A Safe and Effective Way to Perform Wash Steps in the Tray Based FCXM Assay

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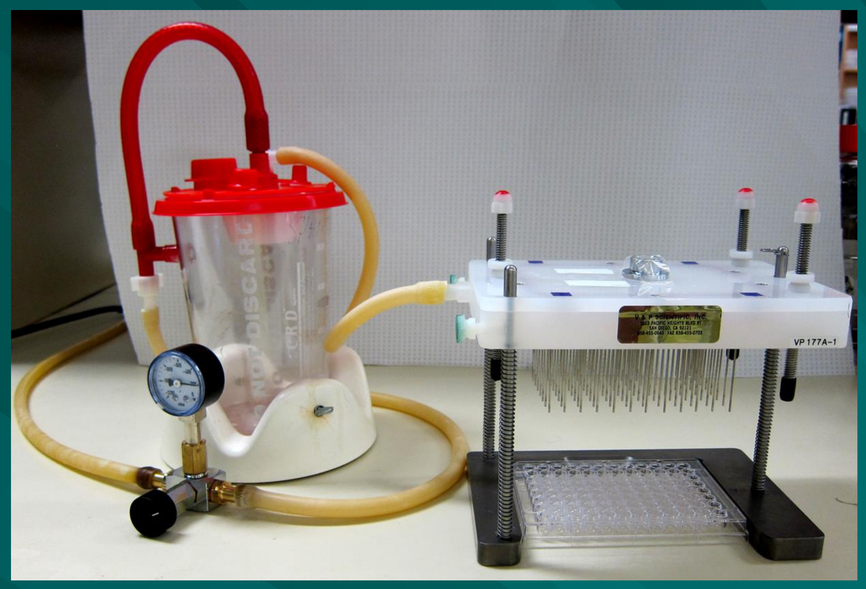
INTRODUCTION

Our laboratory recently changed from a tube based to tray based method for flow cytometry crossmatch (FCXM). A 'flick' wash method is commonly used in a tray FCXM; this technique may create potentially biohazardous aerosols in the laboratory. In our experience, technologist to technologist variation is a concern with the flick wash method. We investigated the use of the VP 177A-1 Aspiration Manifold for FCXM tray wash steps and performed a comparison between the flick wash method and this vacuum manifold wash method.



MATERIALS and METHODS

Setting Up the Vacuum Manifold

A vacuum hose is attached from the manifold to the trap for fluid transfer; two vacuum hoses are connected to a pressure gauge. One is attached to one end to the trap and the other to the vacuum source. The tubing is clamped between the gauge and vacuum source when not aspirating.

Adjusting the Height of the Manifold

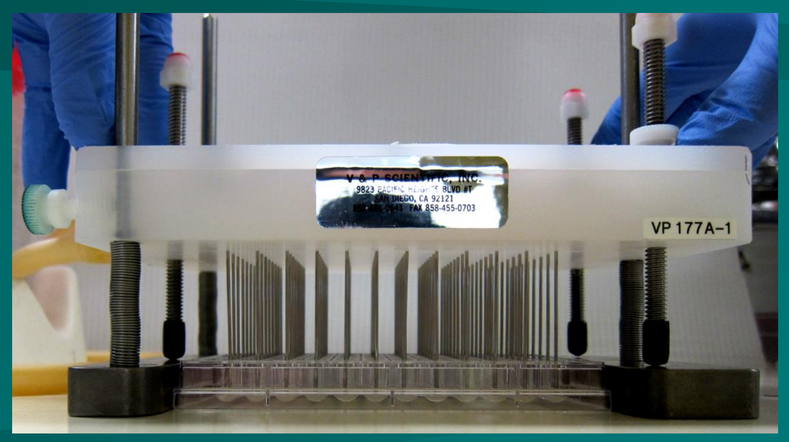
The height of the manifold can be adjusted to achieve the desired dead volume (residual volume post wash) in each well of the tray. This adjustment is easily performed using the screws on the top of the manifold. The manifold was adjusted to leave approximately a 20uL volume behind in each well. The dead volume is routinely assessed to ensure that the wash remains consistent.

Aspirating an FCXM Tray with the Vacuum Manifold

Once the vacuum has been turned on, the tray is placed under the manifold. The manifold is pressed down to the preset height, ensuring that it is level; there is a level on the top of the manifold. Once the supernatant has been aspirated, the manifold is returned to its resting position.

Comparing the Flick Wash to the Vacuum Manifold Wash

Flick Wash and Vacuum Manifold Wash tray crossmatches were performed in parallel using the rapid optimized FCXM method (Liwski et al. ASHI 2011, abstract 45-P). Two separate cell suspensions were used; spleen lymphocytes and PBL lymphocytes. A total of 5 crossmatches were performed, four with spleen cells and one with PBL. Lymphocytes were isolated by Ficoll separation followed by the StemCell™ EasySep™ kit to remove contaminating cells. The crossmatches were performed including duplicate wells of negative control serum as well as four dilutions of a positive pool control .The trays were centrifuged together to eliminate any variation in centrifugation. One set of trays were washed using a flick wash method. The other set of trays were washed using the vacuum manifold to remove supernatant. The residual volumes in each wash method were kept consistent by the addition of 15uL of flow wash buffer to each well of the flick wash trays. Cells were acquired on a BD FACSCanto™ II Flow Cytometer with an HTS tray acquisition system.
Reproducibility was evaluated based on the standard deviation of duplicates (n=14) for each wash method. The median channel value (MCV) shifts for T and B cell FCXM were also determined for each positive control dilution in each wash method by subtracting the negative control serum MCV from the positive control MCV. Sensitivity was determined as the % of increase in the median channel shift of each of the positive pool dilutions.



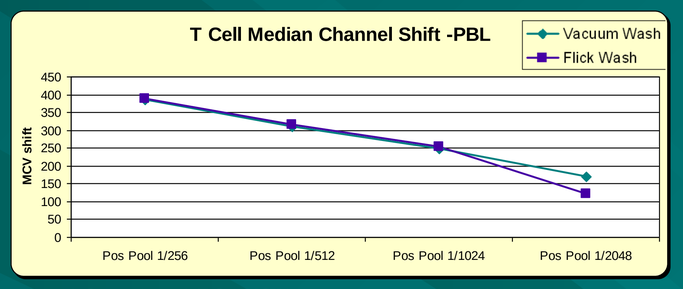


Figure 1. Comparison of T Cell MCV between vacuum wash and flick wash method with PBL

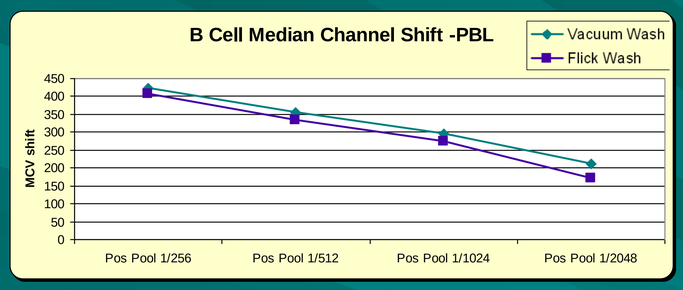


Figure 2. Comparison of B Cell MCV between vacuum wash and flick wash method with PBL

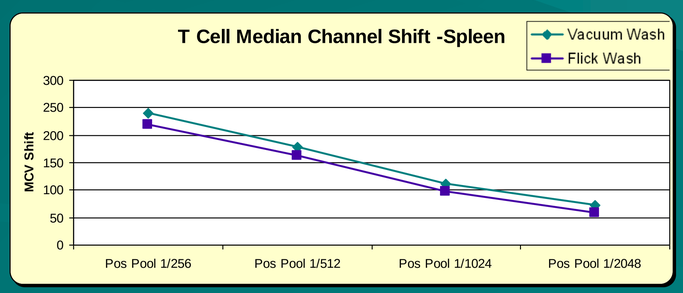


Figure 3. Comparison of T Cell MCV between vacuum wash and flick wash method with spleen

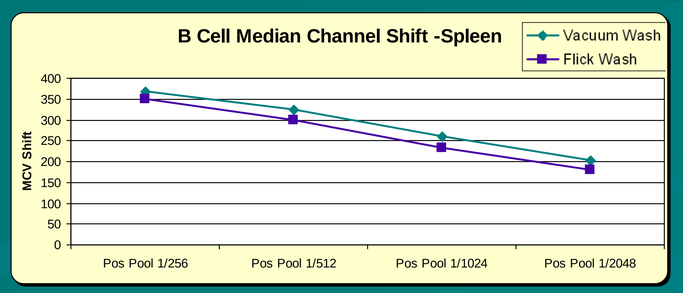


Figure 4. Comparison of B Cell MCV between vacuum wash and flick wash method with spleen

RESULTS

Figures 1 and 2 show the results of the T and B cell median channel shifts from the PBL crossmatch for both wash methods. Figures 3 and 4 show these results for one of the spleen cell crossmatches.

The standard deviation (SD) of the vacuum washed duplicate wells was **4.4** versus **8.9** in the flick wash trays.

The MCV shift is **1.8 – 2.0% more sensitive** with the vacuum wash method as compared to the flick wash method.

DISCUSSION

Figures 1 through 4 demonstrate that the median channel shift of the vacuum wash method are consistent with the flick wash method. These graphs also demonstrate that the sensitivity of the crossmatch is equal to or greater than the flick wash when the vacuum wash method is used. The calculated sensitivity also indicates a slight increase in sensitivity of the crossmatch with the vacuum wash method.

The lower standard deviation of the duplicates in the vacuum wash suggests that this wash method will be more reproducible. The vacuum wash method has been in place in our laboratory since July, 2013. All patient sera are tested in duplicate and these results (not shown) also continue to support this finding.

Training the staff to use the manifold was very simple and this wash method has proven easier for technologists with wrist or shoulder issues.

CONCLUSIONS

Based on this small study, our laboratory chose to implement the vacuum wash method. These results show that this wash method is more sensitive and more reproducible. There is also less likelihood to create potentially biohazardous aerosols.