Unbiased enrichment of circulating tumour cells directly from whole blood

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Abstract

Enrichment of rare circulating tumor cells (CTC) in peripheral blood is required prior to most analytic procedures. The ideal enrichment method would be rapid [<30 minutes (min)], permit a high recovery of viable CTC, and would be independent of surface marker expression, since CTC in the peripheral blood may be undergoing epithelial-mesenchymal transition and may not express epithelial markers. We have developed a new immunomagnetic method (EasySep™ Direct) that fulfills these criteria and can be used directly with whole blood without any preparatory steps. The method was tested using whole blood samples seeded with the adenocarcinoma cell line CAMA to a final concentration of approximately 1%. The CAMA cells were then enriched using either EasySep™ Direct CTC Enrichment (immunomagnetic separation) or RosetteSep™ (immunodensity based control). For immunomagnetic enrichment, the samples were incubated with EasySep™ Direct Enrichment Cocktail for 5 min, then EasySep™ Direct RapidSpheres™ were added and the sample placed in a magnet for 10 min. Magnetically-labeled cells were retained in the magnet and the unlabeled cells poured or pipetted off. RapidSpheres™ were added again and the magnetic incubation and pour-off steps repeated. To perform RosetteSep™ enrichment, the samples were incubated with either the CD45$^+$ cell depletion cocktail or a more extensive CTC enrichment cocktail for 10 min. Samples were then pipetted onto Lymphoprep™ in SepMate™ tubes, centrifuged for 10 min at 1200 x g with the brake on, and poured off. The number of cells recovered with each procedure was counted and the depletion of hematopoietic cells (CD45$^+$/EPCAM$^-$), enrichment of CAMA cells (CD45$^-$/EpCAM$^+$), and depletion of red blood cells (RBCs, Glycophorin A$^+$/CD45$^-$) in each sample were evaluated by flow cytometry. EasySep™ Direct was evaluated on five different samples. Each sample was separated on five different EasySep™ magnet formats (5, 14, and 50 mL single tube magnets and 5 and 14 mL multi-tube magnets) with each condition in duplicate; the two RosetteSep™ cocktails were tested on three of the samples, each in duplicate. There was no significant difference in CD45 depletion, CAMA cell recovery, or residual RBC contamination between samples enriched using the different magnets (Least Squares Fit; Prob F > 0.05) or pooled and compared to the pooled RosetteSep™ controls (Least Squares Fit; Prob F > 0.05). Over all magnet separation conditions and samples, the mean log depletion of CD45$^+$ cells was 2.9 ± 0.4, the mean recovery of CAMA cells was 42 ± 23%, and the residual RBC contamination was ~9,000 RBC/mL of start sample. Rare cells were enriched in 25 minutes using EasySep™ Direct and in less than 40 minutes using RosetteSep™ with SepMate™. CTC can be enriched directly from whole blood with either EasySep™ Direct or RosetteSep™. Since enriched cells are unlabeled there is nothing to interfere with subsequent further enrichment, culture, or evaluation.

Methods

The breast adenocarcinoma cell line CAMA was seeded into 24-hour old whole blood at 0.7 - 1% of total nucleated cells, and tumor cells were then enriched with either EasySep™ Direct Human CTC Enrichment Kit (Cat. #19657; targeting CD2, CD14, CD16, CD19, CD45, CD61, and CD66b), RosetteSep™ Human CD45 Depletion Cocktail (Cat. #15122; targeting CD45 and CD66b) or RosetteSep™ CTC Enrichment Cocktail Containing Anti-CD36 (Cat. #15127; targeting CD2, CD16, CD19, CD36, CD38, CD45 and CD66b). Post-enrichment, the total nucleated cells recovered from each sample were counted, and the samples evaluated by flow cytometry with staining for DRAQ5 (all nucleated cells), CD45 (all nucleated white blood cells), EpCAM (CAMA cells), and Glycophorin A (RBCs). CD45 cell depletion, CTC recovery, and RBC contamination in the recovered fraction were determined. Statistical analysis was performed using Least Squares Fit, JMP software.

FIGURE 1: EasySep™ Direct CTC Enrichment

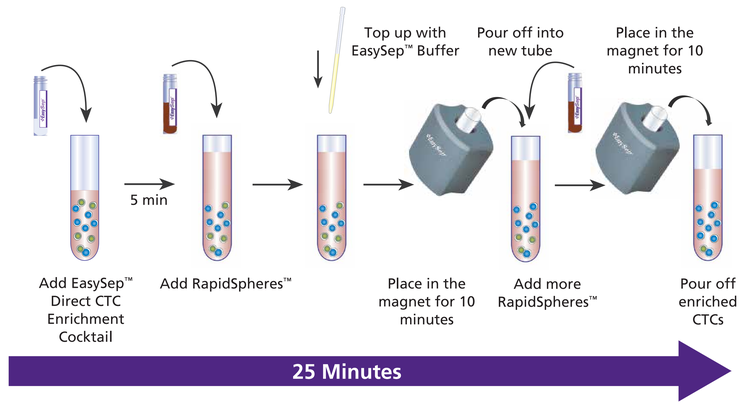
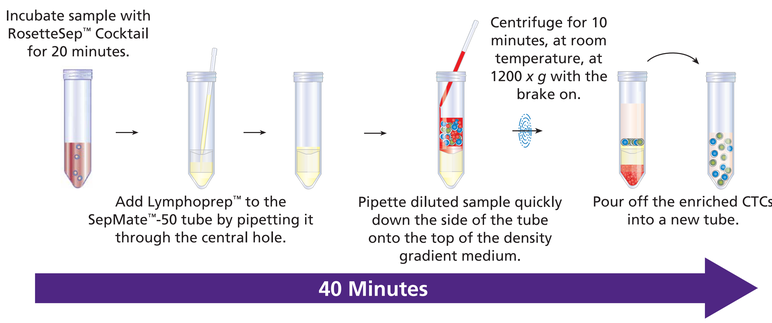
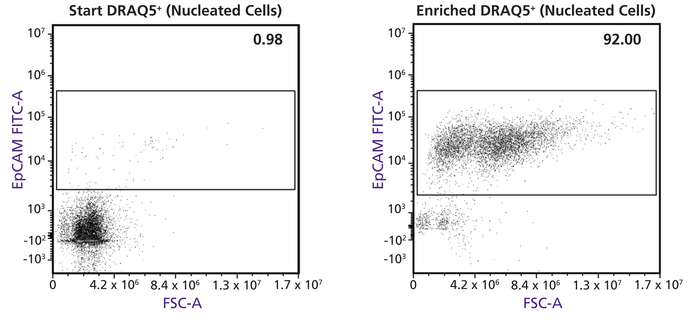


FIGURE 2: RosetteSep™ CTC Enrichment



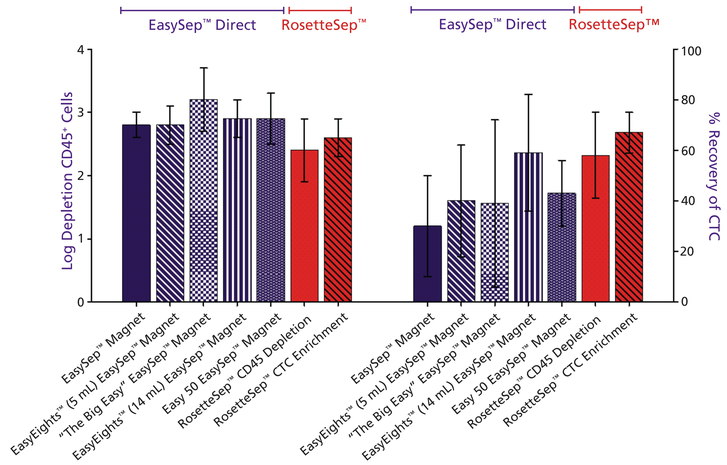
Results

FIGURE 3: Flow cytometric assessment of CTCs before and after enrichment using EasySep™ Direct



Starting and enriched samples were stained with DRAQ5 (to identify nucleated cells) and EpCAM (to identify CAMA cells). Values are the percentage of DRAQ5$^+$ cells that express EpCAM. Plots are representative of results obtained.

FIGURE 4: Enrichment of CTCs with EasySep™ Direct and RosetteSep™



CTCs were enriched using the EasySep™ Direct CTC Enrichment Kit with different magnets or with RosetteSep™. There were no significant differences between the conditions in the log depletion of CD45$^+$ cells or the % of CAMA cells recovered (n = 5; Least Squares Fit analysis).

TABLE 1: Log depletion of CD45 cells, CTC recovery, and RBC contamination with EasySep™ Direct and RosetteSep™

Method	Magnet	Start Volume Range (mL)	Start Volume (mL)	Log Depletion CD45$^+$ Cells	% Recovery CTC	# Nucleated Cells Enriched per mL Start	# RBCs Enriched per mL Start
EasySep™ Direct (n = 5 donors, each in duplicate)	Purple	0.5 - 2	2	2.8 ± 0.2	30 ± 20	27,763 ± 8,343	1,378 ± 927
	EasyEights™ (5 mL)	0.5 - 2	2	2.8 ± 0.3	40 ± 22	35,924 ± 14,916	10,396 ± 9,375
	"The Big Easy"	1 - 7.5	5	3.2 ± 0.5	39 ± 33	29,378 ± 23,912	725 ± 886
	EasyEights™ (14 mL)	1 - 7.5	5	2.9 ± 0.3	59 ± 23	43,168 ± 16,728	9,034 ± 7,263
	"Easy-50"	5 - 25	5	2.9 ± 0.4	43 ± 13	39,200 ± 20,581	27,574 ± 20,931
RosetteSep™ (n = 3 donors, each in duplicate)	CD45 Depletion (15122)	-	5	2.4 ± 0.5	58 ± 17	90,799 ± 44,803	1,733 ± 741
	CTC Enrichment (15127)	-	5	2.6 ± 0.3	67 ± 8	71,122 ± 15,736	124,750 ± 79,738

Summary

- The EasySep™ Direct CTC Enrichment Kit can be used to enrich rare, non-hematopoietic cells directly from whole blood without the need for hetastarch, density gradient centrifugation or ammonium chloride lysis.
- CTCs are enriched with EasySep™ Direct in only 25 minutes.
- EasySep™ Direct can be used on sample volumes of 0.5 mL to 25 mL, depending upon the magnet used.