Improving the Yield of Cell Deposition onto Glass Slides to Detect Target Cells

Continuous Innovation For Pathology

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Abstract

Introduction
The cytocentrifugation protocol for depositing cells from a suspension onto glass slides requires maximal cell yield and highest recovery rate to detect target cells. These deposited cells need to retain their cellular integrity for cell or biomarker identification using antibodies and probes.

Objective
A method was developed to improve the deposition of cells onto glass slides using a new cytocentrifuge.

Conclusion
The cytocentrifugation protocol performed on a Thermo Scientific™ Cytospin™ 4 was improved by leveraging the superior technology of the Sakura® Cyto-Tek® 2500 for deposition of cells onto slides resulting in a higher cell yield and recovery rate.

Materials and Methods

Peripheral Blood and Cancer Cell Lines
Peripheral blood was acquired from the San Diego Blood Bank. Blood counts were performed on an automated hematology analyzer (Sysmex KX-21N, Lincolnshire, IL).

One mL of blood was diluted with buffer, then the diluted sample was depleted of red blood cells using RedSift Cell Processor (Aviva Biosciences, San Diego, CA). The recovered cells were suspended in 0.8mL of AviWash buffer (AVIVA, San Diego, CA) and diluted to 10^6 cells per mL.

Human cancer cell lines (breast, BT-474; colorectal, DLD-1) were cultured under the conditions recommended by the supplier (ATCC, Manassas, VA).

Cytocentrifugation
Three different charged slides were used in this study: Thermo Scientific™ Shandon™ Single Cytoslide™ (coated), Matsunami Platinum Pro (Pro-01), and Thermo Scientific™ Superfrost™ Plus.

Thermo Shandon™ CytoSpin™ 4
Slides were assembled according to manufacturer's instructions. Slides were put into Single Cytofunnel™ assemblies (metal Cytoclip™ slide clip with Single Cytofunnel or EZ Single Cytofunnel™ and capped). All samples were loaded and cytocentrifuged at 600rpm for 5 minutes and then air-dried.

Sakura® Cyto-Tek® 2500
Slides were assembled according to manufacturer's instructions. Slides were put into Fluid Chamber assemblies (1 mL chambers, filter, clips and cap). All samples were loaded and cytocentrifuged at 600 rpm for 5 minutes and air-dried.

Benzidine Wrights Giemsa (BWG) Staining of Peripheral Blood Cells
Slides containing blood cells were:
- Fixed with methanol for 5 minutes
- Incubated with 1% benzidine for 1.5 minutes
- Incubated in peroxide solution for 1.5 minutes
- Rinsed
- Incubated in Wrights Giemsa solution for 10 minutes
- Rinsed and air-dried

Identification of Cancer Cells Using Immunohistochemistry (IHC)
An antibody cocktail was used to detect cancer cells by IHC using the Tissue-Tek Genie® Advanced Staining System (under development, Sakura Finetek USA, Torrance, CA) to identify target cells.

The slides were coverslipped using the Tissue-Tek Film® Coverslipper (Sakura Finetek USA, Torrance, CA) and images were taken using VisionTek® M6 Digital Microscope (at 40x (0.138 µm/pixel), Sakura Finetek USA, Torrance, CA).

Results

Experiments Using Thermo Scientific Cytospin 4

Figure 1. Comparison of cells deposited on different charged slides with sample diluted to 100 µL:

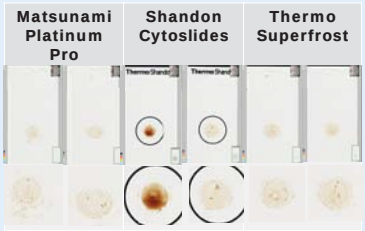


Figure 2. Repeat comparison of cells deposited on charged slides with Shandon Cytoslides and sample diluted to 100 µL:

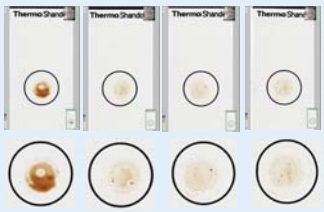
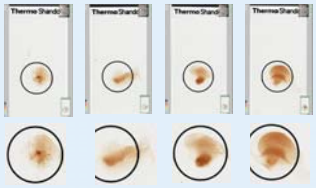


Figure 3. Repeat comparison of cells deposited on charged slides with Shandon Cytoslides and sample diluted to 500 µL:



NOTE: 500 µL is large volume with excess liquid remaining on the slide after cytocentrifugation. Excess liquid results in a smear

Figure 4. Images of two open assemblies using Cytospin EZ Cytofunnel (left) versus Cyto-Tek Fluid Chamber (right):

Experiments Using Sakura Cyto-Tek 2500

Figure 5. Comparison of cells deposited on different charged slides with sample diluted to 500 µL:

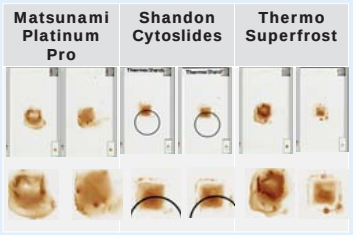


Figure 6. Repeat comparison of cells deposited on charged slides with Shandon Cytoslides and sample diluted to 500 µL:

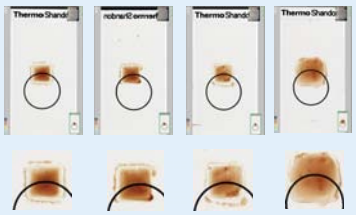


Figure 7. BWG staining of deposited cells:

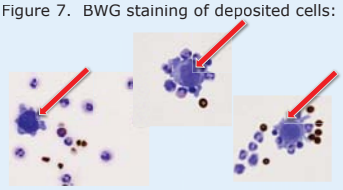


Figure 8. IHC staining of deposited cells:

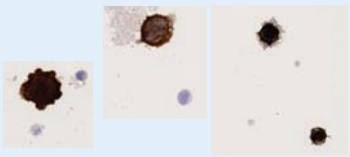


Figure 9. Sakura Cyto-Tek 2500:

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Conclusions

- Consistent cytocentrifugation of red blood cells was observed using Sakura's Cyto-Tek 2500
- Differences were observed in the deposition of cells using the two cytocentrifuges
- The deposited cells were stained using BWG or IHC technologies using Sakura Cyto-Tek 2500
- Large volume (500 µL) left liquid on the slides and resulted in cell spot smears on slides using Thermo Shandon's Cytospin 4
- Only Sakura's fluid chambers did not break or leak during loading or cytocentrifugation
- Only Sakura's Cyto-Tek 2500 could handle large sample volume (500 µL) without cell spot smears

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