**PRINCIPLES OF IMMUNOLOGY**

**Introduction**

            In hydrodynamic focusing, when two different fluids with varying flow rate run side by side and in similar direction into a cell, then laminar flow tends to be created. The sample stream is normally focused and surrounded by the sheath fluid. In the process, the shape and the size of the cell are crucial to the process of hydronamic focusing and conventionally, the cell is normally shaped like a nozzle. However, the designers of the flow cell tend to engineer the microscopic grooves together with the location of the nozzle to channel the sheath fluid for purposes of surrounding the focus stream (Rose, Milgrom & Van Oss, 2014). In flow cytometer, the fluid pressure of the sheath is normally constant at the same time the same fluid is normally adjusted. Hence, when one manipulates the pressure differences, one can easily get the right cross sectional region. The function of the hydrodynamic focus is to align the cells in one to one measure at the point where the laser analysis starts. Without this crucial step, the flow cytometer will not be able to achieve the right resolution. Therefore, it is highly advised that one should never change the data’s resolution (Cushing & Campbell, 2010). Along similar case, the mechanical problems that happen in the pump that is meant to deliver a constant pressure will lead to similar issues. Therefore, the current flow cytometers are normally equipped with double pumps to make sure that the pressure and rate of fluid flow is consistent.

**Background and Principles**

             Flow cytometry is one of the valuable instruments and tools used in analyzing both the physical and the chemical features of a cell. The laboratory methods and features uses the antibodies which are highly conjugated with the fluorochrome for analyzing cells. The process of developing the specialized flow cytometer started when the HIV pandemic hit the world. The process pave way to a wider application of the concept like the modification of research, immunophenotyping, and analyzing the intracellular antigens. Monoclonal antibodies is one of the valuable components of a flow cytometry. They are normally generated to fight biological molecles like the glycolipids. The development of those antibodies helps in detecting the activation states of protein which has enabled people to use the flow cytometries in studying the function of cells (Hall & Yates, n.d.). Because the monoclonal antibodies have the ability of recognizing and binding the antigenic epitopes, they are normally used in identifying various molecules that have specific epitopes.

**Method**

During the practical, the team ensured that the safety glass, laboratory gown, single use lancet, and a blue flow cytometer were present. A site on a finger was selected for capillary puncture. The finger was the gently massaged 4 to 5 times to help in the flow of blood. Alcohol swab was then used in cleansing the selected site on the finger for purposes of removing the surface bacteria. With a help of a single use lancet, skin was then punctured and then discarded to a yellow sharps container. The finger was then squeezed in order to stimulate the flow of blood. This was done through the milky action. The finger was then placed with the drop of blood over the top of the cytometry that contains PBS (Hawley & Hawley, 2011). PBS was then used in washing blood in the tube. In a situation where the blood spills, the area was decontaminated immediately. At least one percentage of blood in the cytometry tube was gotten. The adhesive bandages were available to tape the finger at the punctured region. Immediately, the gloves were put on after the finger prick procedure was done.

During the immunophenotyping activity, two blue flow cytometry were taken and labeled in a clear manner, the first tube was labeled one while the other was labeled as 2. 250 micro liters of the diluted blood were added to the two tubes. 5 micro liters of the first reagents was added to the first tube and also to the second tube. The caps or strips of the sticky tapes were applied to close the tops of the two tubes. The tubes were then incubated in a rack for purpose of protecting it from the sunlight for a period of fifteen minutes. The lysing solutions were then added to the tubes and then gently mixed. In the process, the red cells were checked if they have actually lysed before adding the paraformaldehyde solution. The tubes were then run on the cytometer flow.

The FC500 flow cytometer had two lasers, comprising of the 488nm which is blue green and 635nm which is a red one. The FC500 analyzed the five fluorochrome colors over the two laser spectrums with their respective laser configuration together with their forward and their side scatter. Each of the measurements was perceived to be parameters. During the experiment, blood samples were stained and later analyzed for the lymphocyte surface markers from the T, B and NK subsets.

**Table of Results and Interpretation**

From the results, T-Sum was the percentage summations of CD3’CD4’ and CD3’CD8’ which were equal to the number of CD3. In terms of lymphosum, the total lymphocyte was percentage of CD3+ CD19 + CD3CD56+. The lymphocyte % should be greater than 95%. CD3’ intrapanel check differences between CD3’ cell percentages in replicate tubes should be less than 3.5 percentages.

**Discussion**

        Flow cytometry is the process of analyzing cells and molecules in liquid suspension thus the use of flow and cytometry. Using laser lights and fluorescence with the help of a flow cytometer, various parameters and properties of cells was determined within a shorter time. Immunophenotyping was used in finding out the availability of the leucocyte surface molecules commonly known as the markers. The monoclonal antibodies were tagged with stains especially to the suspension of the cell, the laser lights were scattered and then used in staining the cells at a given wavelength for fluorochrome. The practical used various monoclonal antibodies which were calibrated with various fluorochromes that emitted fluorescent light at a given wavelength. Various parameters were determined from the cell samples. To enable individual investigation cells and particles passed the single file (Darzynkiewicz, Robinson & Crissman, 1994). This happened in the analyzer’s flow cell with various pressures of surrounding the fluid forces in to a hydrodynamic focusing. Hence, the suspension of the cell needs to be free of the clumps of tissue. The fluorescence labeled antibodies was used in identifying the cell antigens, the internal complexity of the cells were determined by the scattering of laser beams. Light scattering happened when cell deflected the laser light. The forward scattered measured as a surface area determinate. The side scatter and the forward scatter provided enough information in order to be separated and getting to know the leucocyte populations on the plot.

**Conclusion**

In order to detect the molecules that are found in the cell surface, the fluorescent dyes were conjugated into a monoclonal antibodies for such molecules. The cell molecules like this were assigned a cluster of differentiation that seeks to catalogue their properties. The specific monoclonal antibodies detected cells in the sample based on what markers are assigned in the sample. Various fluorochrome are present, each are different for the given wavelength at the absorption spectrum and the wavelength where the fluorescence are emitted which is commonly referred to as the emission spectrum. The number of fluorochromes was confined by the signal detectors found at the flow cytometer and reduction of the spectral overlap that exists at various fluorochromes. The necessary selection of the monoclonal antibodies and the fluorochromes allowed for various markets in order to be detected and then compared in a single sample when it is incubated in a single tube.

**References**

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