Scope

This procedure applies to White Blood Cell and PBMC counts from whole blood post Ficoll-Paque gradient centrifugation.

Overview

This is a quick guide that leverages the Hanc HIV/AIDS Network Coordination (HANC) SOP for preparing whole blood samples prior to running a PBMC count.

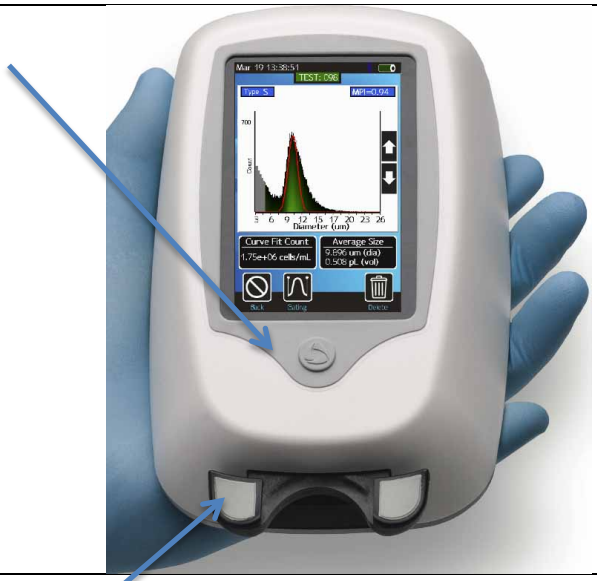
Total Procedure Time

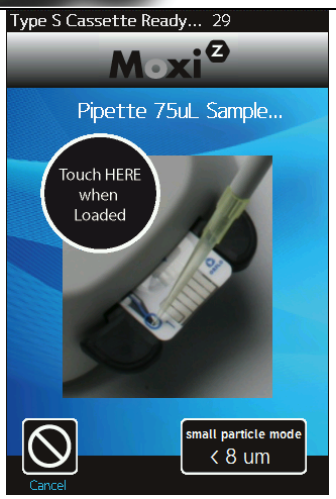
Minutes

Materials

Description	Catalog number	Vendor	Quantity Per Test
Moxi Z Cell Analyzer	MXZ001	Orflo	1
Moxi Z S-Cassette	MXC003	Orflo	1
Orflo Diluent (new 1L bottle	MXA010	Orflo	0.45
10-100ul Pipette	AP-100 (0.2% CV)	Accupet	1
10-100ul Pipette Tips	UR-100	Accupet	1
100-1000ul Pipette Tips	UR-1000	Accupet	1
2ml centrifuge dilution tube w/ cap	22-283R	Genesse Scientific	1
Tube rack			

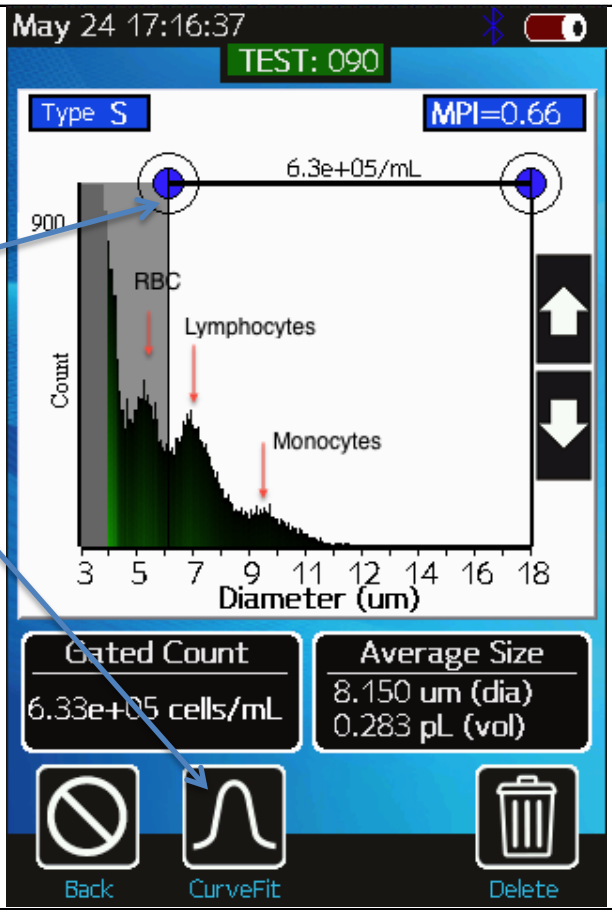
Process

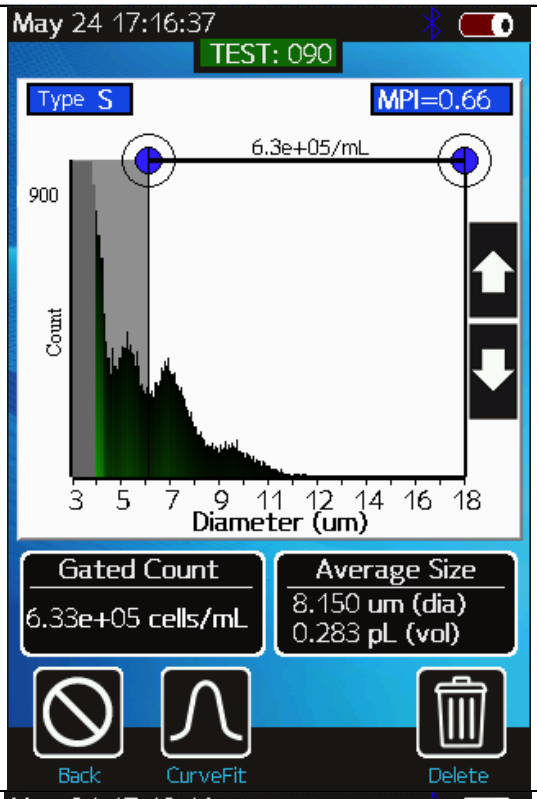
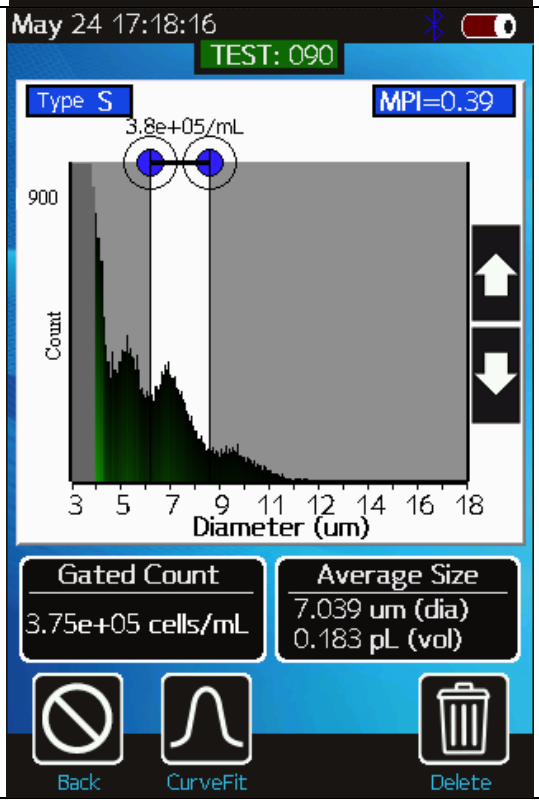
Procedure	Graphic
1. Prepare sample as per Chapter 16 of the "Cross-Network PBMC Processing SOP" V4.0, Effective Date 2011-10-03	
2. (17.3.1) Determine and record the WDR counting re-suspension volume (V) accurate to within 0.1mL 3. Dilute an aliquot of the PBMC sample with ORFLO Diluent (~10-20X) 4. (17.3.4) Mix cells gently, but thoroughly, by pipet trituration 5X	Example: For 10X dilution, add 50ul of PBMC to 450ul of ORFLO Diluent. Mix well.
5. Turn on Moxi Z, by pressing button	
6. Insert cassette by pressing down on grey tab and sliding cassette into open	

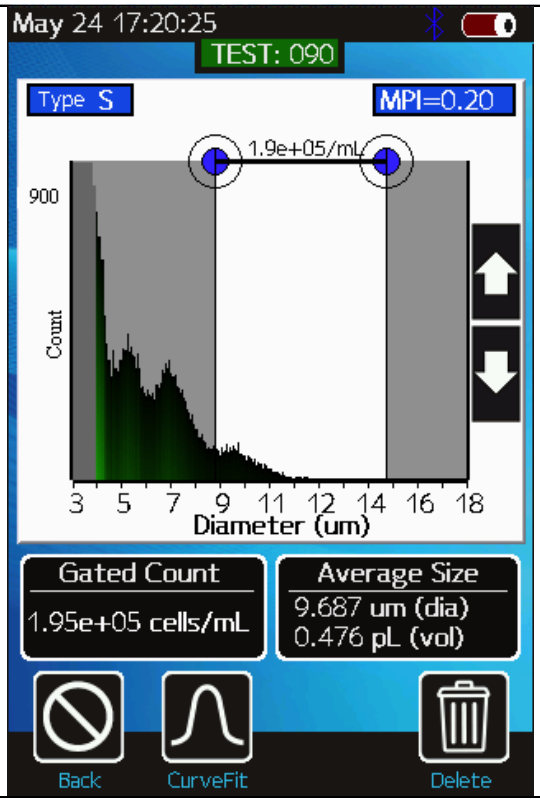
slot	
7. Pipette 80ul of diluted sample into loading port	
8. Touch screen to start test in Normal Mode (anywhere besides the bottom corners of the screen)	

9. Re-scale x-axis to 3-18um diameter range

10. Toggle to Gating Mode to manual gate around the peak of interest, two blue dots will appear



11. For total PBMC counts – gate around both lymphocyte and monocyte peaks	
12. For lymphocyte counts – gate around the peak at ~7um diameter	

13. For monocyte counts – gate around the peak at ~10um diameter	
14. Multiply the gated concentration by the dilution factor	True Conc = Gated Conc. x Dilution Factor
15. (17.3.8) Calculate the cell yield in cells/mL of usable whole blood using the following formula:	Cell Yield (10^6/mL)= T/ Usable Whole Blood Volume
16. (17.3.7) Calculate the total number of cells using the following formula:	T=C x V T= Total number of cells C= Concentration (10^6/mL) determined in counting method V = Count re-suspension volume of WDR in mL