

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.

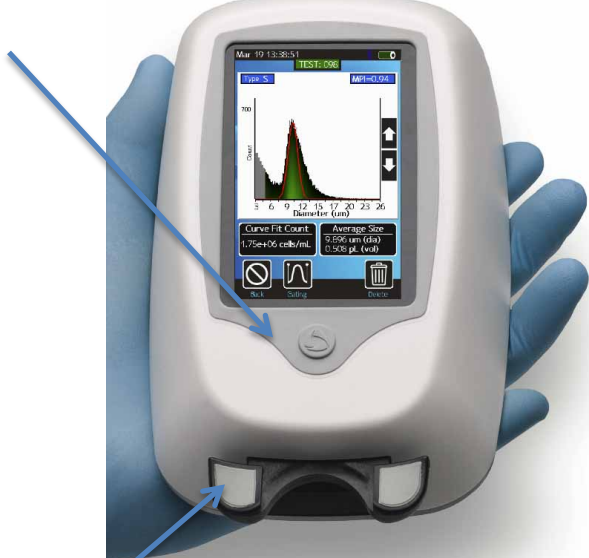

Total Procedure Time



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

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

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

The screenshot shows the Moxi Z software interface. At the top, it displays the date and time 'May 24 17:16:37' and a test ID 'TEST: 090'. Below this, the 'Type S' and 'MPI=0.66' are shown. The main display is a histogram of 'Count' versus 'Diameter (um)'. The x-axis is scaled from 3 to 18 micrometers. The histogram shows three distinct peaks: 'RBC' (Red Blood Cells) at approximately 6-7 micrometers, 'Lymphocytes' at approximately 8-10 micrometers, and 'Monocytes' at approximately 10-12 micrometers. Two blue dots are placed on the x-axis at approximately 6.5 and 17.5 micrometers, indicating manual gates. A blue arrow points from the text 'two blue dots will appear' to these dots. Below the histogram, a 'Gated Count' box shows '6.33e+05 cells/mL' and an 'Average Size' box shows '8.150 um (dia)' and '0.283 pL (vol)'. At the bottom, there are three icons: 'Back' (a circle with a slash), 'CurveFit' (a bell curve), and 'Delete' (a trash can).

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

<p>13. For monocyte counts – gate around the peak at ~10um diameter</p>	
<p>14. Multiply the gated concentration by the dilution factor</p>	<p>True Conc = Gated Conc. x Dilution Factor</p>
<p>15. (17.3.8) Calculate the cell yield in cells/mL of usable whole blood using the following formula:</p>	$\text{Cell Yield (10}^6\text{/mL)} = \frac{T}{\text{Usable Whole Blood Volume}}$
<p>16. (17.3.7) Calculate the total number of cells using the following formula:</p>	$T = C \times V$ <p>T= Total number of cells C= Concentration (10⁶/mL) determined in counting method V = Count re-suspension volume of WDR in mL</p>