Total White Blood Cell (WBC) Count Protocol for use with the Moxi Flow or Zepi Flow

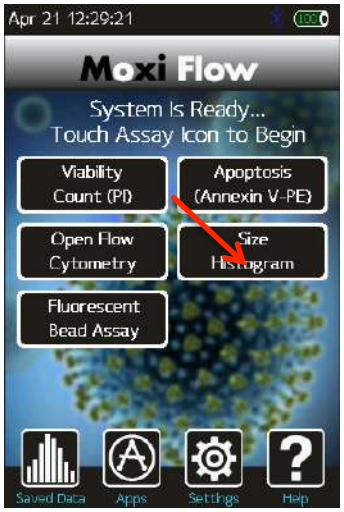
Overview

This approach is a nucleated cell counting method. The technique works by diluting the whole blood, lysing all the cells (including the WBC's), and counting the remaining nuclei. It is designed to provide a rapid means for counting the total WBC counts in a sample. *Note: There is an inherent error in the counts due to contributions of nucleated red blood cells (RBC'S). Ordinarily, this contribution is not significant. However pathological conditions could contribute to higher-than normal nucleated RBC's in blood.*

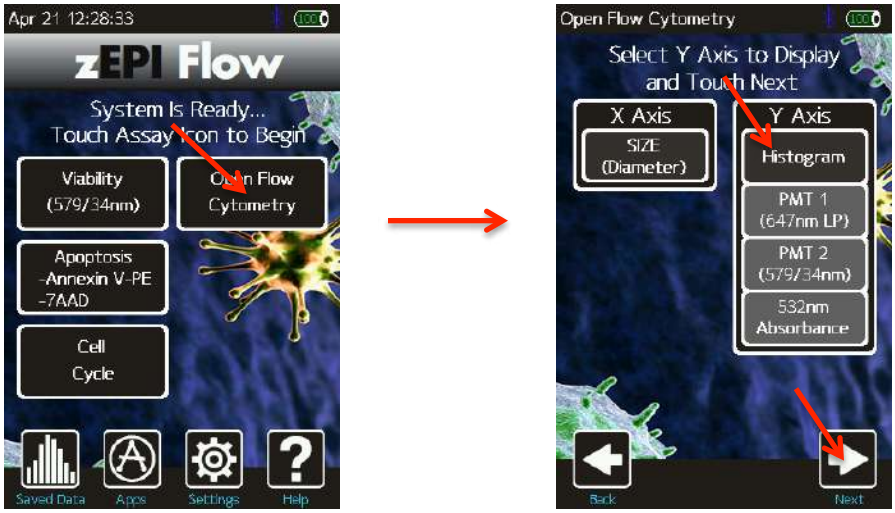
Reagents

- Zap-oglobin II Lytic Reagent ((Beckman Coulter, Part # 7546138))
- Ca^{2+}-free and Mg^{2+}-free PBS or the equivalent
- Anti-coagulant (e.g. Heparin, EDTA, sodium citrate) coated collection tube
- MFS Cassettes (Orflo Prod #MXC020)

Protocol



- Collect >40µL of whole blood into an anti-coagulant coated capillary tube.
- Add exactly 40µL of whole blood to 5mL of PBS/Media.
- Add 3 drops of Zap-oglobin II Lytic Reagent
- Mix the sample several times (10x) through inversion-mixing and allow the sample to sit for a minimum of 3 minutes.
- Turn on the Moxi Flow or Zepi Flow
- Select the appropriate test for the instrument:
  - *Moxi Flow* – Touch "Size Histogram" (red arrow in image to the right)
  - *Zepi Flow* – Touch "Open Flow Cytometry", select "Histogram" for the Y-Axis column, and touch "Next" (see images below)



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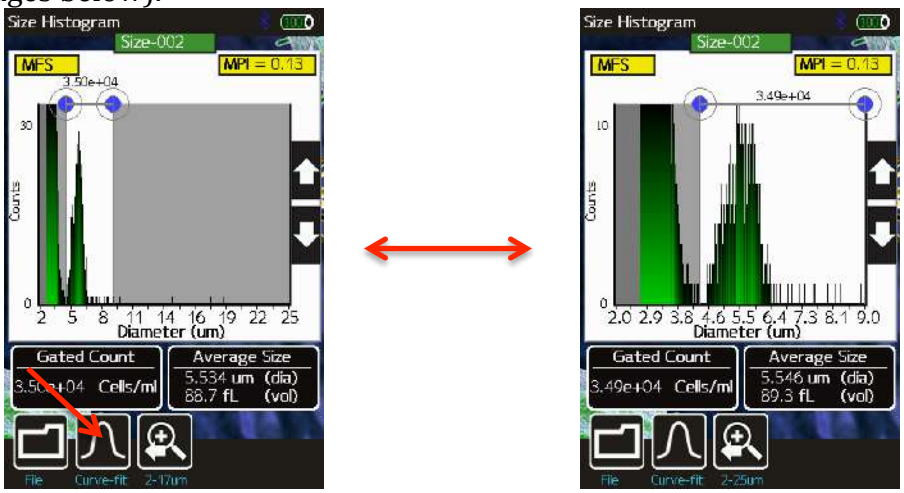
- Load an MFS cassette and close the door(s).
- Prior to loading the sample, mix the sample through slow inversions to ensure proper mixing.
- When the instrument indicates to load the sample (text in the black bar at the top of the screen), open the door and load 75µL (minimum) of sample into the cassette loading well.
- Close the door. The test will automatically start.
- Upon test completion, re-scale the axis to 3 – 10µm by touching the "3-18µm" (magnifying glass icon), and then the "3-10µm" (magnifying glass icon, see images below).



- Gate around the nuclei population (typically 4.5 – 10µm size range).
- The total, dilution-adjusted counts can be determined by muliplying the Moxi Z concentration by 128 (5.12 mL/.04 mL). Note: This includes ~80µL of Zap-oglobin II reagent.