immunization of rabbits induces the production of virus-neutralizing antibodies. The neutralization index is 2.0-3.0 logarithm (lg).

**Virulent and toxigenic properties:** Intracerebral infection of outbred laboratory mice causes herpetic encephalitis and death of animals. The negative logarithm of titer $LD_{50}$ (lethal dose 50%) is no less than 4.0 lg/ml.

**Storage condition:** Store at -20ºC.

**Cultivation condition:** The Strain is cultivated in cell culture with a miltiplicity of infection ranging from 1.0 to 0.01 BOE per cell. Incubation is performed 1.0 hour at 37ºC, after which the inoculum with unadsorbed virus is removed. The plaque-forming result represents the number of viral particles per milliliter (BOE/ml). Eagle's MEM with 5-10% bovine serum as the supporting media.

**Condition for obtaining stock culture:** After 2-3 passages, the strain in dry condition with infectious titer in cell culture 4.5-5.5 BOE/ml is bottled in ampoules and lyophilized.

For sulfitization, we used a technological method that involves introducing sulfur dioxide $SO_2$ into the vaccine. A 2.0 gr of sodium metabisulfite $Na_2S_2O_5$ crystals was dissolved in 100.0 ml of water and used to neutralize formaldehyde in the herpes simplex virion vaccine. The concentration of formaldehyde is shown in Table. The herpes virion vaccine contained formaldehyde at 500.0 μg/ml. After formaldehyde neutralization, the concentration decreased to 27,0 μg/ml, representing a reduction of 473.0 μg/ml.

**Table**

**Blood test of rabbit after herpes simplex vaccination M±m, n=12**

| **No** | **Tested component** | **Group I** (control group), without vaccine neutralization formaldehyde | | **Group II** (tested group), vaccine after neutralization formaldehyde | |
|---|---|---|---|---|---|
| | | Normal range, % | Tested result, % | Normal range | Tested result |
| 1. | Lymphocytes % | 30-85 | 48±2.31 | 30-85 | 53±1.14 |
| 2. | Phagocytic activity of neutrophils % | 35-92 | 64±2.43 | 35-92 | 57±1.82 |
| 3. | Formaldehyde concentration Without neutralization (μg/ml) | 500.0 μg/ml | 500.0 μg/ml | - | - |
| 4. | Formaldehyde concentration after neutralization (μg/ml) | - | - | 50.0 μg/ml | 27.0 μg/ml |
| 5. | Test of inhibition of lymphocytes to HSV antigen % | up to 8% | 14±0,68 | up to 8% | 12±0,47 |
| 6. | Titre of antibodies to HSV 30 days after vaccination | > 1:32 | 1:1536 | > 1:32 | 1:1024 |
| 7. | Titre of antibodies to HSV 60 days after vaccination | > 1:32 | 1: 8192 | > 1:32 | 1:4096 |

The analysis of antibody titres in rabbits with and without formaldehyde neutralization in herpes vaccine showed unexpected results (see Table).

In control group - I, 30 days after vaccination with herpes vaccine without formaldehyde neutralization, antibody titres in rabbits increased to 1:1536, while in test Group II, antibody titrers after formaldehyde neutralization were lower at 1:1024.

After 60 days, Control Group I vaccinated with herpes vaccine without formaldehyde neutralization, antibody titres of 1:8192, while Tested Group II showed titers decreased by half. The formaldehyde neutralization affects vaccine quality and reduces its immunogenicity. This vaccine can be used for both therapeutic and prophylactic purposes. The glycoproteins of herpes virus are the main antigens for triggering immune response against HSV-1 and HSV-2. These viral glycoproteins play

essential roles in virion attachment to various cellular receptors, and virus entry, either through fusion of the viral envelope with the plasma membrane, or receptor-mediated endocytosis. Herpes virus glycoprotein B (gB) is the most highly conserved of all surface glycoproteins and functions primarily as a fusion protein.

Before beginning treatment with herpes simplex virion vaccine, a blood test is necessary to determine the patient's lymphocyte inhibition to the HSV antigen must be checked. If the inhibition percentage is greater than 8%, desensitization with triple-diluted herpes vaccine is required first. Only after the lymphocyte inhibition percentage decreases to 8% or less and active immunization with pure vaccine begin a dose 0.3 ml. Active immunization can be repeated six months after first course. Desensitization involves intradermal forearm injections of triple-diluted herpes vaccine with stepwise increased dodes, comprising a course of 10 injections.

**Conclusions.** Formaldehyde neutralization affects specific herpetic antibodies. Research results showed that in the control group without formaldehyde neutralization, HSV-1/2 antibody titers increasrd 1:1536 after 30 days of vaccination. In the group II, with formaldehyde-neutralized vaccine, titers reacted to 1:2024. Comparative assessment of antibody titers after 30 and 60 days showed maximum antibody increases in the group without formaldehyde neutralization. The sulfitation process likely negatively affects herpes virus glicoproteins, resulting of decreased immunogenicity.

**Keywords:** formaldehyde, antibody titre, herpes simplex virus, virione vaccine, formaldehyde concentration, neutralization of formaldehyde, titre of antibody.