Reviewer's report

Title: LeukoCatch, a quick and efficient tool for the preparation of leukocyte extracts from blood

Version: 1 Date: 27 May 2011

Reviewer: Al Jesaitis

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The work described in this manuscript aims to easily produce a partially purified preparation of leukocytes from whole blood. The leukocytes are trapped in commercially available "Leuko-Catch Filters" composed of a proprietary hydrophilic fibrous matrix, relatively free of erythrocytes and platelets. The method entails aspirating a defined volume of EDTA-treated whole blood into a 10 ml syringe containing multiple layers of LeukoCatch filters. The authors suggest that better purification is obtained if several filters are stacked upon one another between "stoppers". They examine the retention of erythrocytes and platelets from whole blood as well as cultured MONO-MAC cells as surrogates for WBC by monitoring flow through samples and residual "eluted" markers for RBC and WBC. Overall, the procedure and its description should be a simple matter, however several serious problems exist with the descriptions within this paper that significantly detract from its usefulness.

1. Figure 1. is inadequately described. In A, the stacked configuration of the filtering system should be shown. Better yet photographs of the system with multiple filters should be shown. In B, it is unclear what the sequence is. Are the syringes being aspirated (in and out?) multiple times? Or is the material deposited back into the tubes. Is there serial application to new filters or to the same filters?

Overall, this figure is quite confusing. Arrows should show the direction of the flow and whether there have been multiple applications to the repeatedly depleted 2 ml samples, new samples for accumulation of material in the filters, or washes of the filters? There should be a much more detailed description in the legend.

2. Figure 2. What does NT (non-treated?) mean and how does it differ from "0"? Is this Figure representative of serial passage of the flow through to two filters? Or through the same filter? The westerns should be quantitated and given as percentage of the starting material.

3. Same criticism as Figure 2. Is this Serial passage through 1-5 new or the same syringes with a stack of five filters?

4. How can RBCs increase beyond the NT control? Are there cellular fragments from the cells that are being mistaken as RBCs?

5. Figure 5. After 5 passages through the same (or new?) filters the WBC flow-through stabilizes. How does one know that these cells are representative of
the entire population or just those that express adhesion receptors that allow them to stick to filter fiber surfaces differentially, and thus be retained by the filters? The populations of WBCs should be examined by various markers and compared to control cells. One should look for MEL-14, CD14, CD11a,b,c/CD18.

6. Figure 6. What is washed blood? What is the standard centrifugation? In D what is sample 7-8? All of this type of information should be in the legends.

The terminology used in this paper is somewhat confusing and insufficiently descriptive. If one is using the chromatography term elution then it would be more useful to use flow through to identify what is coming through the filter and the retentate for that which is retained by the filter. There should be a materials section where the source and nature of the antibodies used is mentioned. The legends need to comprehensive and precise. Hemoglobin in RBC is at a concentration of 5 mM and about half the volume of whole blood is RBCs. A 90% reduction of hemoglobin suggests that it will still be in the mM range and a huge contaminant in these preparations.

In the discussion it states that this system can deplete 99.99% of the leukocytes in the blood. Figure 4 suggests that only about 30-40% are retained. Where in this paper is this shown? If only part of the WBCs are captured, that population needs to be characterized before the method is useful for the applications implied.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests