The dye is developed in Milkotronic Ltd (synthesized and produced) and its basic parameters surpass repeatedly the dye that is usually being used. With the standard microscopic method, see / Direct microscopic analysis, as a control method was conducted according to ISO 13366-1:2008 «Milk –Enumeration of somatic cells – Part 1: Microscopic method (Reference method)/ the dye which is used is Propidium Iodide. Our dye has the trade name SofiaGreen and its scientific name is YO-DAM1. We also apply a comparative analysis with Propidium Iodide and the best dye created so far - YO-YO 1 of Molecular Probe.

An absorption spectrum of the newly obtained fluorescent dye (YO-Dam-1) with the use of the spectrophotometer JENWAI 6900 is made. From figure 1 it can be seen that the dye has an absorption maximum at 478 nm. After that an emission spectrum of the dye with the spectrophotometer Esclipe is made. It is estimated that the pure dye shows no emission peak and the background signal is barely 5 fluorescent units at a concentration of the dye 50mg/ml. From figure 2 it can be seen that, by adding of 12,5 parts of DNA with a concentration of 25μg/ml to one part of the dye with a concentration of 50mg/ml an emission maximum is manifested at 511 nm.

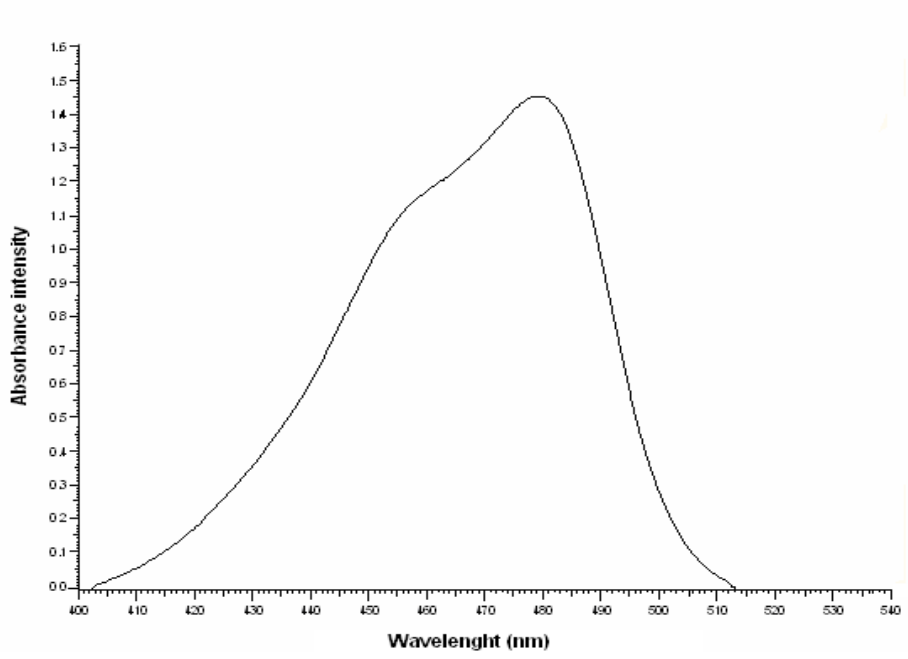


Fig.1. Absorption spectrum of YO-Dam-1

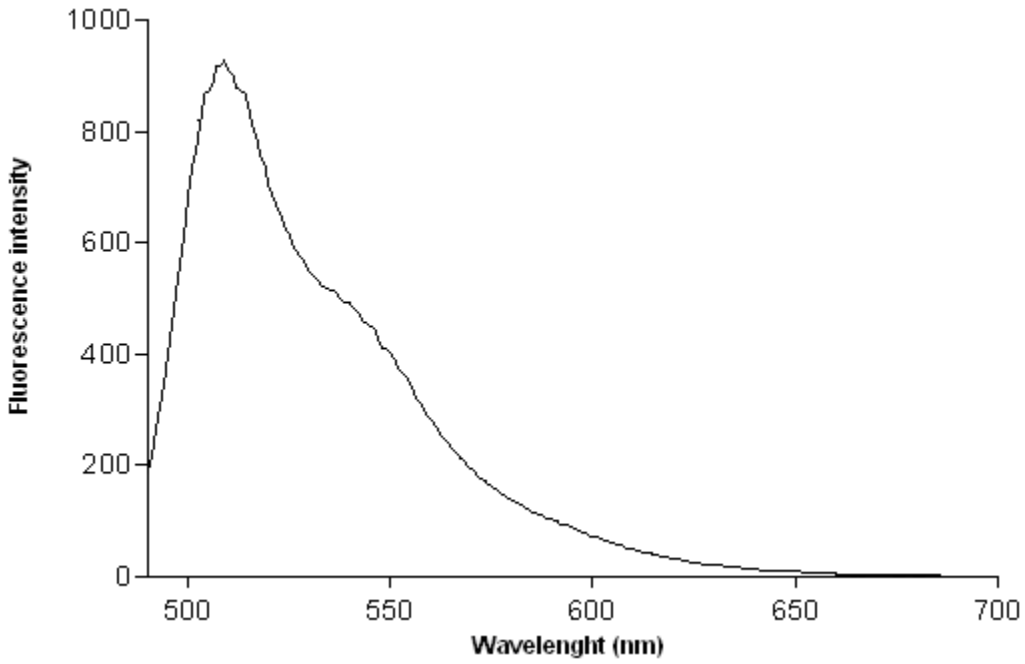


Fig.2. Fluorescent spectrum of YO-Dam-1+DNA (50 mg/ml + 25µg/ml), ratio dye:DNA = 1:12,5

A comparison of the time for reaching a balance of the connection of the synthesized YO-Dam-1 dye with the DNA balanced times for connecting the other two fluorescent dyes -YOYO-1 and Propidium Iodide (PI) is done. The results are shown at figures 3, 4 and 5. The experiments are made at equal concentrations of dyes and DNA and equal ratio of mixing. From the figures it can be seen that the speed of connection of YO-Dam-1 and DNA is very high. The balance is reached after 30 sec. This speed is similar to the speed of connection of Propidium Iodide and DNA. The connection of DNA and YOYO-1 is the slowest one. From Fig. 4 it can be seen that even at 340 min a full balance is still not reached. The full balance is reached after 6 hours. Moreover, at the 6^{th} hour (at an occurred balance) the intensity of the fluorescent signal at the connection of YOYO-1 and DNA is 1,5 times higher than the fluorescent intensity of YO-Dam-1 and DNA also at an occurred balance (at 30 sec).

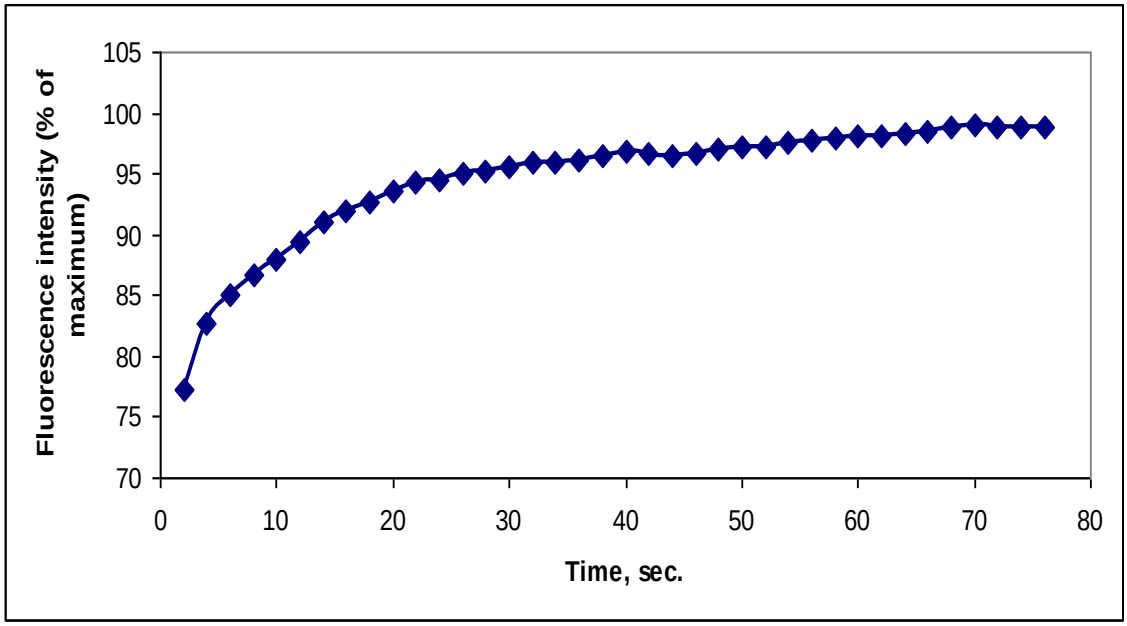
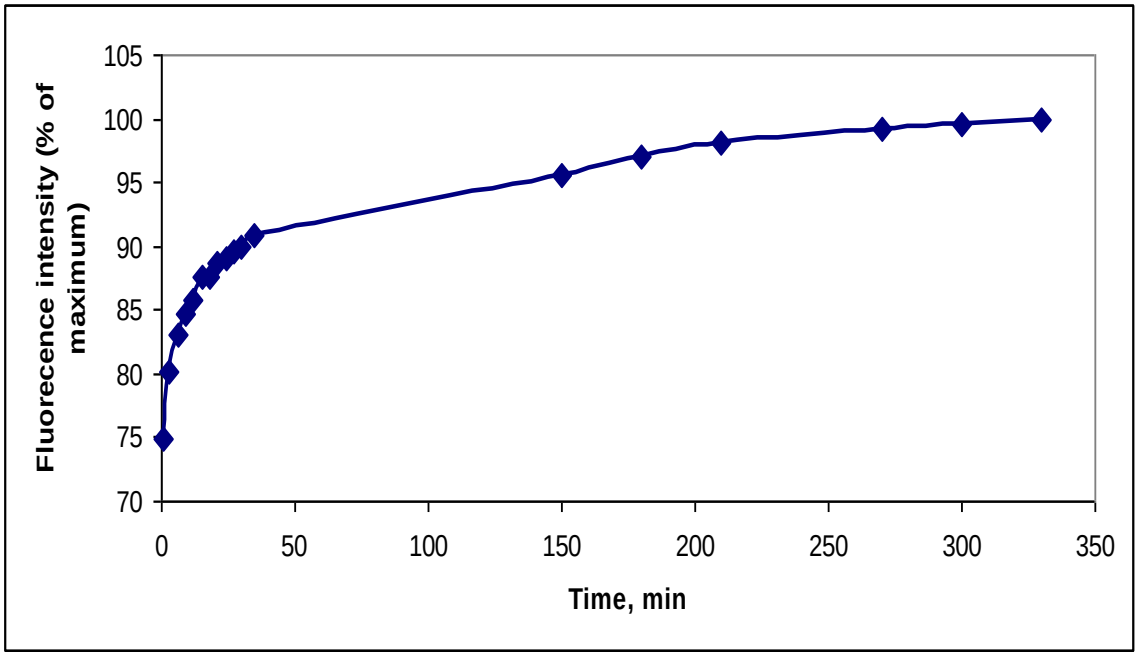


Fig.3. Influence of the time on the fluorescent intensity of the connected synthesized dye YO-Dam-1 with DNA. YO-Dam-1+DNA (50 mg/ml + 25µg/ml), ratio dye: DNA = 1:12,5.



Фиг.4. Influence of the time on the fluorescent intensity of the connected synthesized dye YOYO-1 and DNA. YOYO-1+DNA (50 mg/ml + 25µg/ml), ration dye: DNA = 1:12,5.

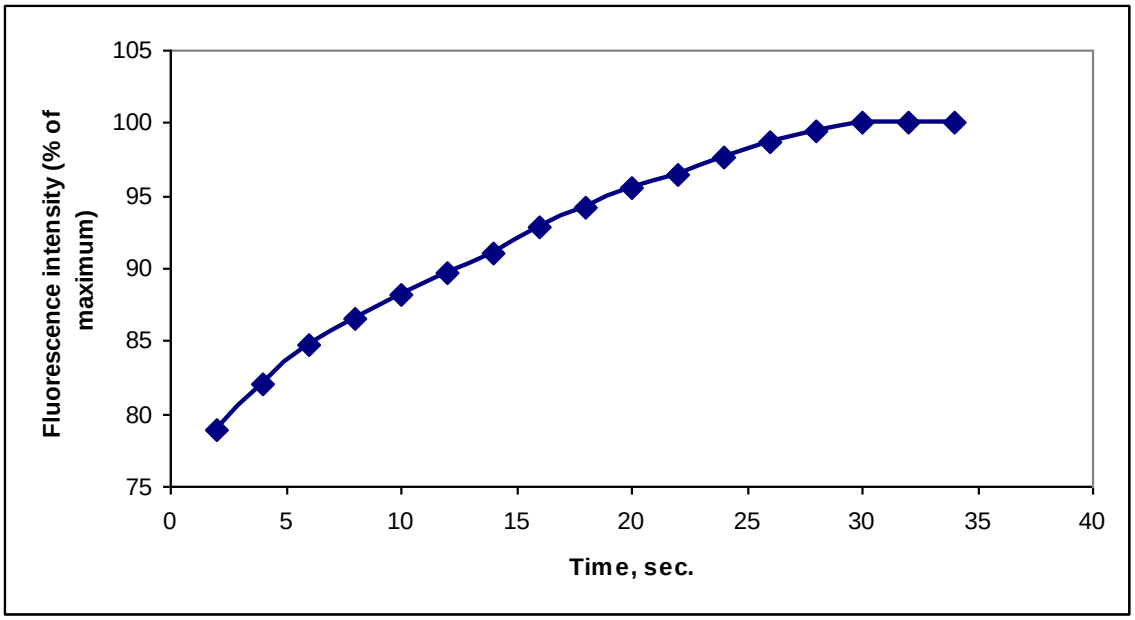


Fig.5. Influence of the time on the fluorescent intensity of the connected synthesized dye Propidium Iodide with DNA. PI+DNA (50 mg/ml + 25µg/ml), ratio dye: DNA = 1:12,5.

The influence of the concentration of DNA on the intensity of the fluorescent signal of the three dyes, measured at 30 seconds is being observed (fig.6). The measurements are made at 30 sec, because the speed of coloring of the cells with the fluorescent dyes is a very important parameter for the analysis. The experiments are made at equal concentrations of the dyes and equal ratio of mixing. The concentration of DNA varies from 5-50µg/ml. From figure 6 it can be seen that the fluorescent intensity of Propidium Iodide is very low at all concentrations of DNA, in comparison with the fluorescent intensity of the other two dyes. So, compared to YO-Dam-1, the signal is around 20 times weaker, and compared to YOYO-1 around 1,5 times, measured at 30 sec. The newly synthesized dye has the highest fluorescence intensity for all the concentrations of DNA at 30sec. For all the three dyes it can be noticed that after 25µg/ml of the DNA, the fluorescent intensity becomes constant around 1,5 times, measured at 30 sec. Therefore, the optimal concentration of DNA is 25µg/ml.

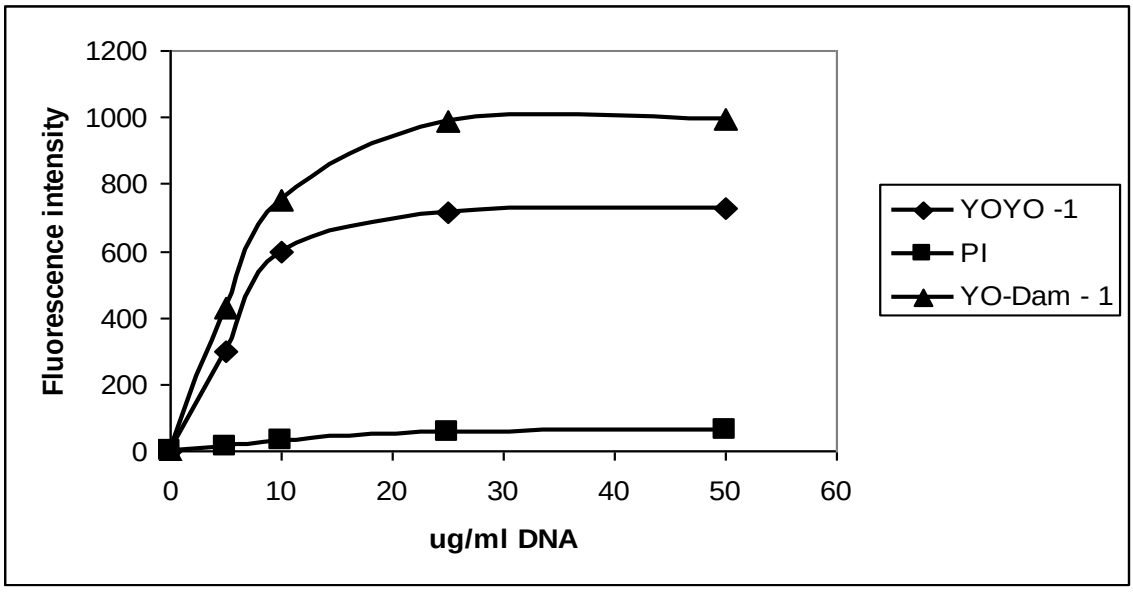


Fig.6. Influence of the concentration of DNA on the fluorescent intensity of the dyes + DNA (50 mg/ml + 25µg/ml), ratio dye: DNA = 1:12,5.

Apart from connecting with the DNA, the newly synthesized dye can be used also for coloring of dead or dying cells – somatic cells (SCC), neutrophils and macrophages. At figure 7 are shown microscope photos of colored with dye macrophages, isolated from the mastitis infected cow, with SCC 900 000 cells/mL of milk and from the healthy cow, with SCC 80 000 cells/mL.

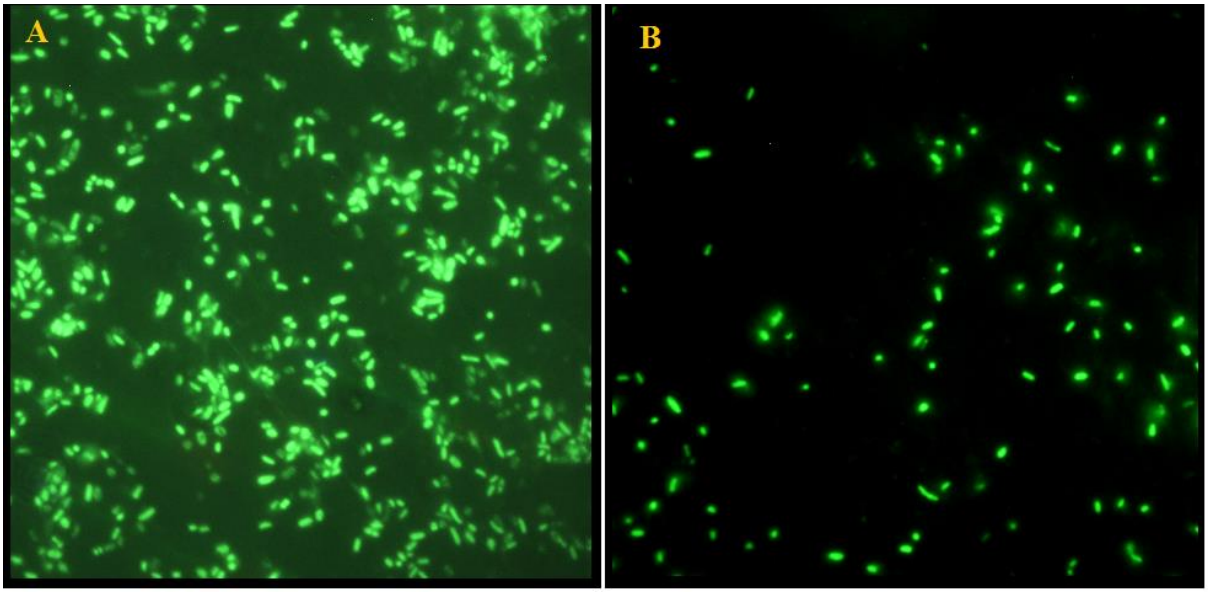


Fig.7. Microscope photos of colored YO-Dam-1 isolated macrophages from the somatic cells with an increase of 1000 x. (A) Mastitis milk with SCC 900 000 cells/mL; (B) Milk from a healthy cow with SCC 80 000 cells/mL

From figure 7 it can be seen that the newly synthesized dye has a very good capacity of coloring the dead cells. So the synthesized dye can be used in a combination with other types of dyes as well, for the purpose of differential counting of dead and live cells.