Title: Moxi V – System Check Bead Procedure

MATERIALS REQUIRED

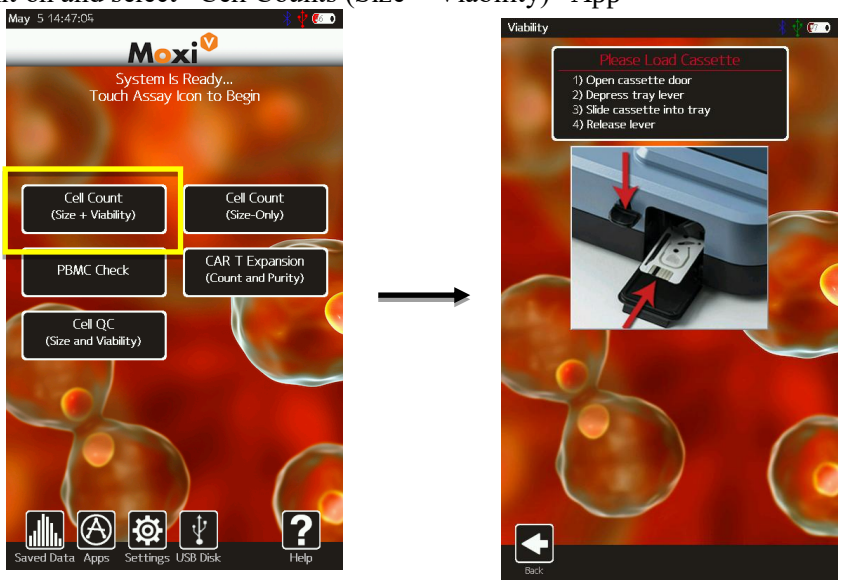
- Moxi V (Cat #MXV102)
- System Check Beads, Orange/Red Channel (Cat# MXA028-3)
- Cassettes: Type S+ (Cat# MXC030-3)

PREPARATION

1. Ultrasonicate Both Check beads for 60 seconds at room temperature (22.5°C)
2. Vortex the beads at the highest speed setting for 30 seconds.
3. Inversion mix (turn the vial upside down, then right side up) the vial, 20-30x prior to running a test.
4. If the "Fluorescent Gain" setting has been changed, change it back to "Default" (Go to "Settings" from the Home screen. Touch "Fluorescence Gain" field to toggle)

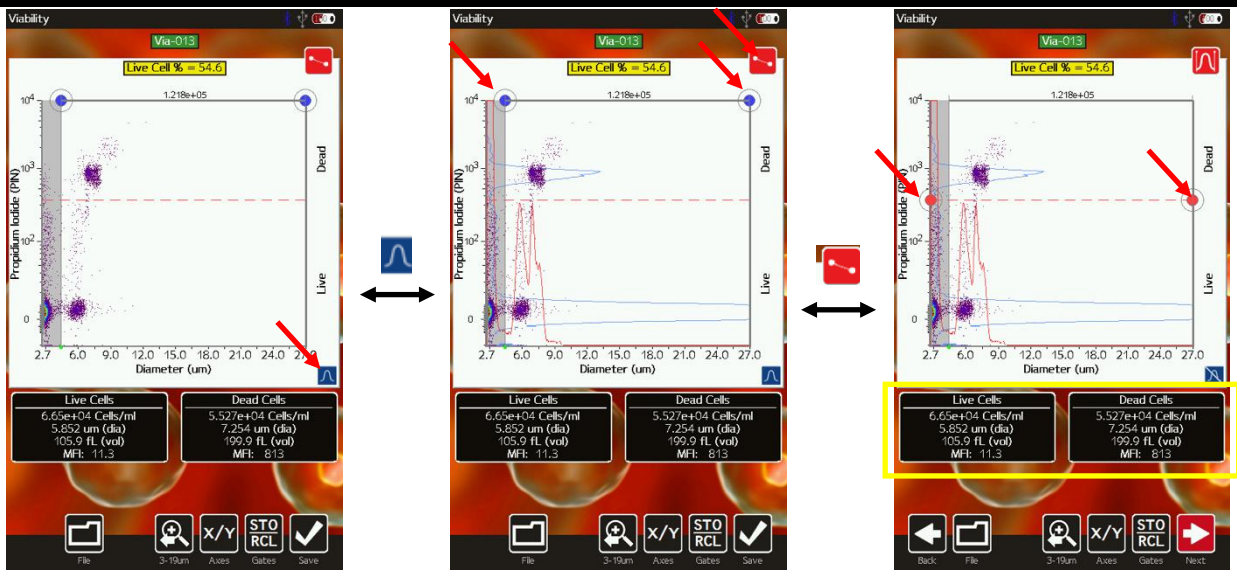
RUNNING THE TESTS

1. Mix beads by slowly (~once every second) inverting the bottle 30x initially (5x between runs, if repeats are being performed)
2. Turn Moxi V unit on and select "Cell Counts (Size + Viability)" App



3. Insert a cassette and wait for the laser cassette alignment to complete.
4. When the alignment completes (and prompted "Enter the Sample" at the top left black bar), pipette 60µL bead sample into the loading well in one fluid motion.
5. Immediately close the door (try not to delay longer than a second or two or the beads will begin to settle) and the test will automatically run.

RESULTS ANALYSIS



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1. The results will be automatically gated by the system if the "Auto Gate" setting is on. If not, or to fine-tune the gate locations, follow the steps above (and described below).
    a. Touch the blue histogram overlay icon at the bottom right of plot, to overlay the size (red, x- axis) and fluorescence (blue, y-axis) histograms
    b. Adjust the size gates (blue vertical markers) to place it in the "valley" (minima) of the red histogram, to the left of the bead peaks (see image above/middle).
    c. Toggle to enable fluorescent gating by touching the red gate icon (top right of scatter plot in the image above/right). Place the fluorescent gate just below the upper (Bright) bead population (below the upper peak on the blue histogram)
2. With this gating, you can record all relevant stats
    a. The total concentration is listed at the top of the scatter plot, between the blue/size gates.
    b. Dark Bead concentration and size is listed in the "Live Cells" black box to the lower/left of the scatter plot.
    c. Bright Bead concentration and size is listed in the Dead Cells" black box to the lower/right of the scatter plot.
3. Verify the reported concentration and size to the expected values on the bottle. The margin of error should be 15% or less for both concentration and size. ***Note: For exact lot size and concentration data, please email tech_support@orflo.com with the bead lot number to obtain a COA for that lot.***

FACTORS THAT CAN AFFECT RESULTS

1. Temperature of the bead solution or environment can slightly affect the reported diameter. Bead solution and environment should be within 20-25°C.

2. Microbial contamination of the bead solution can also affect the reported diameter. Avoid contamination by using a clean pipette tip to aliquot the solution. Store the beads in 2-8°C when not in use.
3. Improper mixing can affect the reported concentration. Slow inversion of the bottle after sonicating/vortexing is key to ensuring single-bead suspension.

Document #	Revision #	Reason
MXV.SYS.005	2.0	Updated company information from Orflo to Gemini Bio Simplified protocol instructions, updated system photos, revised CV acceptance criteria
	2.1	Updated company information from Gemini Bio to ORFLO. Updated part numbers for new kits.