Introduction
A minimally invasive method of monitoring disease status before, during and after treatment benefits both patient and physician. Whole blood serving as a ‘liquid biopsy’ can provide a means of monitoring the status of an event (e.g., pregnancy or cancer) using a minimally invasive needle stick. A simple blood draw is the catalyst for retrieving rare, clinically relevant cells such as tumor cells (cancer) or fetal cells (pregnancy), which have been shed and circulate freely within the bloodstream. These circulating cells have implication in diagnosis and minimal residual disease (MRD) monitoring. The enrichment and detection of rare circulating cells may provide a valuable, more tolerable alternative to an invasive solid tissue biopsy procedure, like amniocentesis or chorionic villus sampling (CVS).

Materials and Methods
Manual Fetal Cell Enrichment and Identification
A manual procedure (using magnetic beads and filtration chips) was developed after evaluating different enrichment technologies. Table 1 to enriched fetal cells from whole peripheral blood (Advanced Biosciences Resources, Alameda, CA with IRB approved protocol). Fetal nucleated red blood cells (nRBCs) were detected using manual immunohistochemistry (IHC) in combination with fluorescent in situ hybridization (FISH) using chromosome enumeration probes, CEP X Aqua and CEP Y Orange (Abbott Molecular, Des Plaines, IL; Schueler, P.A., et. al. Placenta 22: 688, 2001). Slides were coverslipped and images were taken using a fluorescent digital microscope.

Automated Cancer Cell Enrichment and Identification
Using the fetal cell enrichment procedure as a model for cancer cell enrichment, cultured cancer cells were spiked into whole peripheral blood. An automated cell enrichment kit was used to enrich the spiked cancer cells (AVIVA Bioscience, San Diego, CA). The recovered cells were put onto slides and fixed. A new antibody cocktail was used to detect cancer cells by IHC and counterstained with hematoxylin to identify non-target cells. The slides were coverslipped using Tissue Tek Film® Coverslipper (Sakura Finetek, Torrance, CA) and images were taken using VisionTek® Digital Microscope (Sakura Finetek, Torrance, CA).

Results

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<td>Recovery depends on antibody and magnetic bead capture.</td>
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<td></td>
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<td>Recovery is dependent on slit or hole width. Optimization required for target cell type.</td>
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Cancer Cell Lines
Human cancer cell lines (breast, BT-474; colorectal, DLD-1; lung, H526) were cultured under the conditions recommended by the supplier (ATCC, Manassas, VA).

Automated Cancer Cell Enrichment and Identification

Using the fetal cell enrichment procedure as a model for cancer cell enrichment, cultured cancer cells were spiked into whole peripheral blood. An automated cell enrichment kit was used to enrich the spiked cancer cells (AVIVA Bioscience, San Diego, CA). The recovered cells were put onto slides and fixed. A new antibody cocktail was used to detect breast cancer cells by IHC and counterstained with hematoxylin to identify non-target cells. The slides were coverslipped using Tissue-Tek Film® Coverslipper (Sakura Finetek, Torrance, CA) and images were taken using VisionTek® Digital Microscope (Sakura Finetek, Torrance, CA).

Conclusions
- It was possible to enrich rare target cells from peripheral blood (e.g. fetal cells or cancer cells).
- Antibody cocktail to fetal or epithelial antigens was able to detect circulating fetal nRBCs or spiked cultured cancer cells.
- The automated cell enrichment and identification procedure had similar target cell recoveries compared to manual procedure, thereby reducing the potential for human error with complex procedures.
- "Liquid biopsy" may be a valuable alternative to invasive procedures to identifying rare circulating target cells.
- In the future, "liquid biopsy" and automated enrichment and identification instrumentation may help a clinician monitor patient status.

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