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Rapid centrifugal proteomic and hematological assays for point-of-care radiation biodosimetry

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An effective emergency response to a major nuclear accident or radiological terrorist attack requires early (< 48 hours) and rapid radiation dose assessment for a large population to separate individuals needing immediate treatment from the much larger "worried well" population. Established lymphocyte chromosome aberration-based dosimetry techniques are restricted to a much later time window (days to weeks) and cannot test more than a few people at a time, creating an urgent need for fast and effective screening. Using extensive animal research, AFRRI has developed a novel panel of >10 protein and blood cell biomarkers to accurately predict radiation dose from hours to days post-exposure (4, 5). Biomarker-based evaluation of dose is a highly demanding medical diagnostics application that requires rapid simultaneous measurement of multiple proteins and white cell counts from a drop of whole blood. We present a novel "lab on a disk" technique for multiplexed whole blood protein assays and white cell counts amenable to fully-automated integration in a point-of-care device (1).

A key advantage of this approach over previously reported disk based immunoassays (2, 3) is that blood separation, washing, and amplification steps are combined by using an unconventional assay process, leading to rapid sample-to-answer times and improved sensitivities. Protein capture beads and secondary label are incubated directly in whole blood then sedimented through a density medium (Fig 1). During sedimentation, beads are removed from contaminants by Stoke's flow, resulting in highly effective washing and low background signal. In the same step, beads are separated from blood cells due to differences in size and density, and beads become concentrated in a signal-rich pellet (Fig 2). Multiplexed measurement of IL-6 and C-reactive protein with a limit of detection of 6.7 picomoles/L is demonstrated (Fig 3).

The same principles are used to label and sort white blood cells by density and size. We demonstrate quantification of bands of neutrophils and lymphocytes separated from whole blood to obtain a differential white blood cell count (Fig 4). The overlapping architecture for each assay technique facilitates parallel proteomic and hematological screening from drops of whole blood samples. In order to most effectively implement this panel, we are developing an integrated disk and platform to assess radiation dose at sites of medical triage. A fully integrated disk containing phase change valves, magnetic mixing elements, and all reagents needed for operation is presented. Accurate measurement of 6 different radiosensitive biomarkers is demonstrated in human and mouse derived samples. The combination of fast assay time (15 minute), small sample volume (1 microliter/test), sensitive quantification, simple operation, and ability to directly process whole blood make this technique uniquely suited for biodosimetry applications.

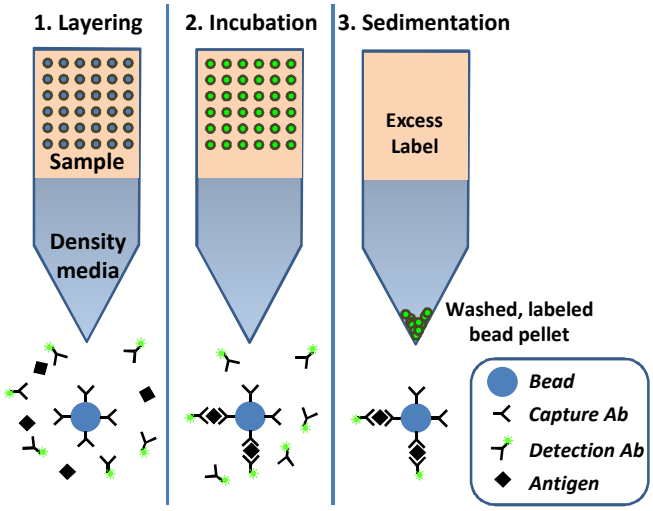

Figure 1. Diagram of the basic bead sedimentation immunoassay technique.

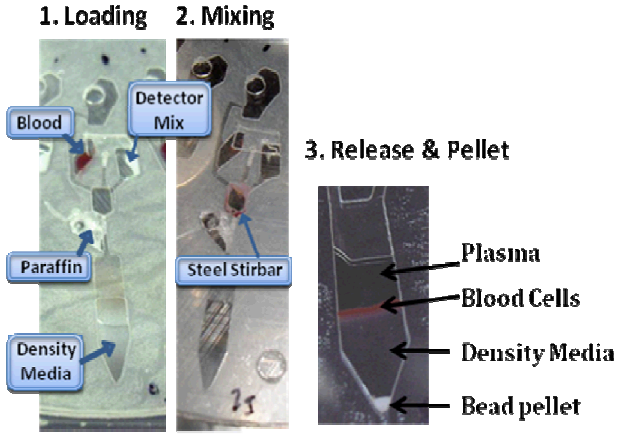

Figure 2. Integrated three step whole blood analysis.

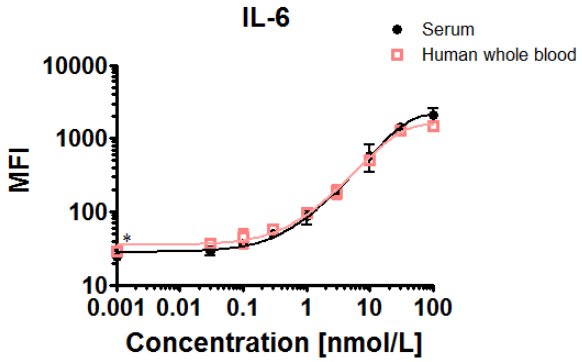

Figure 3. IL-6 detection in whole blood vs serum

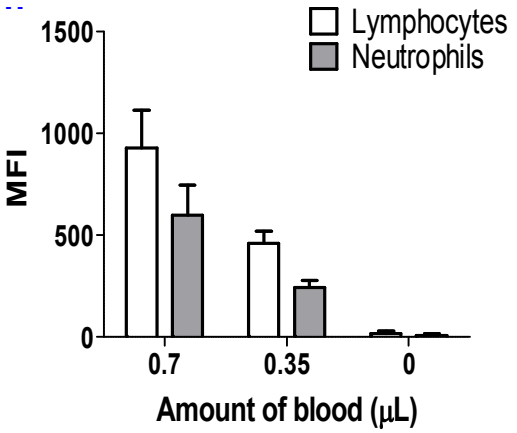

Figure 4. On-disk quantification of lymphocyte and neutrophil populations from whole blood

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