Fully Automated Label-Free Microfluidic System For Circulating Tumor Cell Enrichment

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Clearbridge BioMedics
Bringing clarity to cancer

ClearCell® FX System

Clearbridge BioMedics has developed a highly robust Circulating Tumor Cells (CTC) enrichment platform using spiral microfluidic biochips. The ClearCell® FX System is one of the world's first label-free CTC isolation systems that is able to isolate and retrieve wholly intact CTCs in their native state.

The **ClearCell® FX System** is used together with the company's proprietary **CTChip® FR** – biochips using inertial microfluidic technology. The system overcomes the limitations of current available isolation techniques which are limited to antibody affinity and low sample volumes.

The CTChip® FR uses inherent Dean vortex flows present in curvilinear channels, termed Dean Flow Fractionation (DFF), to isolate CTCs based on size and inertia. The larger channel dimensions within the chip reduces issues arising from channel clogging, as well as sample processing speeds.

CTChip® FR1

Key Features

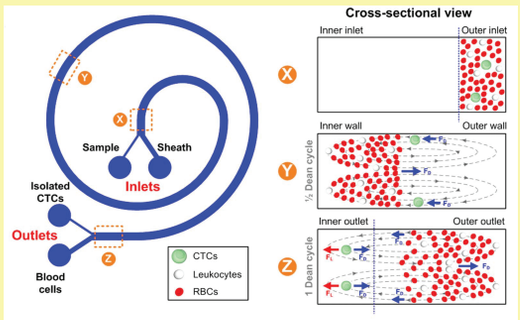
CTChip® FR

- ✓ Label-free isolation method
- ✓ Enrichment of CTCs from blood based on *size & inertia*
- ✓ Retrieval of wholly *intact* and *viable* cells
- ✓ High purity, *4log$_{10}$ depletion of WBCs*
- ✓ Process high blood volume of *7.5ml* in less than an hour
- ✓ Much less susceptible to channel clogging
- ✓ Continuous flow process for isolation of CTCs

ClearCell® FX System

- ✓ Fully automated CTC enrichment platform
- ✓ Small footprint
- ✓ Enrichment of CTCs from blood

Isolation by Size



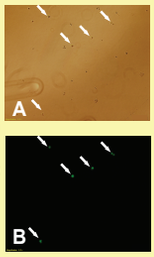
- Smaller hematologic cells [red blood cells (RBCs) ~8µm; leukocytes ~8-15µm] are affected by the Dean Drag and migrates to outer wall after one Dean cycle.
- Larger CTCs (~15-20µm) experience strong inertial lift forces as indicated by the red arrows and is focused along the microchannel inner wall.

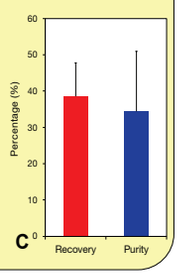
High Purity Protocol

The new high purity protocol offers a single step enrichment solution capable of providing PCR and NGS ready CTC purities. Key features include:

- Recovery >40% with spiked samples
- Total leukocyte contamination <1000 cells per 7.5 mL
- Total processing time on ClearCell® FX ~ 2 hours

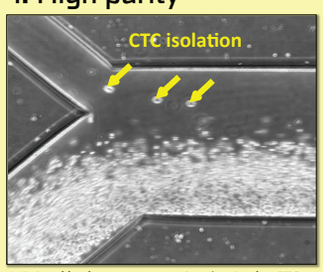
(A, B) Representative bright field and fluorescent images of the output of the new protocol showing the spiked H1975 cells (pre labelled with Green dye) and the background leukocyte contamination. (Scale bar 100 µm). (C) Bar graph indicating the average recovery and purity achieved with 200 H1975 cells spiked into 7.5 mL blood; we can consistently achieve ~40% recovery and >30% purity .





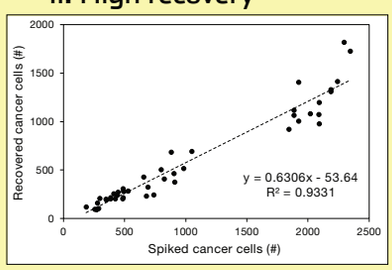
Results

I. High purity



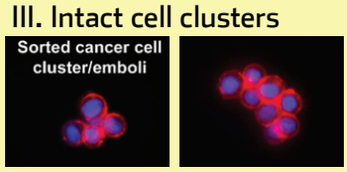
Minimal leukocyte contamination in the CTC outlet.

II. High recovery



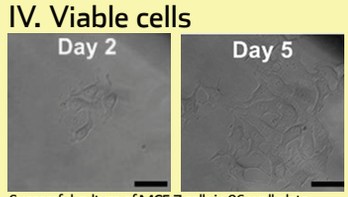
Consistent and high separation efficiency of >60% for varying spiking concentrations in 7.5mL blood.

III. Intact cell clusters



Intact MCF-7 clusters isolated successfully due to short transit time within the channel. Clustered cells may yield important prognostic significance.

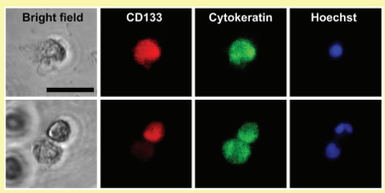
IV. Viable cells



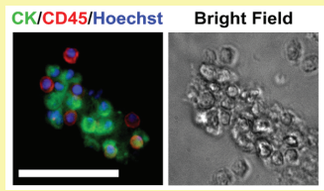
Successful culture of MCF-7 cells in 96-well plate. Scale bar is 50 µm.

V. Clinical samples

Subjects	Number of samples	Range of CTCs/mL				Samples with >5 CTCs/mL (%)
		0-5	5-20	20-50	50-100	
		Number of samples (%)				
Healthy (control)	20	20 (100)	0	0	0	0
Lung cancer	20	0 (0)	5 (25)	6 (30)	9 (45)	100

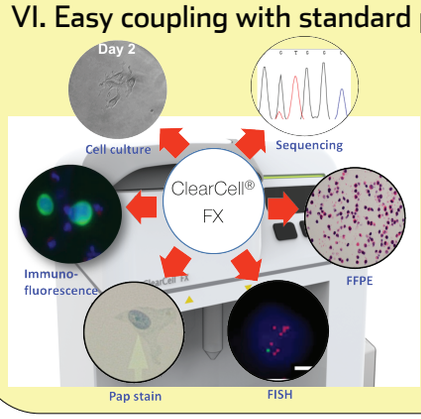


Bright field and fluorescence images of isolated CTCs stained for stem cell marker CD133, cytokeratin and Hoechst. Scale bar 20 µm.



Optical images of an isolated CTC cluster using DFF. Scale bar 50 µm.

VI. Easy coupling with standard pathology assays



Retrieved cells remain untagged without any antibodies and hence are more viable for propagation and other downstream analysis including cell culture, immuno labeling, Pap staining, formalin-fixed paraffin embedded (FFPE) block, fluorescence in-situ hybridization (FISH), polymerase chain reaction (PCR), and next-generation sequencing (NGS).