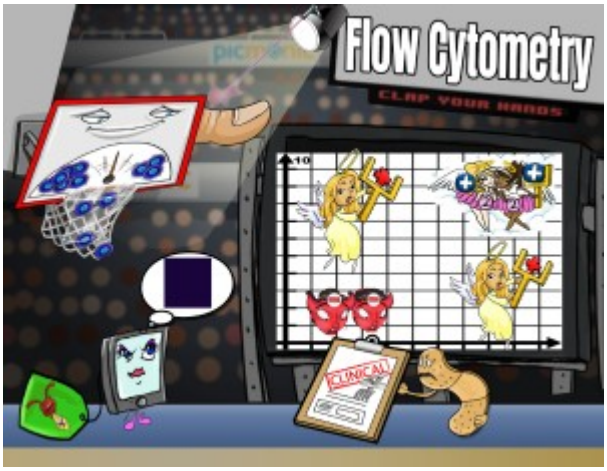
Flow Cytometry



PLAY PICMONIC

Definition

Individual Cell Analysis Technique

Single Cell Analyzing

Flow cytometry is designed to allow the detection and characteristic assessment of individual cells in a sample. It can provide information on cell size, granularity, and immunophenotype.

HOW DOES IT WORK?

Laser and Light Source

Laser and Light

The flow cytometer consists of three systems that enable single-cell analysis. First, the sample is suspended in sheath fluid and passes individually through the fluidic system of the flow cytometer. Next, the sample is pressurized and focused at the optics intercept, where each cell undergoes analysis through collection (light) and excitation (laser) optics systems. The generated visible and fluorescent light signals from each cell are then detected and converted by the cytometer's electronic system into digital signals. These digital signals can be read by the computer and used to analyze the morphological and immunophenotype characteristics of each cell in the sample.

Antibody Tag

Ant-tie-body Tag

Differential protein expression of each cell allows them to be tagged with antibodies specific to surface or intracellular proteins. The antibodies are usually pre-tagged with a unique fluorescent dye, allowing fluorescent sorting of cell populations according to their immunophenotype.

ANALYSIS

Data Plot

Data Plot-chart

Data analysis is the final and most crucial step of the flow cytometry technique. Usually, data is plotted into a two-parameter histogram or scatter plot, allowing for the distinction of different cell populations in the same sample by comparing their immunophenotype regarding the presence or absence of the two compared antibody tag fluorescence.

Furthermore, keep in mind that as several new parameters are being included due to the use of multiple laser systems, as well as technique combination with microscopy and mass spectrometry, amongst others. Flow cytometry data analysis is moving towards the use of data analysis

algorithms for data mining. However, dot plot is still the main conventional data analysis method used.

Double Negative

Double Negative-Devil

Cells in the lower-left quadrant of the two-measure scatter plot are labeled "double negative" because they exhibit a negative detection for both compared antibody tags. This indicates that this group of cells lacks any of the immunophenotype characteristics associated with the compared antibody tags.

Double Positive

Double Positive-angel

Cells in the right upper quadrant of the two-measure scatter plot are referred to as "double positive" because they exhibit positive fluorescence detection for both compared antibody tags.

Single Positive

Single Positive-angel

Cells in the upper left and lower right quadrants of the two-measure scatter plot exhibit positive detection for only one of the compared antibody tags. They are referred to as "single positive" cells, indicating a distinct immunophenotype from double positive or double negative cells, representing a different cell population.

APPLICATION

Clinical Application

Clinical-clipboard Application

Flow cytometry is an important laboratory technique due to its clinical application in diverse areas like hemato-oncology (e.g., diagnosis of leukemia or paroxysmal nocturnal hemoglobinuria) and infectology (e.g., CD4+ cell count in HIV). Flow cytometry serves not only as a diagnostic aid tool but also for disease monitoring.