

Development of non-biohazardous p24 antigen expressing cell mimics as a reference control alternative to HIV+ donor samples

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ABSTRACT

Flow cytometry provides a practical and rapid way to monitor anti-retroviral therapy and predict disease progression. However, the traditional approach of using HIV-infected cells as reference controls presents enormous bio-safety risks to lab personnel, which results in the need for costly bio-safety equipment and segregated lab space. Moreover, patient donor blood can introduce variability and limit consistency in results when used as a reference control. HIV-1 p24 expression can be used as a marker for translation-competent HIV-infected cells. However, low or variable expression of p24 antigen in patient samples also poses an additional challenge to reproducible results.

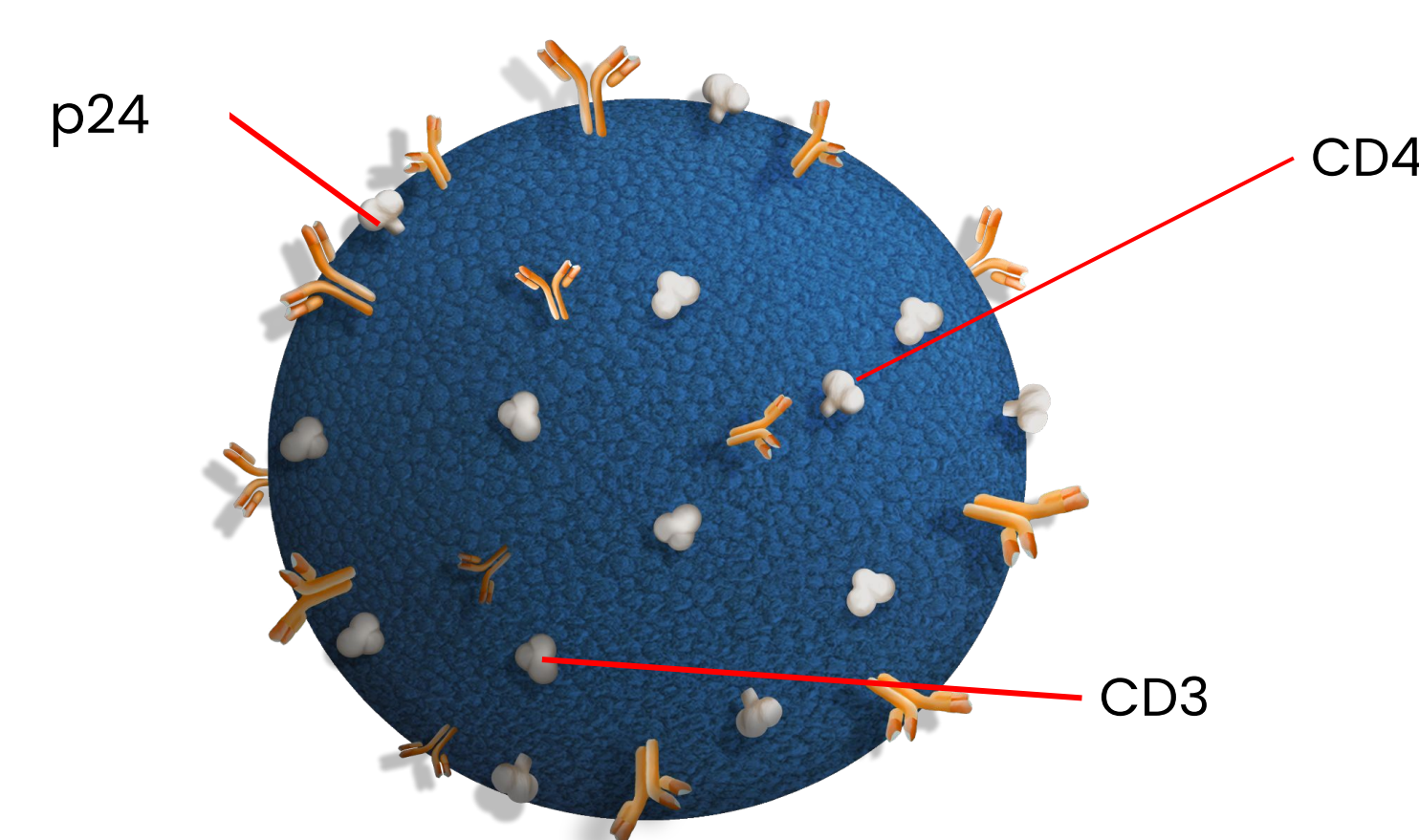
To address these challenges, we leveraged the principles of biochemistry, high-precision manufacturing and polymer chemistry to engineer a p24-expressing, non-infectious T cell mimic that could safely, reproducibly, and conveniently be used as a cellular reference control for detecting p24 antigen-positive T-cells in HIV+ patients. Here we show that we were able to generate varying p24 antigen expression levels as well as match the antigen density of patient samples. In addition to p24 the engineered cell mimic also expressed CD3 and CD4 for T-cell identification. These custom cell mimics can improve safety in the laboratory by reducing opportunities for accidental infection. Furthermore, they eliminate the need for bridging studies and cell line maintenance, a vast improvement and significant cost savings over the use of traditional cell-based reference material, which is subject to variable marker expression as seen among blood samples from different donors or cell lines.

INTRODUCTION

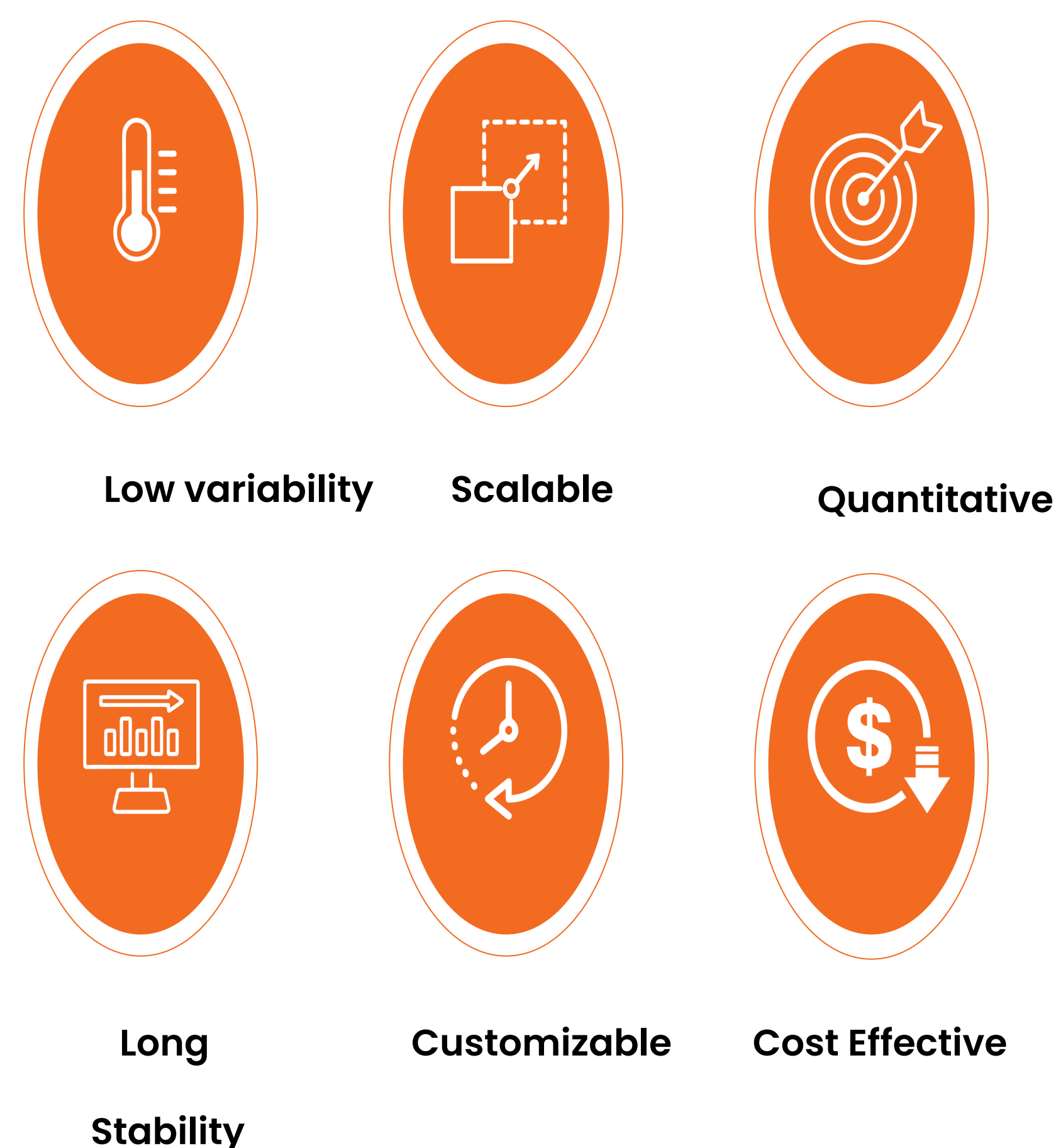
- HIV research requires the use of translation-competent HIV-infected cells commonly sourced from viremic HIV+ blood donors, thereby presenting biohazard risks and limitations around sample variability.
- It is time consuming and costly to acquire and store viremic HIV+ donor blood or create biological p24-antigen-expressing cell lines. This often includes bridging studies that require extensive regulatory paperwork and reporting
- Slingshot utilizes next-generation technology to create cell mimics that address these challenges and support the advancement of HIV therapeutics

SLINGSHOT TECHNOLOGY

Slingshot Biosciences produces TruCytes™ synthetic cells with tunable size, morphology, and biochemical profiles that can be matched to any cell type. Using this technology, the Slingshot team produced a synthetic T cell mimicking p24, CD3, and CD4.



Clear Benefits over HIV 1+ Donor Control



RESULTS

As shown in Figure 1, Slingshot Biosciences was able to match the desired antigen expression levels of p24. The Slingshot team also generated varying concentrations of p24 with different antigen expression levels on p24+ TruCytes (Figure 2).

The availability of Slingshot's engineered T-cell p24 antigen expressing synthetic cells eliminated the need to use HIV+ patient donor blood as an assay reference control. These custom synthetic cells improved safety in the laboratory by reducing opportunities for accidental infection.

The previous method of analysis required the use of HIV+ viremic T cells and involved extensive processing, including a magnetic enrichment step to isolate T cells from whole blood samples. This process takes significant effort and time which together bears high reagent costs.

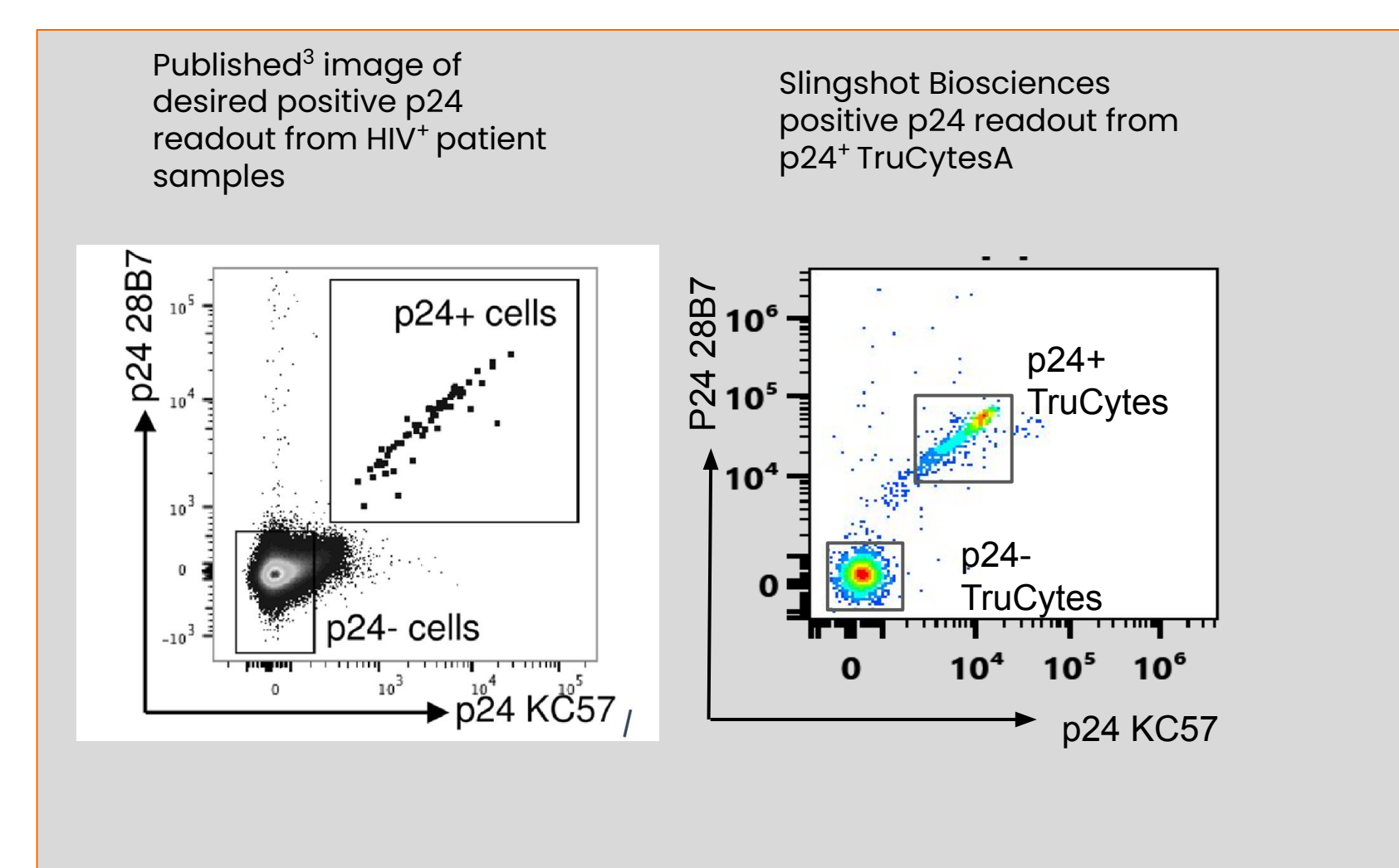


Fig 1. Slingshot Biosciences generated a p24+ synthetic cell line that matches the desired scatter profile and p24 antigen expression.

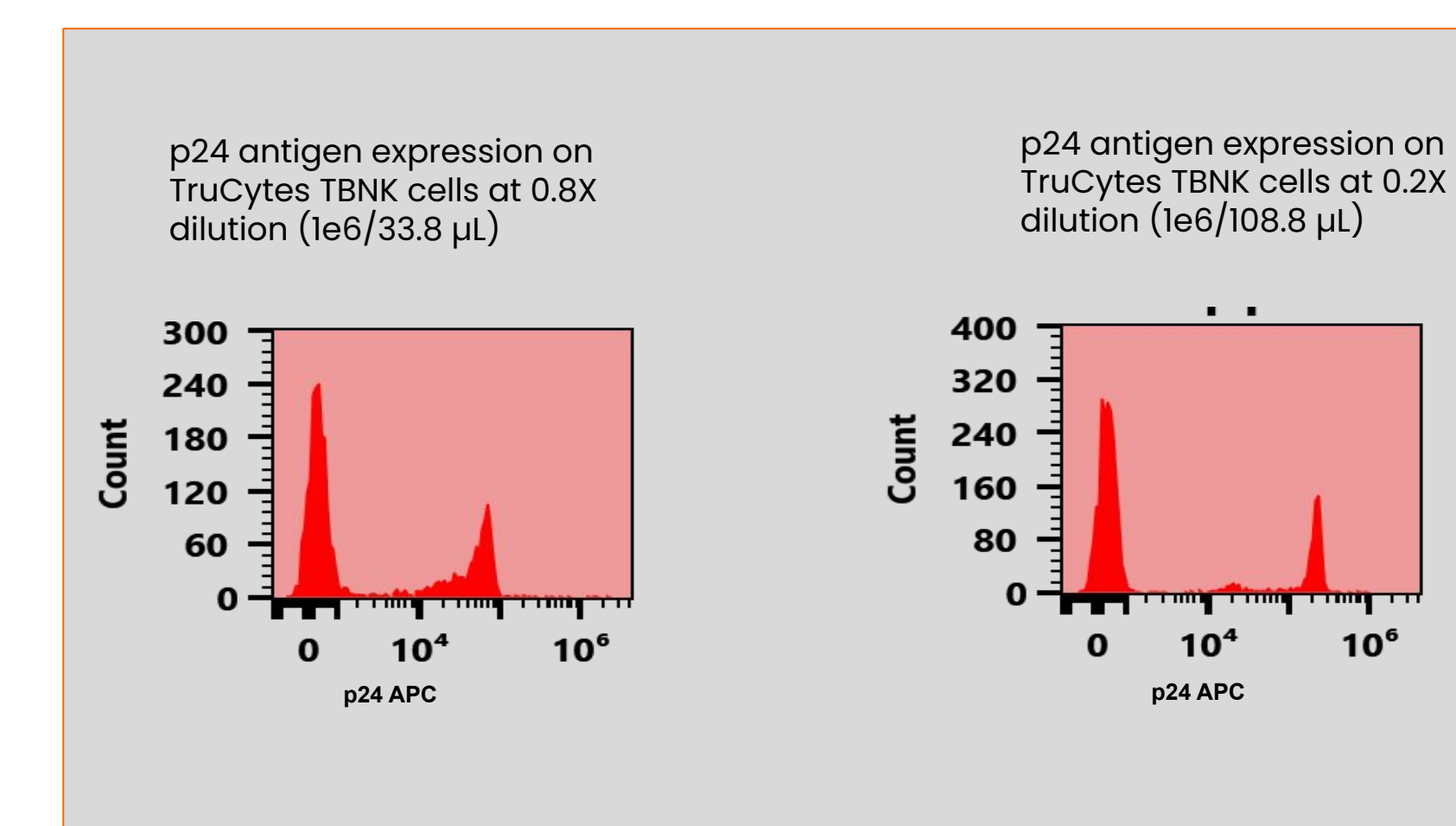


Fig 2 Expression of p24 antigen at two different concentrations

CONCLUSIONS

- Slingshot p24+ synthetic T cell mimics are lyophilized synthetic cells that are quickly reconstituted in a buffer, saving considerable time, effort, and cost.
- Operator safety was improved by eliminating the risk of infection during processing and handling.
- The market-leading lot-to-lot consistency of Slingshot's cell mimics has the added benefit of yielding a stable signal over time, eliminating the need for bridging studies and cell line maintenance

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