ClearLLab Control Cells

A Process Control for ClearLLab 10C Application

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IN THIS PAPER YOU WILL

Discover the first IVD process controls compatible for use with ClearLLab 10C Panels.

Learn about the ClearLLab Control Cells, a liquid preparation of stabilized human erythrocytes and leukocytes (lymphocytes, monocytes and granulocytes).

Experience the robustness and stability of the ClearLLab Control Cells, a process control for immunophenotyping using the ClearLLab 10C panels.

Introduction

This white paper is to introduce ClearLLab Control Cells. Flow Cytometry immunophenotyping is a commonly used technology as an aid in diagnosis of hematolymphoid malignancies. ClearLLab 10C Panels are composed of 4 independent immunophenotyping reagents including 27 unique makers which are consistent with WHO and Bethesda guidelines. To be compliant, the performance of reagents and staining procedures needs to be verified using positive control materials. ClearLLab Control Cells were developed to support ClearLLab 10C Panels and include Normal Control and Abnormal Control Cells. ClearLLab Control Cells are whole blood based products that can consistently provide process/positive controls for all the markers/antigens in ClearLLab 10C Panels.

Methods

Multiple lots of both fresh (*T0M) and aged (*T3M+Op-1M) Control cells were stained with each of the four ClearLLab 10C Panels to assess the staining signal, resolution of each antigen as well as specimen stability. In addition, unique gating strategies have been developed for Control Cell data acquisition and analysis to provide an easy, simple and consistent assessment for each relevant cell population.

Figure 1. Control Cells follow the same sample prep and cell acquisition procedure as for the whole blood specimens defined in ClearLLab 10C application. Data is analyzed using Kaluza CSW.

Normal or Abnormal Control Cells

- Pre-wash control cells three times with PBS+2% FBS (0.45mL:12mL)
- Stain washed cells (100µL) with ClearLLab 10C T, B, M1 or M2 Cell Tubes
- Lyse cells with IOTest3 Lysing Plus Fixative Solution (2mL per sample)
- Spin and aspirate, Wash again with 1x PBS (3mL per sample)
- Spin and aspirate, suspend cell pellet with 1x PBS (0.5mL per sample)
- Acquire sample with Navios or Navios EX Cytometer Instrument
- Analyze Data with Kaluza C Software version 1.5

* Fresh (T0M): Control cell specimen was tested within 2 weeks of post production date; Aged (T3M+ Op-1M): Control Cell was tested 3-month close-vial plus a 1-Month open-vial post production date.

Results

ClearLLab Control Cells are liquid preparations of stabilized human erythrocytes and leukocytes (lymphocytes, monocytes, and granulocytes) that have lysing, light scatter, antigen expression, and antibody staining properties representative of those found in human whole blood specimens: The erythrocytes function as the lysable component of ClearLLab Control Cells. The leukocytes function as the positive cell component having surface antigens present on the targeted cells that bind to the antibodies included in ClearLLab 10C Panels, including CD34, CD117 and CD123 (Figure 2).

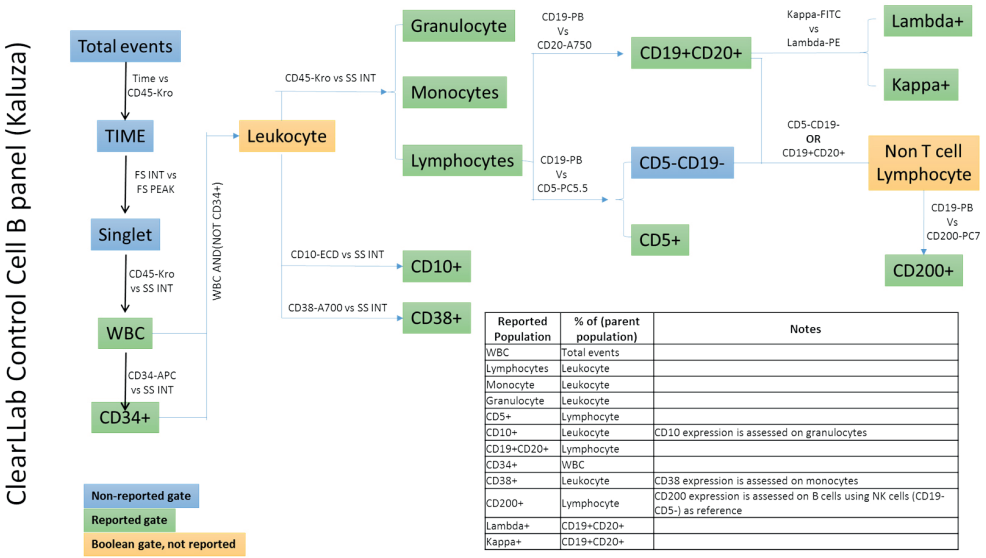
Figure 2. ClearLLab 10C Panels.
The four ClearLLab 10C Panels (B, T, M1 or M2 Cell Tubes) contain 27 different markers/antigens.

	405 nm		488 nm					633 nm		
ClearLLab 10C Panels	PB (1)	Krome Orange	FITC	PE	ECD	PC5.5	PC7	APC	APC-A700 (2)	APC-A750 (3)
B Cell Tube	CD19	CD45	Kappa	Lambda	CD10	CD5	CD200	CD34	CD38	CD20
T Cell Tube	CD3	CD45	TCR-γδ	CD4	CD2	CD56	CD5	CD34	CD7	CD8
M1 Cell Tube	CD11b	CD45	CD16	CD7	CD10	CD13	CD64	CD34	CD14	HLA-DR
M2 Cell Tube	CD19	CD45	CD15	CD123	CD117	CD13	CD33	CD34	CD38	HLA-DR

(1) Pacific Blue (2) APC-Alexa Fluor 700 (3) APC-Alexa Fluor 750

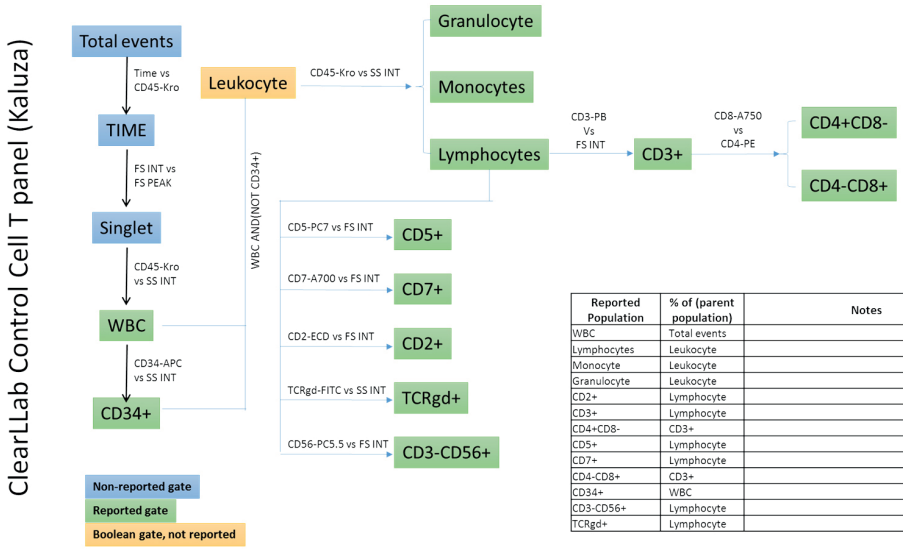
Figure 3. ClearLLab Control Cells Gating Strategy for B, T , M1 or M2 Cell Tubes:
The gating strategies are developed specifically for control cells to provide easy, simple and consistent assessment of every relevant cell population. The same population/marker is gated in a similar way. ClearLLab Control Cells include Normal and Abnormal controls - the only difference is that the Abnormal Control has a blast-like population that is CD45dim, and expresses CD34, CD123 and CD117. The same gating strategy is applied to both Normal and Abnormal, B Cell Tube (3A); T Cell Tube (3B), M1 Cell Tube (3C) and M2 Cell Tube (3D).

3A: B Cell Tube



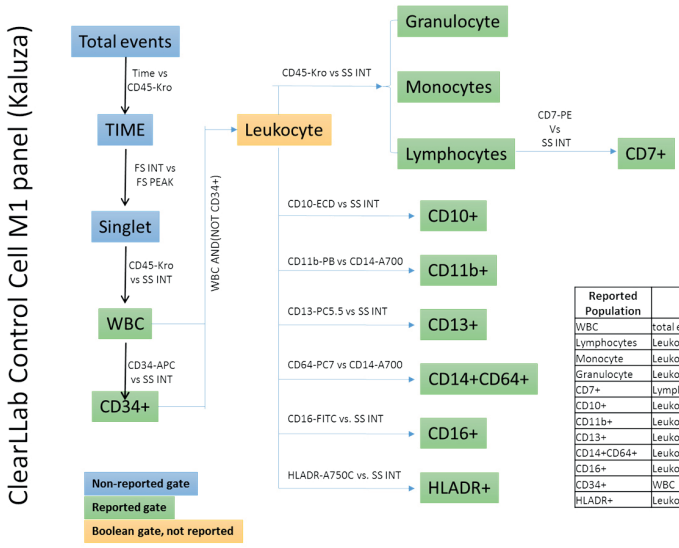
Reported Population	% of (parent population)	Notes
WBC	Total events	
Lymphocytes	Leukocyte	
Monocyte	Leukocyte	
Granulocyte	Leukocyte	
CD5+	Lymphocyte	
CD10+	Leukocyte	CD10 expression is assessed on granulocytes
CD19+CD20+	Lymphocyte	
CD34+	WBC	
CD38+	Leukocyte	CD38 expression is assessed on monocytes
CD200+	Lymphocyte	CD200 expression is assessed on B cells using NK cells (CD19-CD5-) as reference
Lambda+	CD19+CD20+	
Kappa+	CD19+CD20+	

3B: T Cell Tube



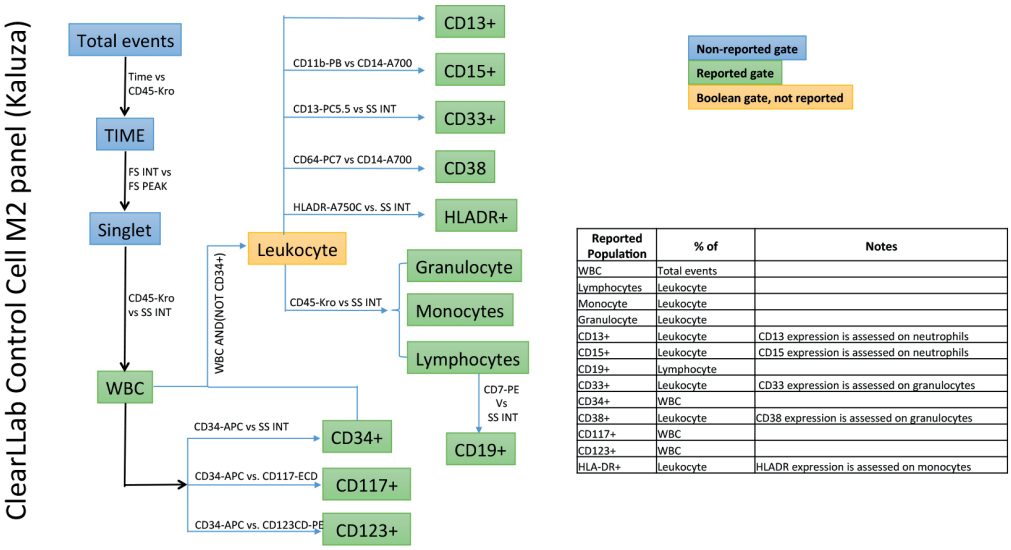
Reported Population	% of (parent population)	Notes
WBC	Total events	
Lymphocytes	Leukocyte	
Monocyte	Leukocyte	
Granulocyte	Leukocyte	
CD2+	Lymphocyte	
CD3+	Lymphocyte	
CD4+CD8-	CD3+	
CD5+	Lymphocyte	
CD7+	Lymphocyte	
CD4-CD8+	CD3+	
CD34+	WBC	
CD3-CD56+	Lymphocyte	
TCRgd+	Lymphocyte	

3C: M1 Cell Tube



Reported Population	% of	Notes
WBC	total events	
Lymphocytes	Leukocyte	
Monocyte	Leukocyte	
Granulocyte	Leukocyte	
CD7+	Lymphocyte	
CD10+	Leukocyte	CD10 expression is assessed on granulocytes
CD11b+	Leukocyte	CD11b expression is assessed on monocytes
CD13+	Leukocyte	CD13 expression is assessed on monocytes
CD14+CD64+	Leukocyte	CD14 & CD64 expression is assessed on monocytes
CD16+	Leukocyte	CD16 expression is assessed on neutrophils
CD34+	WBC	
HLADR+	Leukocyte	HLADR expression is assessed on monocytes

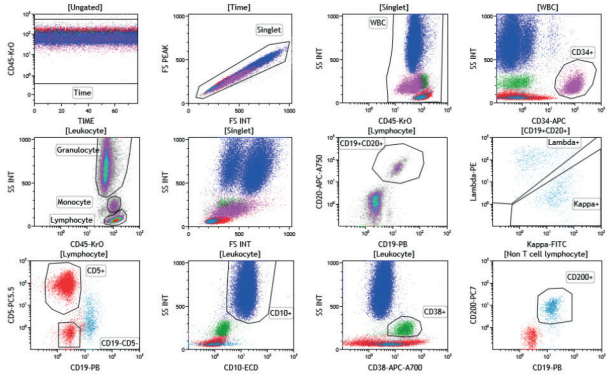
3D: M2 Cell Tube



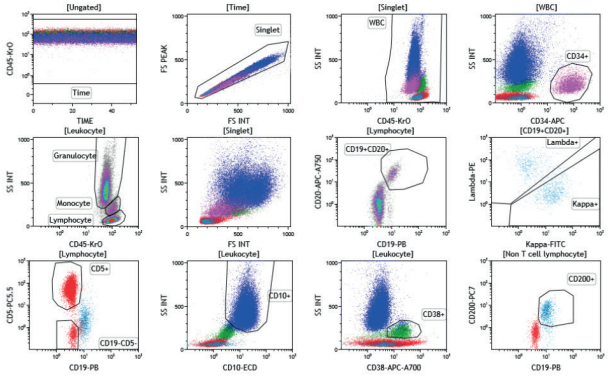
Reported Population	% of	Notes
WBC	Total events	
Lymphocytes	Leukocyte	
Monocyte	Leukocyte	
Granulocyte	Leukocyte	
CD13+	Leukocyte	CD13 expression is assessed on neutrophils
CD15+	Leukocyte	CD15 expression is assessed on neutrophils
CD19+	Lymphocyte	
CD33+	Leukocyte	CD33 expression is assessed on granulocytes
CD34+	WBC	
CD38+	Leukocyte	CD38 expression is assessed on granulocytes
CD117+	WBC	
CD123+	WBC	
HLA-DR+	Leukocyte	HLADR expression is assessed on monocytes

Figure 4. ClearLLab Control Cell Staining Profile:
Representative dot plots of ClearLLab Control Cells Normal and Abnormal: No significant difference was observed between fresh (T0M) and aged (T3M+Op-1M) control Cell specimen except that aged cells have dropped side scatters and decreasing separation of blast and mono population. Neither impacts control cell application. The results had confirmed the consistent staining performance of Control Cell.

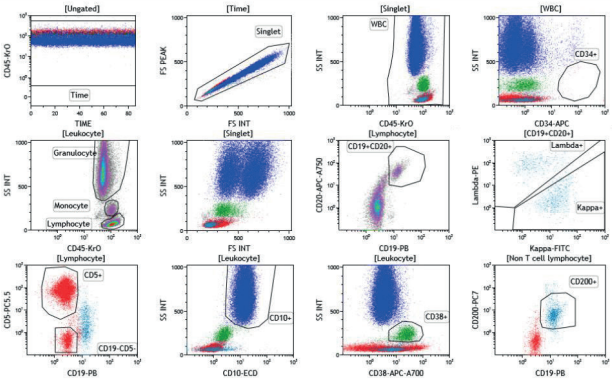
B Panel-Abnormal (TOM)



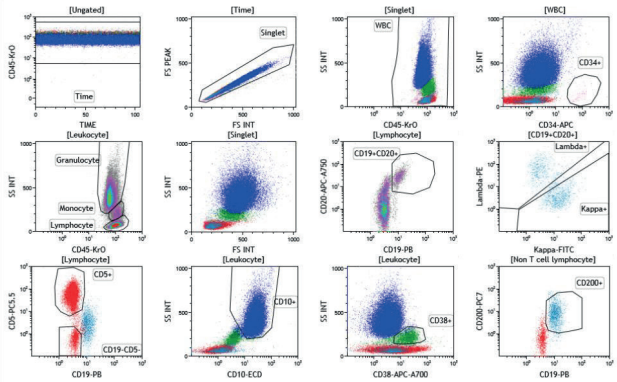
B Panel-Abnormal (T3M+Op-1M)



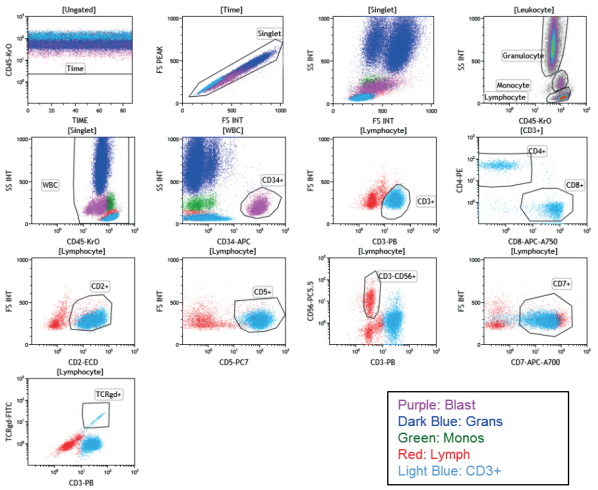
B Panel-Normal (TOM)



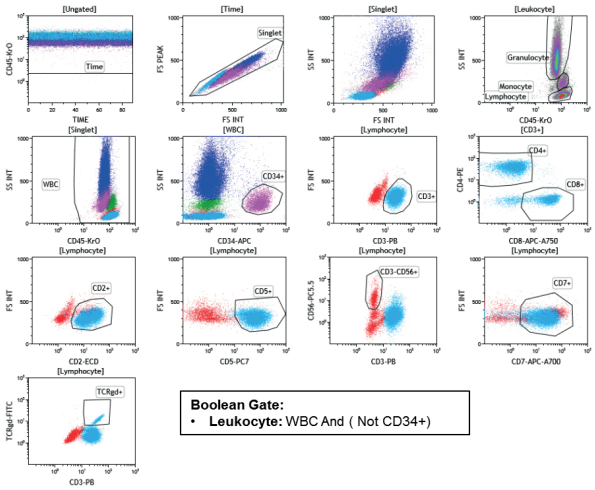
B Panel-Normal (T3M+Op-1M)



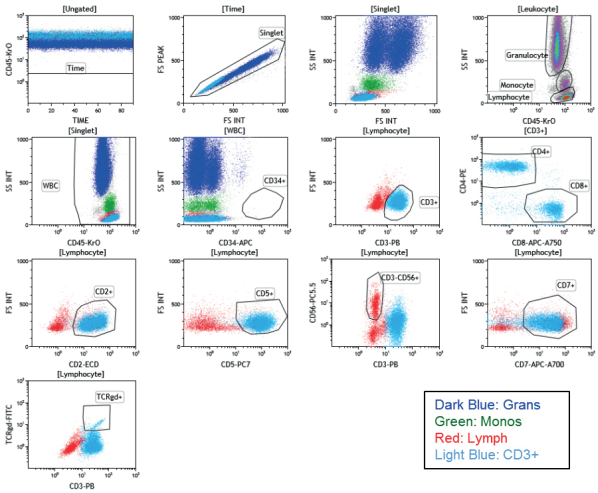
T Panel-Abnormal (TOM)



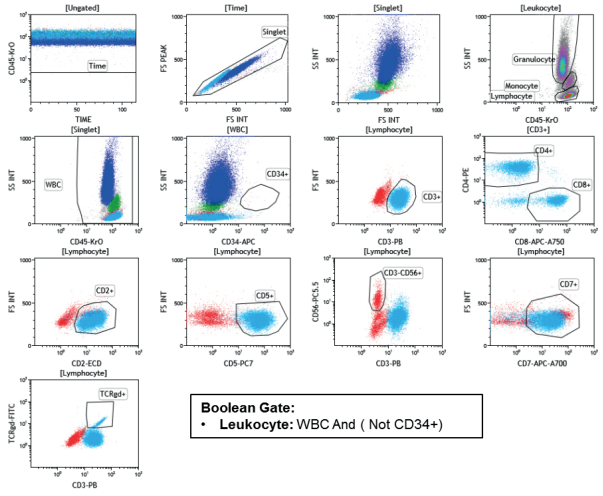
T Panel-Abnormal (T3M+Op-1M)



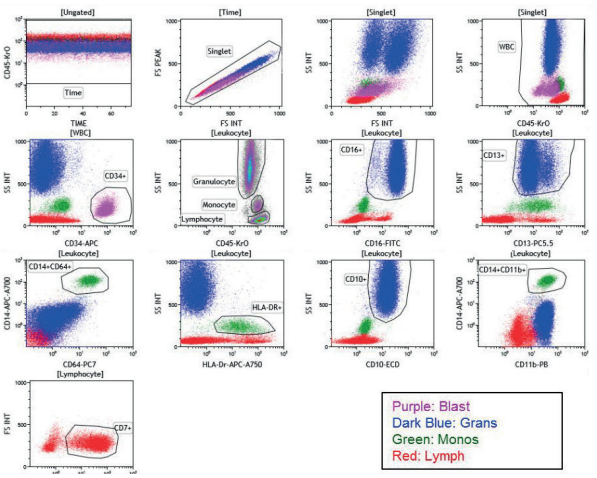
T Panel-Normal (TOM)



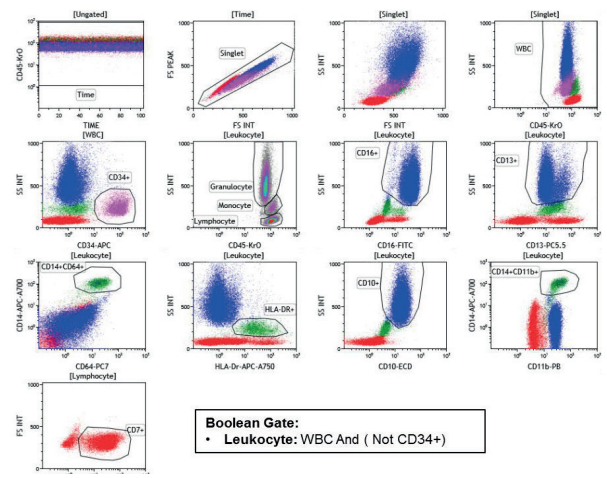
T Panel-Normal (T3M+Op-1M)



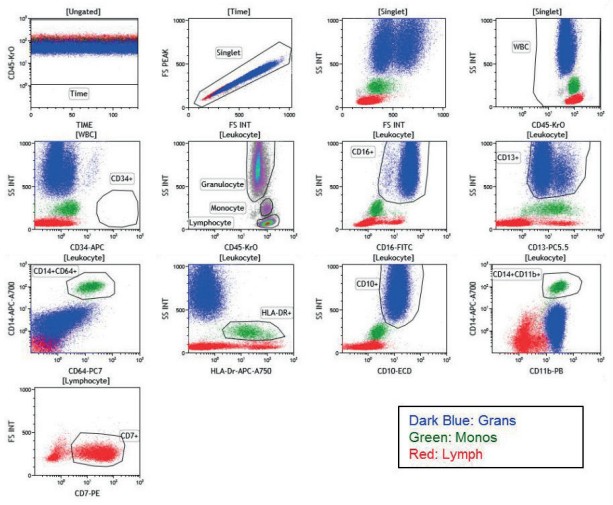
M1 Panel-Abnormal (TOM)



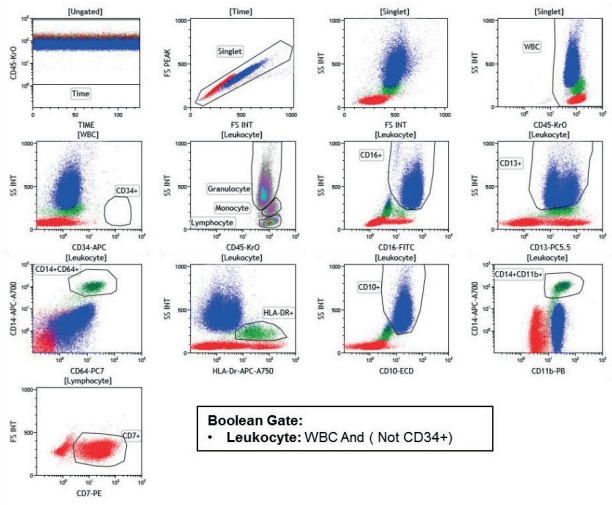
M1 Panel-Abnormal (T3M+Op-1M)



M1 Panel-Normal (TOM)



M1 Panel-Normal (T3M+Op-1M)



Conclusions

- The ClearLLab Control Cell Normal and Abnormal showed prominent expression of the 27 antibodies contained in the ClearLLab 10C Panels, with acceptable cell population resolution.

- The Control Cells have demonstrated similar property as whole blood specimens in terms of antibody staining performance, erythrocyte lysing and flow cytometric analysis as process control for ClearLLab 10C application.

- The gating strategies specific for ClearLLab Control Cells provide easy, efficient and consistent assessment of relevant cell populations/markers of ClearLLab 10C Panels.

Acknowledgments

We are grateful for the following support: Robert Magari, Karen Lo & all members of Apollo 10C project team.

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FLOW-5732WP07.19