

January, 1999

From: The NIAID Flow Cytometry Advisory Committee; the Guidelines Subcommittee
To: NIAID DAIDS Flow Cytometry Laboratories
Subject: Comparison study information for labs wishing to switch to 4-color measurement of percent CD3+CD4+ and CD3+CD8+ cells

Laboratories measuring T cell subsets for ACTG studies have expressed an interest in using 4-color combinations of antibodies to arrive at percentage values for CD3+CD4+ and CD3+CD8+ cells. The Flow Cytometry Advisory Committee (FAC) agrees that using 4-color combinations may have certain advantages (see below). Therefore, laboratories with knowledge and experience in 4-color flow cytometry are allowed to switch to 4-color if an acceptable comparison study is performed.

PLEASE DO NOT IMPLEMENT 4-COLOR PHENOTYPING FOR THE NIAID STUDIES UNTIL YOU HAVE RECEIVED APPROVAL FROM THE FLOW CYTOMETRY ADVISORY COMMITTEE.

Comparison Study

Whenever a laboratory is considering a change in procedures, there is a need to document the effect (or lack of effect) that change will make on reported laboratory values. Therefore, the FAC is requiring that a comparison study be conducted.

At least 60 different sequential patient specimens with CD3+CD4+ \leq 30% must be analyzed using your current 2-color or 3-color method and using your proposed 4-color method. Please do not submit your 60 "best" samples or your 60 "worst" samples. It is important that this data be reflective of the data generated in your laboratory and not a select subset of comparisons. PLEASE KEEP LIST MODE FILES FROM THIS STUDY IN CASE THERE IS A NEED TO LOOK AT THEM.

Advantages of 4-color Immunophenotyping

1. Cost may be less than with a larger 2-color or 3-color panel
2. Time for specimen processing is less
3. There are fewer tubes to aliquot and handle
4. Isotype controls are not needed

Disadvantages of 4-color Immunophenotyping

1. Spectral compensation for 4-colors is more complicated than for 2-colors or 3-colors
2. Added expertise is needed for instrument set-up, data collection, and data analysis

4-Color Panel Recommendation

The Flow Cytometry Advisory Committee recommends the following 4-color panel. There are currently no recommendations for use of particular fluorochromes for the monoclonal antibodies.

CD3/CD4/CD8/CD45

Advantages:

1. Easy to distinguish lymphocytes based on CD45 and SSC
2. Isotype control is not needed
3. "Bright" CD45 as a gating parameter in the tube helps to ensure inclusion of only lymphocytes, thus eliminating the need to correct for gate purity

Disadvantages:

1. Sometimes gating is not clear-cut as there are dim CD45+ cells that have low side scatter
2. Use of only one tube does not allow for QC check with CD3
3. Added expertise is needed versus 2 or 3 color methods

Please note that CD19 (to enumerate total B cells) must be included in the panel for all pediatric patients. (Please include CD45 in the 4-color combination).

THREE-COLOR VS FOUR-COLOR COMPARISON STUDY FOR APPROVAL TO SWITCH TO FOUR-COLOR FLOW CYTOMETRY

Institution: _____

Department: _____ ACTG Lab Code:

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Name of Person Completing Form: _____

Title: _____

Phone Number: _____ Date Form Completed: _____

Please complete the following information

Model of flow cytometer used 3-color:

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 Model of flow cytometer used 4-color:

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- 01-EPICS-C
- 02-EPICS Profile I
- 04-FACS Analyzer
- 05-FACScan
- 09-Other, Specify (if not listed below) _____
- 10-EPICS Profile II
- 11-EPICS Elite
- 12 EPICS XL
- 13 FACStar
- 14-FACStar Plus
- 15 Ortho Cytoron Absolute
- 16-FACSCalibur
- 17-FACSort
- 18-FACS Vantage
- 19-EPICS Altra

Method of cell preparation used 3-color:

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 Method of cell preparation used 4-color:

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- 1-Whole Blood Lysis using Ammonium Chloride
- 2-Whole Blood Lysis using FACS Lysing Solution (BD)
- 3-Whole Blood Lysis using Immuno-lyse (Coulter)
- 4-Whole Blood Lysis using Immuno-Prep (Q-Prep, Coulter)
- 5-Whole Blood Lysis using Lyse and Fix (GenTrak)
- 6-Whole Blood Lysis using Ortho-mune Lysing Reagent (Ortho)
- 7-Whole Blood Lysis using OptiLyse B Lysing Solution (AMAC)
- 8-Whole Blood Lysis using OptiLyse C Lysing Solution (AMAC)
- 9-Other, Specify _____
- 10-Whole Blood Lysis using FACS Lysing Solution (BD) – NO WASH

Institution _____

ACTG
Labcode

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Using the list of antibody manufacturers (MAb Mfg) and fluorochromes on the next page, please fill in the following information

Three-Color Tubes Used to Collect CD3+4+% and CD3+8+%

Please enter the markers for each tube in row one, antibody manufacturer (MAb Mfg) in row two, fluorochrome for each marker in row three, and whether a premix was used (Y or N) in row 4. If the antibody manufacturer or fluorochrome codes are not listed on the next page, please use code 99 then fill in the information on the line below the appropriate box.

	TUBE 1			TUBE 2		
1)	CD		CD		CD	
2)	MAb Mfg				MAb Mfg	
3)	Fluorochrome				Fluorochrome	
4)	Premix (Y or N)				Premix (Y or N)	

Four-Color Tube Used to Collect CD3+4+% and CD3+8+%

Please enter the markers for the tube in row one, antibody manufacturer (MAb Mfg) in row two, fluorochrome for each marker in row three, and whether a premix was used (Y or N) in for 4. If the antibody manufacturer or fluorochrome codes are not listed on the next page, please use code 99 then fill in the information on the line below the appropriate box.

	TUBE 1			
1)	CD		CD	
2)	MAb Mfg			
3)	Fluorochrome			
4)	Premix (Y or N)			

Codes for antibody manufacturers (MAb Mfg):

AA	Antigenix America	IS	Immunosource
BD	Becton Dickinson	IN	In-house
BI	Biodesign International	LN	Leinco
CT	Caltag	OR	Ortho
CL	Coulter	PM	PharMingen
DK	Dako	SG	Sigma
EX	Exalpha	SR	Serotec
GT	GenTrak	99	Other, specify
IM	Immunotech		

Codes for fluorochromes:

1-FITC (Green)	10-Cy3 (Yellow)
2-PE (Yellow)	11-PE-Cy5 (Far Red)
3-RD1 (Yellow)	12-Alpha Red (Red)
5-PerCP (Far Red)	13-Quantum Red (Red)
6-ECD (Red)	14-Tricolor (TC) (Far Red)
7-Cy-Chrome (Far-Red)	15-PC5 (Far Red)
9-RPE (Yellow)	16-APC (Far Red)
	99-Other, specify

**THREE-COLOR VS FOUR-COLOR COMPARISON STUDY FOR APPROVAL TO SWITCH
TO FOUR-COLOR FLOW CYTOMETRY**

**BE SURE ALL SAMPLES HAVE A THREE-COLOR CD3+4+% ≤ 30%
PLEASE USE 60 SEQUENTIAL SAMPLES MEETING THIS REQUIREMENT**

Institution: _____

ACTG Lab Code:

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	Sample Number	Date	3-color CD3+4+%	4-color CD3+4+%	3-color CD3+8+%	4-color CD3+8+%
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2	<input style="width: 100%; height: 20px;" type="text"/>	<input style="width: 100%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>
3	<input style="width: 100%; height: 20px;" type="text"/>	<input style="width: 100%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>
4	<input style="width: 100%; height: 20px;" type="text"/>	<input style="width: 100%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>
5	<input style="width: 100%; height: 20px;" type="text"/>	<input style="width: 100%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>	<input style="width: 50%; height: 20px;" type="text"/>
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Institution: _____

ACTG

Lab Code:

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	Sample Number	Date	3-color CD3+4+%	4-color CD3+4+%	3-color CD3+8+%	4-color CD3+8+%
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Institution: _____

ACTG
Lab Code: -

	Sample Number	Date	3-color CD3+4+%	4-color CD3+4+%	3-color CD3+8+%	4-color CD3+8+%
42	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
43	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
44	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
45	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
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47	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
48	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
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60	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

PLEASE SEND COMPLETED FORMS TO CINDY WILKENING, STATISTICAL AND DATA
ANALYSIS CENTER, HARVARD SCHOOL OF PUBLIC HEALTH FXB 549-A,
651 HUNTINGTON AVE, BOSTON, MA 02115-6017 OR
FAX THE FORMS 617-432-3163
QUESTIONS? CALL CINDY WILKENING AT 617-432-2809