# RAPID AND EASY ESTIMATION OF APOPTOTIC CELLS FOR CLINICAL DIAGNOSTICS

## Description

Apoptosis (programmed cell death) is a physiological process intended to maintain an appropriate quantity of cells in tissues and organs of the multi-cellular organism. Apoptosis is characterized by a sequence of distinct events ultimately leading to cell death, and is the major process responsible for the breakdown and elimination of cells in tissues and organs. In this way, apoptosis plays a crucial role in the renewal of aged cells and removal of damaged, "sick" and virus-infected cells. The disturbances in this process lead to different pathological states such as autoimmune disorders or cancer.

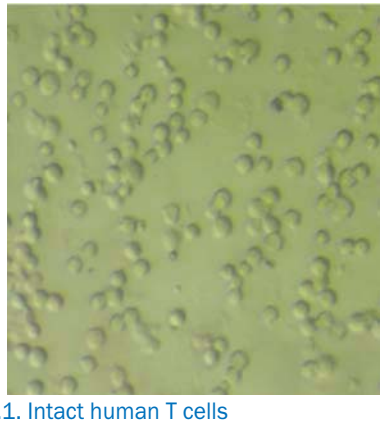
A set of characteristic features attributable to apoptosis were discovered and some of them were used for the development of practical approaches for apoptosis detection. Most of these features belong to biochemical markers of apoptosis, located in the nucleus, cytoplasm or mitochondria of the cell. Measuring such markers inside the cell is time- and resource-consuming procedure.

Recently, we discovered a novel biomarker of apoptosis in the plasma membrane (cell surface) of cell. The utilization of this biomarker for apoptosis detection does not require disruption of cell integrity. We have proved that plasma membrane of the apoptotic cells contains an increased amount of б-D-mannose and в-D-galactose-rich glycoproteins. Such an increase in the level of above mentioned glycoproteins was proved to be a universal feature of the apoptotic cells, independent on their tissue origin, or the way of apoptosis induction. This feature of the apoptotic cells was clearly detected as early as 12 hours after apoptosis induction.

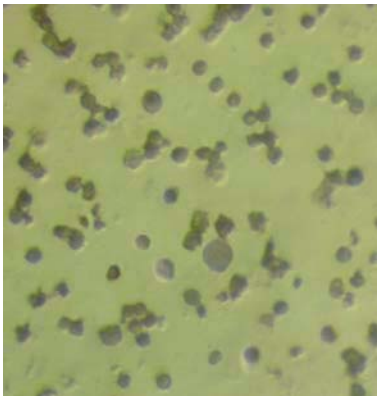
Specific lectins (carbohydrate binding proteins) were applied for selective detection of the mentioned glycoproteins. Before use, they were labeled with horseradish peroxydase, fluorescent dye, or in another way for better apoptotic cell detection. Besides, the nanoparticles conjugated with specific lectin were used for isolation of the apoptotic cells from their mixed population. Lectin-induced agglutination test was also developed for express detection of apoptosis in cell samples in clinics.

## Innovative Aspect and Main Advantages:

Unlike the competitor methods, the proposed technology does not require target cell destruction in the process of apoptosis detection, since it uses cell surface biochemical markers of apoptosis. Besides, the technology provides high speed and high reproducibility of the measurements. Another known biomarker of apoptosis is an externalization of cell membrane phosphatidylserine, described by Fadok et. al., (J. Immunol, 1992.). An ability of protein annexin V to bind
specifically phospatidylserine was used for the development of method for apoptosis detection (US Patent 5,834,196). One analysis within our technology is much cheaper (~0.2 $) then the competitor's Annexin V binding test (~6$).



Pic.1. Intact human T cells



Pic. 2. Apoptotic hu-man T cells,
Detection of б-D-mannose-containing glycoproteins in plasma membrane of the cells (lectin-peroxydase staining).

## Areas of Application:

Express measurement of cell apoptosis for clinical diagnostics (ex. oncology, immunology, others).

## Stage of Development:

The technology is partially protected by the USA Patent Application 67789-368. It was tested at the Department of Immunology and Allergology of the Diagnostic Center. It is ready for introduction.

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