The experimental substantiation of the shelf-life of “Candidocyde” vaccine

Because of the difficult situation with the diagnosis and therapy of candidiasis many researchers propose to use vaccines as an alternative to anti-fungal drugs for preventing and treating candidal infections. The authors have developed “Candidocyde” vaccine against candidiasis.

**Aim.** To determine experimentally the shelf-life of the solution of “Candidocyde” vaccine.

**Materials and methods.** “Candidocyde” vaccine was stored at two temperature regimes: in the refrigerator at the temperature of 2-8 °C and at the room temperature of (15-25 °C). Antibody titer of the solution of “Candidocyde” vaccine were assessed every three months to prevent and treat candidiasis. For this purpose the reagent kit for immunoassay detection of antibodies of class G to *C. albicans* using the test system ELISA “Vector-best” was applied.

**Results and discussion.** When conducting the studies of the vaccine stored in glass vials protected from light at the temperature from 2 ºС to 8 ºС it was found that the activity of the solution was preserved for 2 years and 3 months when preventing and treating candidal infections. The titer of antibodies in animals remained at the level of 1 : 600-1 : 4000 when preventing and treating candidiasis. The positive results were recorded in all animals in the group, i.e. all animals were healthy. It indicates that the solution of “Candidocyde” vaccine is stable for 2 years.

**Conclusions.** When studying the shelf-life of the solution of “Candidocyde” vaccine based on antigens of cells of *C. albicans* and *C. tropicalis* fungi it has been found that it is stable for 2 years of storage in glass vials protected from light at the temperature of 2-8 ºC.

**Key words:** vaccine; antigen; candidiasis; shelf-life; antibody titer
**Candida** causes a wide range of infections: from insignificant diseases of the skin and mucous membranes to invasive processes, which can affect practically the whole human body [1-3].

Because of the difficult situation with the diagnosis and therapy of candidiasis many researchers propose to use vaccines as an alternative to anti-fungal drugs for preventing and treating candidal infections. Similar studies are performed in many countries of the world, and currently the drugs developed undergo preclinical and clinical trials [4, 5]. It should be mentioned that at present no domestic vaccine is produced in Ukraine and no imported vaccines for candidiasis have been registered. Therefore, development of a vaccine against candidal infection is the topical issue for both domestic and foreign modern pharmacy and medicine.

At the premises of the National University of Pharmacy the researchers of the Biotechnology Department and the Department of Microbiology, Virology and Immunology developed the composition and technology of “Candidocyde” vaccine on the basis of **C. albicans** and **C. tropicalis** fungi. Experimentally the following main parameters were determined: the technology of cell cultivation of **C. albicans** and **C. tropicalis** fungi at the temperature of 25 ± 2 °C for 6 days [6]; the technology of fungal cell disintegration using an ultrasonic disintegrator at the frequency of 22 kHz, the intensity of 5 W/cm² and exposure of 15 min [7]; the purification technology consisting of centrifugation with the rotation speed of 1000 rpm for 15 min, prefiltration and sterilizing filtration using filters with the pore diameter of 0.45 µm and 0.22 µm, ultrafiltration through the membrane providing separation of the biological material with the size of 10 kDa [8]. According to the results of our studies of the optimal excipients the composition of the solution of “Candidocyde” vaccine based on the cell-associated antigens of **C. albicans** and **C. tropicalis** fungi with the protein concentration of 3 mg/ml and **C. tropicalis** with the protein concentration of 5 mg/ml in the ratio of 1 : 1 with the solvent (phosphate buffer solution) and phenol as a preservative in the concentration of 0.25% was determined [9, 10]. The vaccine developed shows a pronounced activity when preventing and treating candidiasis in animal experiments.

At the next stage of our research it is necessary to determine the shelf-life of the solution of “Candidocyde” vaccine when preventing and treating candidiasis by the indicators of antibody titers. The aim of the study is to determine experimentally the shelf-life of the solution of “Candidocyde” vaccine.

**Materials and Methods**

For studies the samples of the solution of “Candidocyde” vaccine of different batches were prepared and put for storage in glass vials made of a neutral opaque glass of the first class. The vaccine studied was stored at two temperature regimes: in the refrigerator at the temperature of 2-8 °C and at the room temperature of (15-25 °C). Antibody titers of the solution of “Candidocyde” vaccine were assessed every three months.

The antibody titers were determined in preventing and treating candidal infections in two month white mice weighing 18-22 g (six animals in the control and experimental groups each). They were kept in the same conditions on a standard diet. Before the study the animals acclimatized themselves under experimental room conditions. In studies regarding prevention of candidal infection the mice received a double intramuscular injection of 0.2 ml of the solution of “Candidocyde” vaccine in the upper part of the rear right paw with an interval of 14 days. After immunization the animals were infected intraperitoneally. For this purpose the suspensions of **Candida albicans** fungi in the amount of 20 mln of cells and **C. tropicalis** in the amount of 60 mln of cells in the volume of 1 ml were used; they were introduced with an interval of 1 h. Animals of the control group were injected the sterile 0.9% isotonic saline solution. In 14 days the protective functions of the animals’ body were determined by the titer of specific antibodies of **C. albicans** when conducting enzyme immunoassay (ELISA) according to the SPHU, 1-st ed., art. 2.7.1, p. 55-57. To do this, the reagent kit for immunoassay detection of antibodies of class G to **C. albicans** using the test system...
ELISA “Vector-best” was applied. Since there were no the sets of reagents for immunoassay detection of antibodies of class G to C. tropicalis, identification of antibodies only of class G to C. albicans was conducted.

In studies concerning the treatment of candidiasis the animals were infected intraperitoneally with the suspensions of Candida albicans in the amount of 20 mln of cells and Candida tropicalis in the amount of 60 mln of cells in the volume of 1 ml introduced with the interval of 1 h. In 5 days a double intramuscular injection of 0.2 ml of the solution “Candidocyde” vaccine was introduced to mice in the upper part of the rear right paw with an interval of 14 days. Animals of the control group were injected the sterile 0.9 % isotonic saline solution.

In 14 days the protective functions of the animals’ body were determined by the titer of specific antibodies of C. albicans when conducting ELISA.

To obtain the reliable results of the study all data were statistically processed, the median for all groups and its confidence interval were calculated.

**Results and Discussion**

The results of the study showed that before the treatment of “Candidocyde” vaccine the antibody titers of healthy animals were in the range of 1 : 200-1 : 500. This can be explained by the possible contact with the fungus of Candida genus during the life of animals or animals can be carriers of the fungal species since these fungi are part of the normal microflora of animals. After a double injection of the solution with an interval of 14 days there was an increase in the antibody titer, and it was in the range of 1 : 600-1 : 4000. The titer of antibodies in the control group was in the range of 1 : 400-1 : 1000.

Comparing the results obtained in the experimental and control groups it can be assumed with confidence that introduction of the solution of the vaccine stimulates formation of antibodies that are responsible for humoral immunity.

When conducting these studies of the vaccine stored in glass vials protected from light at the temperature from 2 ºС to 8 ºС it was found that the activity of the solution was preserved for 2 years and 3 months when preventing and treating candidial infections. The titer of antibodies in animals remained at the level of 1 : 600-1 : 4000 when preventing and treating candidiasis. The positive results were recorded in all animals in the group, i.e. all animals were healthy. It indicates that the solution of “Candidocyde” vaccine is stable for 2 years. The research results are given in Table.

<table>
<thead>
<tr>
<th>Temperature, ºС</th>
<th>Beginning of the storage</th>
<th>3 months</th>
<th>6 months</th>
<th>2 years and 3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevention</td>
<td></td>
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<tr>
<td>2-8</td>
<td>1 : 600-1 : 4000</td>
<td>1 : 600-1 : 4000</td>
<td>1 : 600-1 : 4000</td>
<td>1 : 600-1 : 4000</td>
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<tr>
<td>15-25</td>
<td>1 : 600-1 : 4000</td>
<td>1 : 800-1 : 1600</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Control</td>
<td>1 : 400-1 : 1000</td>
<td>1 : 400-1 : 1000</td>
<td>1 : 400-1 : 1000</td>
<td>1 : 400-1 : 1000</td>
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<tr>
<td>Treatment</td>
<td></td>
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<tr>
<td>2-8</td>
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<td>15-25</td>
<td>1 : 600-1 : 4000</td>
<td>1 : 800-1 : 1600</td>
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<tr>
<td>Control</td>
<td>1 : 400-1 : 1000</td>
<td>1 : 400-1 : 1000</td>
<td>1 : 400-1 : 1000</td>
<td>1 : 400-1 : 1000</td>
</tr>
</tbody>
</table>

Notes: n = 6.
REFERENCES


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