# Cell Cycle Effect on HLA expression in HTB-4 and MRC-5

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## Abstract

Human Leukocyte Antigen (HLA) is a gene that codes for cell-surface proteins that are the basis of our bodies' immune response. We observed two cell lines, a cancerous HTB-4 line and a non-cancerous MRC-5 line. Our purpose for this experiment was to observe the effect of the cell cycle on HLA expression for these cell lines. Two flasks were prepared for each cell line: one confluent and one non-confluent. Flow cytometry analysis was performed for each of the four flasks. The flow cytometry results of the MRC-5 cells indicate increased HLA expression in the confluent flask, in the G1 phase of the cell cycle. A smaller amount of expression was found in the 48-hour group. Analysis of the HTB-4 cells revealed that there was more HLA expression in the confluent flask as well. These results show that for both cell lines, HLA appears to be expressed more in confluent cells that are not undergoing large amounts of proliferation. This suggests the cell cycle does have an effect on HLA expression for these cell lines. We would like to repeat it to verify our results and use this new information about HLA expression in drug treatment experimentation.

## Objectives

HTB-4 is a cell line originating from transitional cell carcinoma; it is characterized with hypodiploidy to hypopentaploidy. MRC-5 is a cell line originating from the lung tissue of a healthy fetus and the cells should all be diploid.

The goal of our project was to observe how HLA expression can vary between a cancerous and a non-cancerous cell-line, as well as how HLA expression can change in a nonconfluent and confluent cultures.

Class I HLA expression was compared for cells isolated from non-confluent and confluent cultures. Non-confluent cultures contain many cells in the $G_1$ , S, and $G_2$/M phases of the cell cycle. Confluent flasks tend to contain cells in the $G_1$ phase, especially normal cells that become contacted inhibited at confluency.

We expected that the non-confluent cultures would have higher HLA expression due a higher cell division rate. However, we were also interested in viewing how HLA is expressed in a potentially lower amount in a cancer cell line compared to normal cells.

## Materials & Methods

**I. Preparation of MRC-5 and HTB-4 Cultures**

We prepared two flasks of cells for both the MRC-5 and HTB-4 cell lines, one of which for was fully confluent on the day of the experiment and another for which a 1:3 split was performed 48 hours prior to experimentation such that when the experiment was run there was still ample room for cell proliferation.

**II. Preparation for Flow Cytometry**

On the day of the analysis, all 4 flasks were harvested and a cell count was performed. Using our obtained counts, 1,000,000 cells for each culture were aliquoted into two tubes. The cells were spun down at for 10 minutes at 300g.

**III. Staining of Cells for Flow Cytometry**

The cells were resuspended in 50 µl of flow buffer and 5 µl of FITC conjugated Anti-HLA ABC antibody or control antibody was added to each tube. The tubes were incubated on ice for 20 minutes, protected from light, before centrifuging at 300g for 5 minutes. The supernatant was then removed, 2 ml of flow buffer was added and the spin was repeated. This step was repeated 3 times at which point the solution was resuspended in flow buffer.

**IV. Flow Cytometry Analysis**

At this point, 10,000 cells from each tube were run on the flow cytometer detecting the amount of green fluorescence, thereby allowing us to quantify the amount of Class I HLA ABC expression on each cell population.

## Results

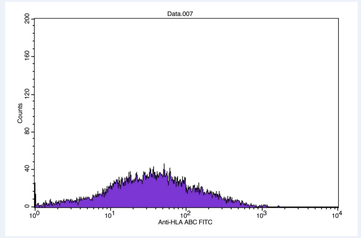


**Figure 1a:** HTB-4: 48 hr + antibody. A mean log value of 70.14 was found for the Anti-HLA-ABC antibody

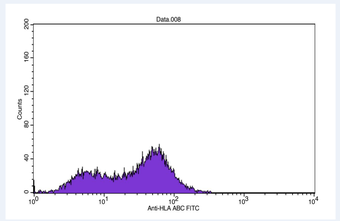


**Figure 1b:** HTB-4: confluent + antibody. A mean log value of 39.56 was found for the Anti-HLA-ABC antibody

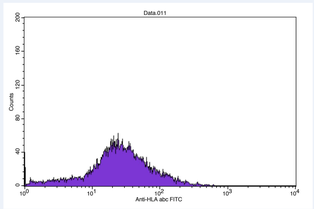


**Figure 2a:** MRC-5: 48 hr + Antibody. A mean log value of 38.81 was found for the Anti-HLA-ABC antibody

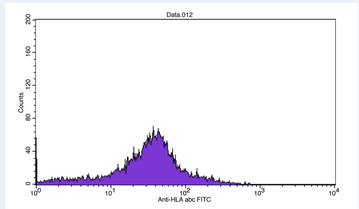


**Figure 2b:** MRC-5: confluent + antibody. A mean log value of 45.67 was found for the Anti-HLA-ABC antibody

## Conclusion

Flow cytometry analysis of the MRC-5 cells illustrated in Figure 2, a and b, indicates an increased HLA expression in the confluent culture compared to the sub-confluent culture. Upon analysis of the HTB-4 cells illustrated in Figure 1, our results showed two peaks of HLA expression for the confluent culture, with the larger peak appearing to be higher than the peak displayed by the 48 hr sample. These results appear to show that for both cell lines, Class I HLA ABC expression is increased in cells from confluent cultures compared to cells isolated from sub-confluent cultures. This leads us to hypothesize that the cell cycle does have an effect on HLA expression for these cell lines where cells in the $G_1$ phase express the HLA proteins more than those in, S and $G_2$/M portions of the cell cycle.

After completing this experiment we would like to perform it multiple times to confirm our results. This experiment was intended to give us an idea of the effect of the cell cycle on HLA expression so that we could begin experimentation on these cell lines with multiple drugs and observe their effects on expression.

## References

Lewis, Jani. *Preparing Cells for PI/FACS Cell Cycle Analysis*. 2018.

"MRC-5 (ATCC® CCL-171™)." *MRC-5 ATCC ® CCL-171™ Homo Sapiens Lung Normal*, www.atcc.org/products/all/CCL-171.aspx.

"T24 (ATCC® HTB-4™)." *T24 ATCC ® HTB-4™ Homo Sapiens Urinary Bladder Transitional*, www.atcc.org/products/all/HTB-4.aspx.

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