Abstract #161.9

One-step Enrichment of Leukocyte Subsets Directly in the Blood Collection Tube

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Summary

Immunologically-based therapies are a promising approach for the treatment of solid tumour malignancies, especially in the setting of minimal residual disease. To develop these strategies, it is necessary to isolate specific leukocyte subsets. The ideal cell isolation approach would be rapidly performed in a single step from whole blood, thereby maximizing the recovery and viability of the purified cells. To meet this goal, we have adapted the RosetteSep™ negative cell selection protocol to be performed in Cell Preparation Tubes ("CPT™"; Becton-Dickinson, Franklin Lakes, NJ). CPT™ are evacuated blood collection tubes containing ficoll below a gel insert and either sodium heparin or sodium citrate as an anti-coagulant above the gel.

Peripheral blood from normal donors was collected into either standard blood collection tubes or CPT™. RosetteSep™ antibody cocktail was added to the whole blood and incubated for 20 minutes at room temperature. The blood collected in the standard collection tubes was then diluted 1:1 with PBS containing 2% FBS, layered over ficoll, and centrifuged for 20 minutes at 1200 x g ("Standard RosetteSep™"). After incubation with RosetteSep™, the blood collected in the CPT™ was simply centrifuged for 20-25 minutes at 1600-1800 x g. Post centrifugation, the enriched cells were collected from the plasma : ficoll interface, washed once, and analyzed by flow cytometry.

The purity of lymphocytes enriched with the Standard RosetteSep™ protocol was slightly higher than the purity of lymphocytes enriched using CPT™. The recovery of the desired cells was the same with either approach. The purity and recovery of monocytes enriched by either protocol was similar. Monocytes enriched with either method were cultured in GM-CSF, IL-4 and TNF-alpha. After 7 days, the cells lost expression of CD14 and expressed markers (CD1a, CD80, CD83) typical of dendritic cells.

The specific advantages of using CPT™ are 1) all procedures are performed in the collection tube; 2) it is not necessary to layer the rosetted blood over a buoyant density medium; and 3) it is very easy to remove the enriched cell layer.

Methods

Figure 1. Procedure for enriching cells in the CPT™ (CPT RS)

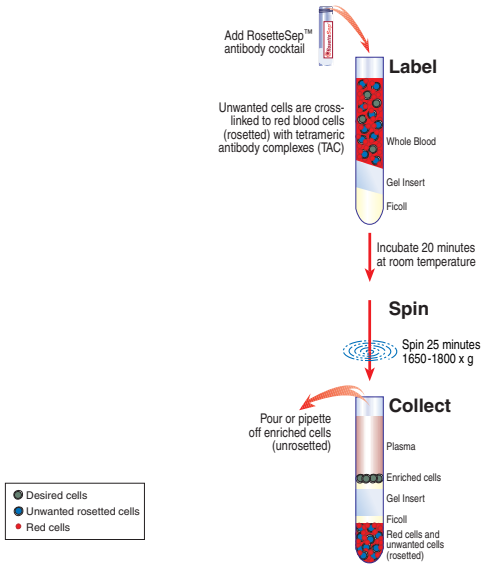
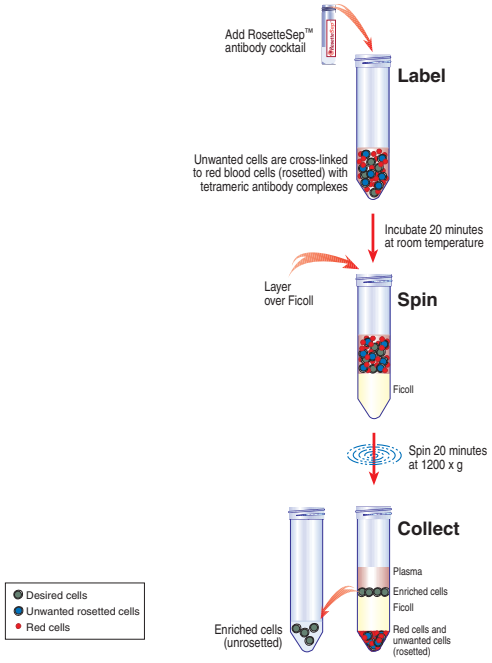


Figure 2. Standard RosetteSep™ procedure for enriching cells directly from whole blood (Std RS)



Enriched cells were analyzed by flow cytometry.

Results

Figure 3. Purity of Enriched Cells Collected in Heparin

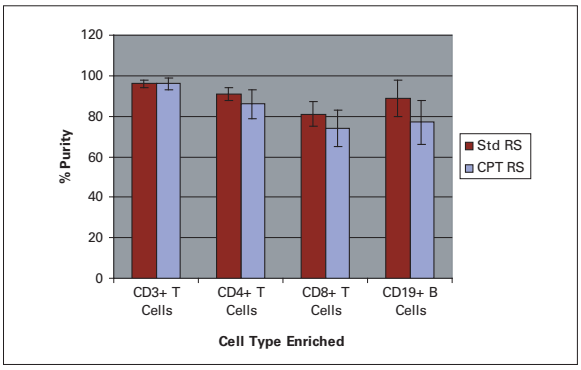


Figure 4. Purity of Enriched Cells Collected in Citrate

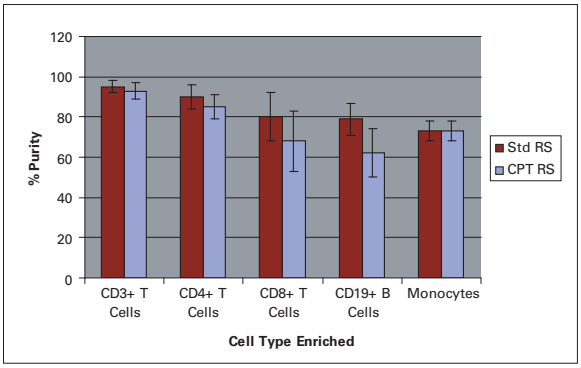


Figure 5. Recovery of Enriched Cells Collected in Heparin

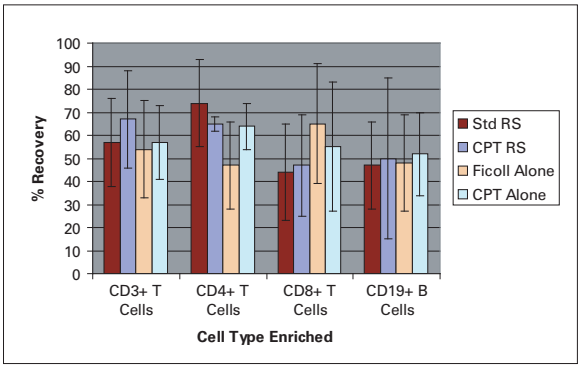
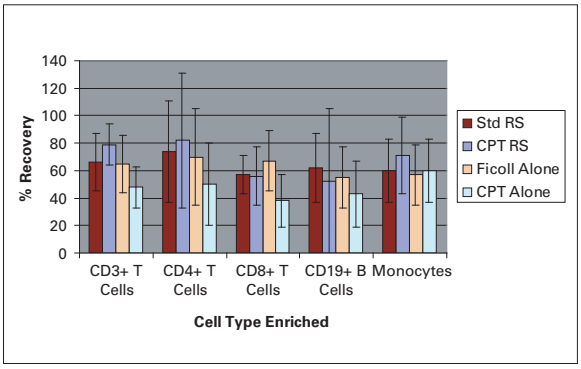


Figure 6. Recovery of Enriched Cells Collected in Citrate



Monocytes enriched with either method were cultured in GM-CSF, IL-4, and TNF-alpha. After 7 days, the cells lost expression of CD14 and expressed markers (CD1a, CD80, CD83) typical of dendritic cells.

Cell Type Enriched	n	Ficoll Alone		CPT™ Alone		Standard RosetteSep™		RosetteSep™ in CPT™	
		% Purity	% Recovery	% Purity	% Recovery	% Purity	% Recovery	% Purity	% Recovery
CD3+ T Cells	7	69 ± 8	54 ± 21	66 ± 7	57 ± 16	96 ± 2	57 ± 19	93 ± 3	67 ± 21
CD4+ T Cells	6**	43 ± 9	47 ± 19	41 ± 10	64 ± 10	91 ± 3	74 ± 19	86 ± 7	65 ± 3
CD8+ T Cells	16	22 ± 5	65 ± 26	20 ± 5	55 ± 28	81 ± 6	44 ± 21	74 ± 9	47 ± 22
CD19+ B Cells	12*	10 ± 3	48 ± 21	11 ± 4	52 ± 18	89 ± 9	47 ± 19	77 ± 11	50 ± 35

Table 1. Purity and recovery of cells collected in heparin and processed using either the standard RosetteSep™ protocol or in CPT™. Results obtained in the absence of the RosetteSep™ cocktail ("Ficoll Alone", "CPT™ Alone") are shown for comparison. *n=11 for recoveries **n=5 for recoveries

Cell Type Enriched	n	Ficoll Alone		CPT™ Alone		Standard RosetteSep™		RosetteSep™ in CPT™	
		% Purity	% Recovery	% Purity	% Recovery	% Purity	% Recovery	% Purity	% Recovery
CD3+ T Cells	7	67 ± 10	65 ± 21	64 ± 11	48 ± 15	95 ± 3	66 ± 21	93 ± 4	79 ± 15
CD4+ T Cells	6	42 ± 7	70 ± 35	51 ± 13	50 ± 30	90 ± 6	74 ± 37	85 ± 6	82 ± 49
CD8+ T Cells	7	23 ± 9	67 ± 22	23 ± 8	38 ± 19	80 ± 12	57 ± 14	68 ± 15	56 ± 21
CD19+ B Cells	10	8 ± 2	55 ± 22	8 ± 2	43 ± 24	79 ± 8	62 ± 25	62 ± 12	52 ± 53
Monocytes	8	14 ± 4	57 ± 22	20 ± 5	60 ± 23	73 ± 5	60 ± 23	73 ± 5	71 ± 28

Table 2. Purity and recovery of cells collected in sodium citrate and processed using either the standard RosetteSep™ protocol or in CPT™. Results obtained in the absence of the RosetteSep™ cocktail ("Ficoll Alone", "CPT™ Alone") are shown for comparison.

Conclusions

- Specific leukocyte subpopulations can be enriched directly in the blood collection tube in less than one hour.
- The purity of lymphocytes enriched with the Standard RosetteSep™ protocol was slightly higher than the purity of lymphocytes enriched using CPT™.
- There was no significant difference in the recovery of lymphocytes enriched with either protocol.
- There was no significant difference in the purity or recovery of monocytes collected in sodium citrate and enriched with either protocol (Monocytes could not be satisfactorily enriched using the heparin CPT™).
- The purity of $CD19^+$ B lymphocytes was significantly better when the blood was collected in heparin, regardless of the protocol used ($p<0.05$).

The specific advantages of using CPT™ are:

- all procedures are performed in the blood collection tube.
- it is not necessary to layer the rosetted blood over a buoyant density medium.
- it is very easy to remove the enriched cell layer.

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