

# Detection of Viral Infections using Flow Cytometry and Molecular Beacons

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## Introduction

Detecting viruses is crucial for the prevention of an infectious disease outbreak. In this project, we are investigating the use of molecular beacons (MB) and flow cytometry as a high-throughput method for detecting an RNA virus in live cells. Molecular beacons are dual-labeled, hairpin oligonucleotide probes designed to fluoresce only when they hybridize to a complementary target. The virus used in this study is bovine Respiratory Syncytial Virus (bRSV), a known model for human respiratory syncytial virus (hRSV) and is an important pathogen in infants [1, 2]

## Materials and Methods

### Cells and Virus

Primary bovine turbinate (BT) cells were grown in DMEM with 5% horse serum and 5% goat serum with 100 U/ml of penicillin and 100 mg/ml of streptomycin. Cells were infected with a multiplicity of infection of 0.2 at a confluence of approximately 50-70 percent and the flow cytometry experiments were performed 7 days post-infection.

### Molecular Beacons and Fluospheres

In order to enhance the sensitivity of the measurements, two MB probes were used for this study: 5'-Cy3-CGACGAAAAATGGGGCAA *TACGTCG*-BHQ-2-3', and 5'-Cy3-CCATGGGGCAAATAAAT*CATGG*-BHQ-2-3' (stem is underlined and hybridization domain is in italics). They designed such that the first MB targets several repeated sequences that occur within the critical gene-end-intergenic-gene-start signals of the hRSV and bRSV genome and the second hybridizes to a nearby region. Having both beacons binding to the same RNA strand allows for a significant increase in fluorescence signal. Molecular Probes, 22 nm diameter, Fluospheres (Nile Red) were used as an intracellular positive control for these studies and were delivered to cells using the same method as the beacons. Nile Red and Cy3 can both be excited at 488 nm and fluoresce at 575 nm.

### Molecular Beacon and Fluosphere Delivery

MBs and Fluospheres were delivered into living cells using a reversible permeabilization method with streptolysin O (SLO)[3]. Cells were incubated with a mixture of 0.2 U/ml of SLO and 2  $\mu$ M of MB or a 1/100 dilution of the stock Fluosphere concentration, in an appropriate amount of serum-free medium for 10 min at 37°C. The SLO/MB/serum-free medium was then removed and replaced with fresh, normal medium. The cells were then incubated at 37 C for 30 minutes prior to being prepared for flow cytometry. After the 30 min., the cells were trypsinized and resuspended in 1 ml of PBS.

## Results and Discussion

Two sets of experiments using the BD LSR flow cytometer were performed; 3000 cells were used for each histogram. The first involved flowing uninfected

cells and uninfected cells with Fluospheres delivered to them. The results can be seen in Fig. 1A and show a factor of 2 increase in the mean of the intensity histogram. In the second experiment, uninfected cells, uninfected cells with MBs delivered to them, and infected cells with MBs delivered to them were also interrogated with the flow cytometer. These results can be seen in Fig. 1B. It can be seen that there is only a 10% increase in fluorescence from the MBs within the uninfected cells but a 60% increase in the fluorescence from the infected cells, therefore detecting the virus. The beads were used as to verify successful delivery and in future studies, dilution assays will be used as a fluorescence standard to quantify the fluorescence from the MB samples.

## Conclusions

Even though there was an increase in the intensities from the infected cells, a high signal-to-noise ratio is necessary to make this assay clinically worthwhile. One reason for the low sensitivity is the autofluorescence induced by the excitation at both 325 and 488 nm, which are always on during the experiments. Future experiments will be performed using Cy5 (633/680) labeled MBs and Dark Red Fluospheres to minimize the effects of autofluorescence.

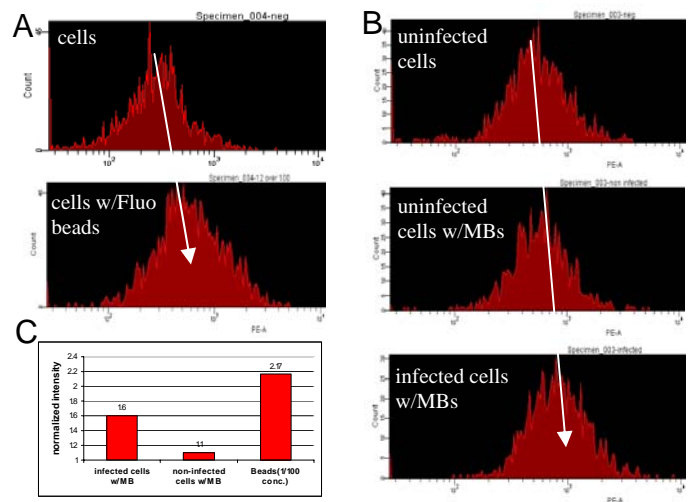


Figure 1. A) Flow results from the intracellular delivery of 22 nm Fluospheres beads. B) Flow results from BT cells, uninfected cells with MBs, and infected cells with MBs. C) Flow cytometer mean intensities normalized to cells without beads or MBs.

## References

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