# PINWHEEL ASSAY FOR COST EFFECTIVE AND LABEL FREE ENUMERATION OF CD4+ T LYMPHOCYTES

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## ABSTRACT

CD4+ T cell count indicates the progress of HIV infection in patients and the effectiveness of antiretroviral therapy (ART). State-of-the-art CD4+ counting methods based on flow cytometry are too taxing for resource-limited regions [1], due to their high technique requirement and cost. Here we present a cost-effective and label-free method, integrating immunomagnetic separation and the pinwheel assay [2], to enumerate CD4+ T cells. Results show that the new method correlates closely with flow cytometry ($R^2$=0.997).

**KEYWORDS**: Cost Effective, Label-free, CD4+ T Lymphocytes, Immunomagnetic Separation

## INTRODUCTION

Worldwide, over 33 million people have been diagnosed with HIV infection, and over 2/3 of the patients live in resource-limited regions. To monitor the disease progression, CD4+ T lymphocytes, which are a type of T cells that express CD4 molecules, need to be enumerated periodically. When this cell count drops below 350 cells per microliter of blood (indicating they are immune-compromised), antiretroviral therapy (ART) should be initiated [3]. The current gold standard for cell counting is flow cytometry. However, the high cost and technical requirements make it inapplicable in resource-limited regions. The urgent need for affordable and technically-simple CD4+ diagnostics is widely recognized. Polymeric DNA will absorb to silica-coated particles under chaotropic conditions and form aggregate given enough DNA.[4] The more DNA present, the tighter the binding and, therefore, the smaller the aggregate size. The DNA concentration can be determined by the aggregate size, and knowing that each cell of the same type contains the same mass of DNA, the cell counting can be determined. A rotating magnetic field (RMF) is used to enhance the rate at which silica-coated magnetic beads intertwine with DNA strands (Figure 1).

## EXPERIMENTAL

CD4+ T cells were first isolated by immunomagnetic separation (Figure 2). Purified CD4 +T cells were then lysed by guanidinium chloride, and the released DNA was quantified by pinwheel assay [2]. The CD4 count was calculated based on the DNA content.

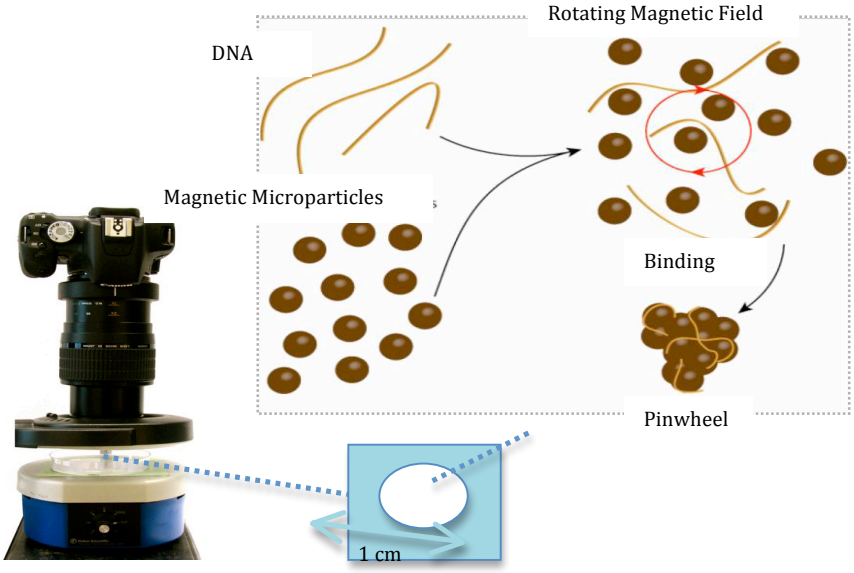


***Figure 1. Pinwheel set up and chemistry**. A high-resolution camera was applied for image capturing and a stir plate was used to generate RMF. The upper right cartoon shows DNA strands absorb on silica coated magnetic microparticles when guanidinium chloride, a chaotropic salt is at presence, and under RMF DNA and magnetic particles will intertwine and form aggregate.*

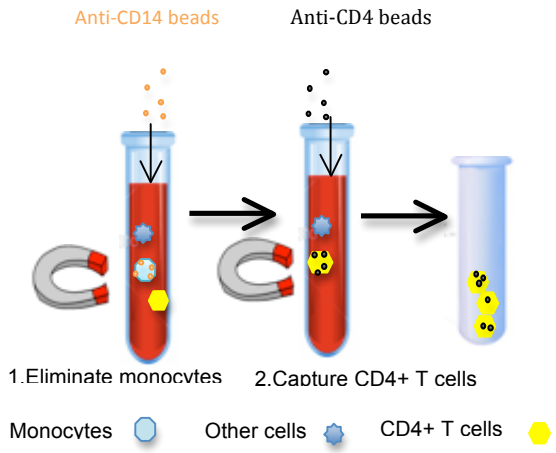


***Figure 2. Schematic of the isolation procedure.*** *1. Depletion of monocytes with anti-hCD14 coated magnetic beads. Monocytes also express CD4 molecules, therefore the major contaminant and were removed at the first step. 2.Positive isolation of CD4+ T cells with anti-hCD4 coated magnetic beads. Captured CD4 T cells were then lysed by 8M GndCl solutions to release DNA.*

## RESULTS AND DISCUSSION

Figure 3 shows cells captured successfully by antibody-coated magnetic beads under microscopy. The DNA concentration correlated inversely with the aggregate size, i.e., the larger the [DNA], the tighter the aggregates and the smaller dark area (Figure 4); the dark area is defined as numbers of pixels that make up the aggregate area, dark area% is normalized against a negative control without DNA but otherwise the same solution. The pinwheel assay was calibrated against a hemocytometer to correlate DNA concentration with cell numbers. Experiment results show an excellent correlation between the pinwheel assay and flow cytometry ($R^2$=0.997) when blood samples are analyzed within 10 hours after collection (Figure 5); aged samples (>48 hours) are poor targets for immunocapture, compromising the isolation efficiency[5] and, therefore, less desirable results are obtained ($R^2$=0.775). However, pinwheel results are still

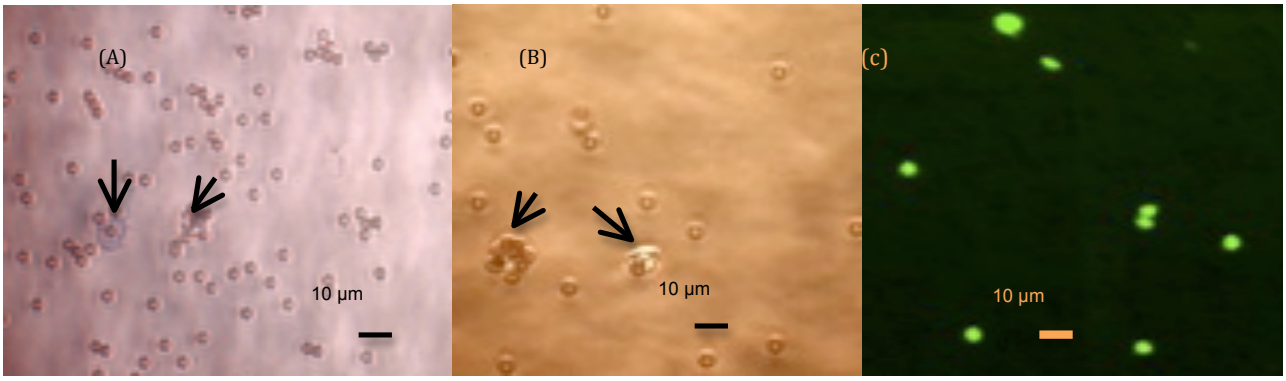


***Figure 3. View of captured cells.*** *A. Captured monocytes under bright field microscope, arrows point to cells enwrapped by beads. B. Captured CD4+ T cells under bright field microscope. C. Isolated CD4 T cells stained by Acridine orange under fluorescence microscope. From A and B we can see the ratio of capturing beads and cell was kept high to ensure efficient cell capture.*

comparable to the hemocytometer results ($R^2$=0.971), which were processed within hours of receiving blood samples (Figure 5B), indicating a robustness for the pinwheel assay. Of 21 blood samples received and analyzed (fresh and aged), using a threshold at 350 cells/μL (the critical value to determine whether to initiate ART), only one sample was called as false positive (Figure 5D); this makes the pinwheel assay 95% accurate relative to the gold standard flow cytometry.

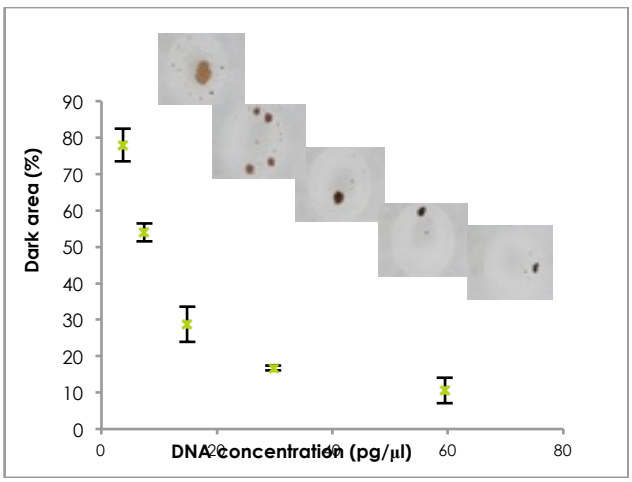


***Figure 4. DNA calibration curve****. More DNA winds micro-particles tighter and forms smaller aggregate as can be seen from the pictures. These pictures were taken by a high resolution camera and processed by a Mathematica algorithm which picks up the dark area in these pictures. Dark are in each picture was normalized over a negative control that contains no DNA. N=3*

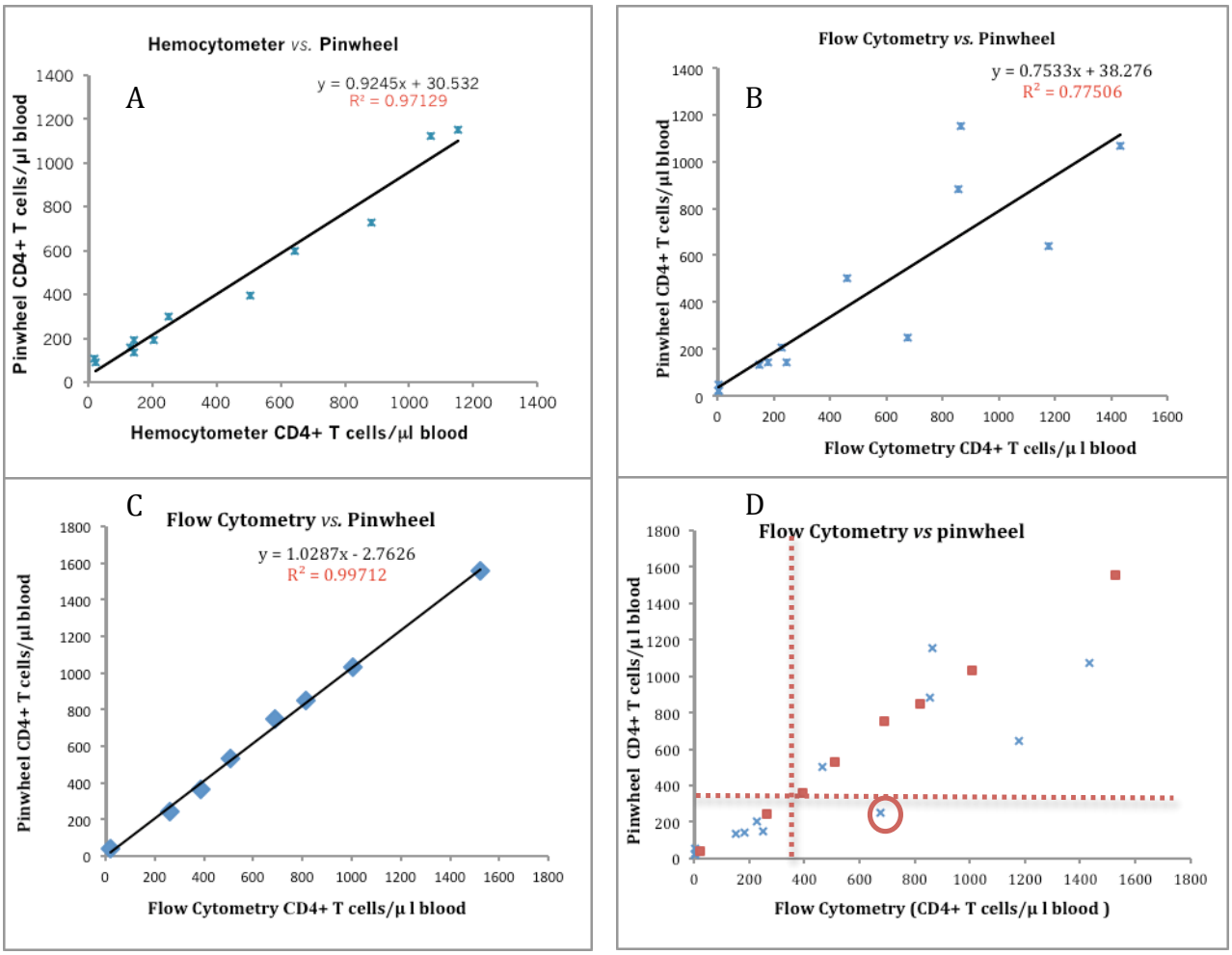


***Figure 5. Pinwheel assay CD4 results A****. Comparison of pinwheel assay versus Hemocytometer shows good correlation ($R^2$=0.971). After isolation steps the captured CD4+ T cells were divided into two aliquots, one was enumerated by hemocytometer while the other quantified by pinwheel assay. Blood samples > 48 hours.* ***B****. Same blood samples as in A, correlation of pinwheel assay versus flow cytometry ($R^2$=0.775). Flow cytometry data were obtained in Mayo clinic while the pinwheel assay results were acquired after receiving samples. A comparison of 5A and 5B indicates the immunolcapture step was the source of discrepancy because samples have degraded and become poor targets.* ***C.*** *Flow cytometry and pinwheel data correlates excellent ($R^2$=0.997) when blood samples are fresh (within 10 hours).* ***D****. The red circle indicates the only false positive sample out of 21 samples studied.*

## CONCLUSIONS

In conclusion, the pinwheel effect is shown to be effective for accurately quantitating CD4+ T lymphocytes in a label-free manner. The low cost and easy-to-operate features of the pinwheel assay rivals with flow cytometry and, hence, is considered a promising alternative for $CD4^+$ T cell counting in resource-limited regions.

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