Rabies is the anthropozoonoses infectious viral disease that is almost always fatal after clinical signs. According to WHO 55 thousand people in the world die from rabies annually.

The only method of controlling the disease are pre- and post-exposure prophylaxis, in which rabies immunoglobulin and rabies vaccine are used.

One of the rabies vaccine production stages is obtaining of the rabies virus suspension. Are used as the substrate for the biological tissue (brain suspension); chicken embryos; cell culture (primary and constant cell culture) propagation of the virus biomass.

With the biotechnological methods development all over the world, the virus suspension propagation of in biological tissues and chicken embryos is almost never used, since that such vaccines can contain a large number of unnecessary agents. The most economically profitable and safe method is the obtaining rabies vaccine using permanent cell cultures.

PJSC “PHARMSTANDART-BIOLEK” (Kharkov, Ukraine) develops a rabies vaccine for humans use based on rabies virus a fixed strain L. Pasteur and a permanent cell line Vero (kidney cells of the green monkey).

The permanent cell line Vero is widely used as a substrate for production of the cultural rabies vaccine against rage. This cell culture meets the requirements of the World Health Organization, State Pharmacopoeia of Ukraine and European Pharmacopeia to substrates for human vaccines production because it is not tumorigenic.

The aim of the study was to master a technique of the rabies virus propagation of the fixed strain L. Pasteur in the Vero cell culture.

The object of the study was a fixed rabies virus strain L. Pasteur the was obtained from the Novi Sad Pasteur Institute (Novi Sad, Serbia) and deposited with the Depository of the State Scientific Control Institute of Biotechnology and strains
(SSCIBS) from 05.01.2017 years (registration number 678), adapted to the permanent cell line BHK-21 clone 13 (kidney cells of a newborn Syrian hamster).

The cells were cultured in vials sterile for the cell cultures in a parietal monolayer 1 day after passaging in DMEM medium with 10% content of blood serum of cattle under conditions of CO₂ incubator at 37 °C and 5% CO₂. To infect a permanent cell line, a sterile rabies virus suspension strain L. Pasteur was used.

The cell culture was infected in the presence of 80% monolayer on the second day after reseeding. Virus suspension was cultivated within 3 days from the moment of infection in the supporting environment (99% of DMEM, 1% of blood serum of cattle) at 33 °C and 5% CO₂. The received virus suspension was merged in sterile centrifugal test tubes and centrifuged in the refrigerator centrifuge at 4 °C for removal of a cellular detritus. The supernatant was poured into sterile containers and frozen at -80°C.

For definition of infectious activity of the accumulated biomass of the virus used a titration method in the culture of BHK-21 clone 13 cells. The viral suspension was titrated with 5-fold dilution in 96-well plates in 5 replicates. The cells were cultured for 48 hours at 37 °C and 5% CO₂.

The cell monolayer was then washed with phosphate buffer, fixed and stained with monoclonal anti-rabies antibodies labeled with fluorescein isothiocyanate. The results were recorded using a laboratory microscope equipped with a luminescent nozzle, with an increase in the objective of 10×, the eyepiece of 10×. At microscopy, a bright green specific glow was taken into account in each well of the plate. The viral titer was calculated using the Spearman-Carber formula and expressed in a decimal logarithm of a 50% infection dose for cell cultures (lg CCID₅₀).

During the conducted researches the technique of accumulation of suspension of a virus of a rabies of the fixed strain of L. Pasteur in a Vero cell culture. A method for controlling the infectious activity of a fixed rabies virus by titration in the culture of BHK-21 clone 13 cells using labeled monoclonal anti-rabies antibodies has also been developed.

The infectious activity of the obtained virus suspension was 5.82±0.08 lg CCID₅₀, which indicates that this method of accumulating a suspension of rabies virus can be used in the technological scheme for producing a rabies vaccine. This will create a drug that will meet the requirements of World Health Organization, State Pharmacopoeia of Ukraine and European Pharmacopoeia.