

Appendix A: Generic HVTN-HPTN PBMC Processing Worksheet

Note: For HPTN and HVTN, use the protocol-specific PBMC Processing Worksheet found in the protocol specific Specimen Processing Laboratory Instructions (SPLI) or Laboratory Manual unless otherwise directed. Complete all fields by hand, using a pen.

Specimen Processing Laboratory:		Protocol:	
Participant ID (PTID/PID):		Visit Number:	Visit Type:
Collection Date:		Collection Time:	
Processing Start Date:		Processing Start Time:	Processed By (Initials):
Reagents	Manufacturer	Lot Number	Expiration Date
DMSO			
FBS			
WDR: HBSS or PBS (circle one)			
Cell Separation Tube (frit)			
Density Gradient Media			
	Volume in mL (record as X.Y)		Expiration Date
CPS	CPS	DMSO	FBS
Data to be Captured During Processing			Sample
Sample tube type (circle one or record "other" tube type)			ACD / HEP / EDT Other: _____
Blood condition (circle one or more; add comments on reverse as needed)			SAT/ HEM / CLT
Measured usable whole blood volume (to the nearest 0.1mL)			mL
Indicate processing method (circle one)			CSTFB / overlay / underlay
Counting Method: Name of specific instrument or manual count (record in field to right)			
Counting resuspension volume of HBSS (or other WDR) (V) (record as X.Y)			mL
Cell count average concentration (C)			x 10 ⁶ cells/mL
Total cell number (T) = C x V			x 10 ⁶ cells
Calculate cell yield/mL of whole blood (QC check)= (T/Usable Whole Blood Volume)			x 10 ⁶ cells/mL
Calculate estimated CPS resuspension vol. (V1)=(T/15x10 ⁶ cells)(1mL)			mL
Calculate final CPS resuspension volume (Vf), (V1 rounded DOWN to nearest whole (X.0) mL)			mL
Calculate actual number of cells per vial N2 = (T/Vf) x V2; (V2=1 mL).			x 10 ⁶ cells/vial
Print and QC LDMS Label content/barcodes (initials of person (s) performing QC)			
Frozen Date and Time (ddMMMyyyy /HH:MM) (Explain in comments section if not within 4 hours of processing start time)			
Number of Cryovials actually frozen Note: Should be equal to final CPS resuspension volume for 1mL aliquots (Vf).			
Complete remaining LDMS entries including total cell count & frozen time (Initials)			

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Specimen Processing Laboratory:

PTID/PID:

Transfer of Cryovials to Freezer Storage Box	
Person who transferred cryovials to storage box locations assigned by LDMS	
Date (ddMMMyyyy)/time cryovials were transferred from controlled-rate freezing device to storage box. (Sample must be maintained at -80°C during transfer)	
Initial (Primary) Review (Initials/Date)	
Final (Secondary) Review (Initials/Date)	

Hemacytometer Counts	Total Count	Viable Cells	Non-Viable
Square #1 (cells/mm ²)			
Square #2 (cells/mm ²)			
Square #3 (cells/mm ²)			
Square #4 (cells/mm ²)			
Average Cell Count per Square (cells/mm ²)			
PBMC Dilution Factor (1:DF*)			
Hemacytometer Factor for cells/mL	10 ⁴	10 ⁴	10 ⁴
Cell count concentration (C) = (Average Cells/mm ²)(DF)(10 ⁴); convert to 10 ⁶ cells/mL	Not applicable	x 10 ⁶ cells/mL	Not applicable
% viability = (Viable cells 4 squares/total cells 4 squares) (100)	Not applicable		Not applicable

***Note:** Dilution Factor (DF) = (parts cells + parts dilution fluid)/ parts cells

Automated Cell Counts (10 ³ /μl=10 ⁶ /mL)	Count #1
Cell Count (C) as cells x 10 ⁶ /mL	
PBMC Dilution Factor (1:DF**)	
Cell Concentration = (C)(DF)	x 10 ⁶ cells/mL
% viability (if applicable)	

****Note:** Dilutions for automated counters are extremely rare. If performing direct counts, enter a 1 in the DF box and complete the column.

Comments, protocol deviations, and additional information not captured elsewhere in this worksheet: