



# EVALUATION OF A SINGLE AUTOMATED PLATFORM FOR THE STANDARDIZATION OF FUNCTIONAL CELL BASED ASSAYS IN PLATES

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## ABSTRACT

The use of cell-based assays in high throughput drug screening as well as biologic development in both research and clinical trial settings has grown due to their relevance to *in vivo* settings. Although there are an increasing number and diversity of functional cellular assays, the utility of these assays has not been fully recognized due to their highly variable nature. The variability arises from a variety of factors ranging from source of the cells, kind of assay, sample processing methodology (isolation, freezing, thawing, and culturing), sample staining protocol and ultimately the bioinformatics.

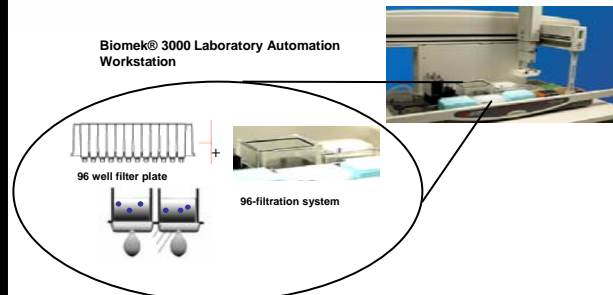
With a view to standardizing the preparation of functional cellular assays in plates, the Biomek3000 automated workstation was evaluated. Untreated peripheral blood mononuclear cells (PBMCs), were fixed and permeabilized on the Biomek 3000 using a validated protocol for intracellular cytokine analysis. The versatility of the platform enabled the integration of the MultiScreen HTS Filter Manifold for non-centrifugal washing and recovery of cells in suspension. To optimize the wash protocol, combinations of many filtration plate formats from Millipore (membrane type, pore size) and mixing mechanisms to recover cells from the filter were also evaluated. The optimum conditions for the assay included the use of PVDF filter membranes with a 0.45µm pore size. The assay enabled cell recoveries of >80% without selective cell loss. The precise pipetting, timing, and washing of cells in suspension on a single platform, in the absence of centrifugation, greatly improved sample processing uniformity and reduced the variability inherent to functional assays as compared to the manually performed procedure. Additionally, the automation enabled a significant reduction of "hands-on" sample preparation and analysis time. The use of automation thus provides a greater degree of standardization in these functional assays, allowing for their use in applied research settings as surrogate markers of efficacy or activity.

## INTRODUCTION

Flow cytometry continues to gain acceptance as an analytical technique for drug screening assays because of its flexibility to simultaneously query genomic, proteomic, and functional profiles in individual cells of *in vitro* model systems. Intracellular cytokine assays are one example where the flow cytometry is used to determine (qualitatively and quantitatively) the response of specific cell phenotypes to immunological challenges. This is a question asked often in vaccine-candidate immuno-toxic clinical trials that require large number of test samples to be processed. This application, in part, has stimulated increasing interest in transitioning common flow cytometry protocols into the field of high throughput testing. As a result, new flow cytometers are able to process samples in 12x75mm test tubes as well as different configurations of 96-well plates. Similarly, sample preparation processes are transitioning from manually driven steps to automated instrument platforms.

This document focuses on the technical challenges and approaches to transition sample washes in 12x75mm test tubes to 96 well plates. Sample washes are common steps in flow cytometry protocols whereby soluble interfering components and/or fluorescence background are separated from fluorescently-labeled target cells via centrifugation, removal of suspension media, and re-suspension of cell pellet in desired solution. The automation of this operation is investigated using the filtration manifold of a liquid handler, Biomek 3000.

## EXPERIMENTAL STRATEGY



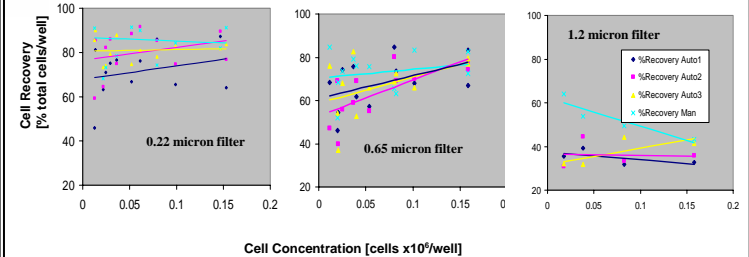
### Input Variables

Cell types (PB28 cells and PBMCs)  
Cell Conc. (25-250 x10<sup>3</sup> cells/well)  
Filter type (PTFE and PVDF)  
Mixing process

### Output Variables

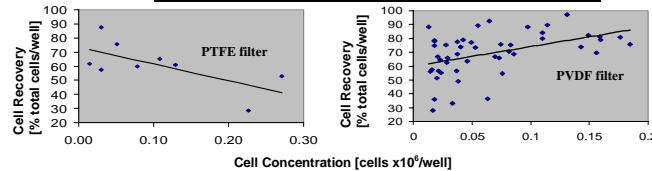
Total cell recovery  
Selective cell losses

## Impact of filter pore size and mixing conditions on cell recovery



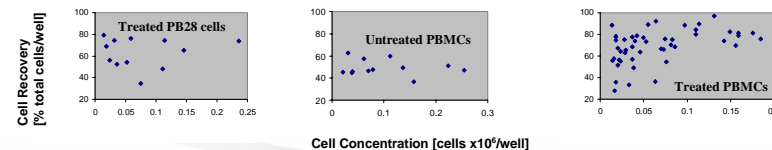
Impact of filter pore size and mixing conditions on cell recovery. Cell recovery was evaluated using treated (fixed and permeabilized) PBMCs and PVDF membranes of 0.22, 0.65, and 1.2µm pore sizes. Mixing conditions were: Auto1 = 200 µl at 100 µl/sec, Auto2 = 100 µl at 300 µl/sec, and Auto 3 = 200 µl at 300µl/sec. Experiments were performed using the filtration system for the Biomek 3000 Laboratory Automation Workstation. Each experimental point is the average of quadruplicate wells. Each well was subjected identical filtering conditions. Well content was manually transferred to a ViCell-XR for cell counting.

## Impact of membrane composition on cell recovery



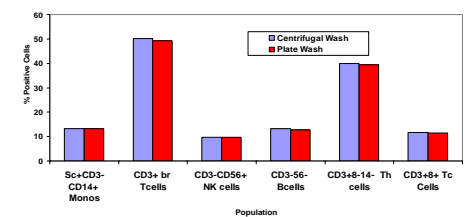
Impact of membrane composition on cell recovery. The impact of 96-well filter, PTFE and PVDF, plates on the recovery of treated (fixed and permeabilized) PBMCs was evaluated using the filtration system of the Biomek 3000 Laboratory Automation Workstation. Each experimental point is the average of quadruplicate wells. Each well was subjected identical filtering conditions, automated mixing, and content transferred to a ViCell-XR for cell counting.

## Cell recovery using different cell types and preparation



Cell recovery using different cell types and preparations. Cell recovery was evaluated using untreated PBMCs and treated (fixed and permeabilized) PBMCs and T cell line (PB28). Experiments were performed using 0.45µm PVDF filter plates and the filtration system for the Biomek 3000 Laboratory Automation Workstation. Each experimental point is the average of quadruplicate wells. Each well was subjected identical filtering conditions and automated mixing. Well content was manually transferred to a ViCell-XR for cell counting.

## Impact of wash method on selective cell losses



Impact of wash method on selective cell losses. Selective cell losses were evaluated using stained treated PBMCs washed by centrifugation and automated filtration method. The automated filtration process consisted of 0.45µm PVDF filter plates and mixing 200 µl at 100 µl/sec. Samples were analyzed using the FC500 flow cytometer. Each experimental point is the average of triplicate wells or sample tubes. Each well was subjected identical filtering conditions.

## CONCLUSIONS

- PVDF filter membranes allowed higher recovery of treated PBMC than PTFE membranes with identical process conditions.
- Both treated PBMC and T-cell line (PB280) showed better recoveries than untreated cells.
- PVDF membranes of 0.22 micron pore size showed better cell recovery that increasing pore sizes (0.45, 0.65, and 1.2 micron).
- Overall cell recovery is dependent on mixing conditions after the filtration process.
- The observed cell losses using treated PBMC do not appear to be selective based on tested markers for monos, NK, and T-cells, and B-cells.