Title: Moxi V – Validation Check Bead Procedure

MATERIALS REQUIRED

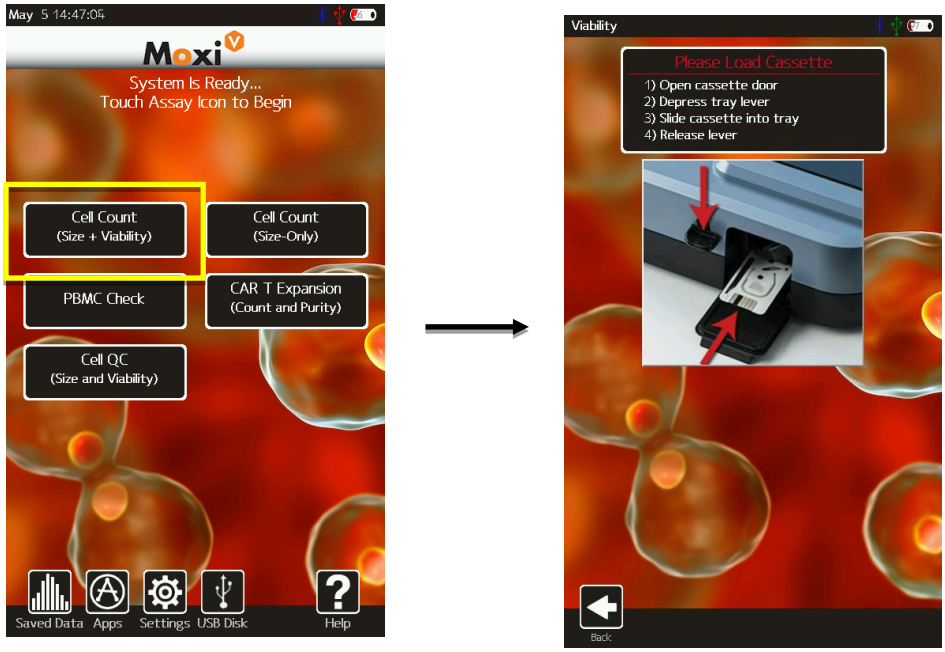
- Moxi V (Cat #MXV102)
- Validation Check Beads, Orange/Red Channel (Cat# MXA028-3)
- Cassettes: Type S+ (Cat# MXC030-3)

PREPARATION

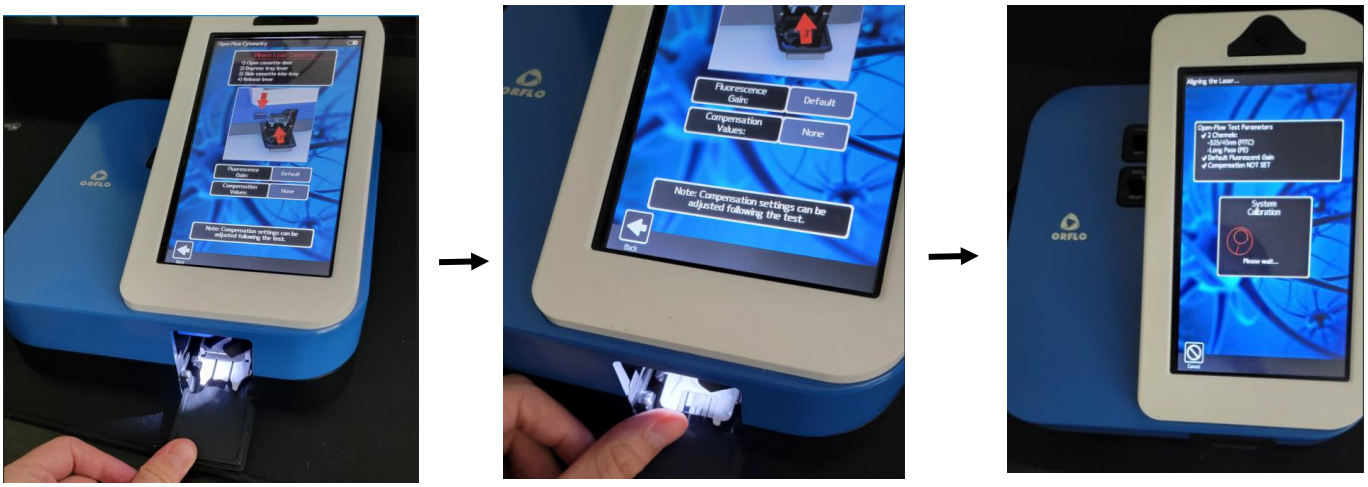
1. Ultrasonicate the validation check beads for 60 seconds at room temperature (22.5°C)
2. Vortex the beads at the highest speed setting for 30 seconds.
3. Inversion mix the vial (turn the vial upside down, then right side up), 20-30x prior to running a test.
4. If the "Fluorescent Gain" setting has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

RUNNING THE TESTS

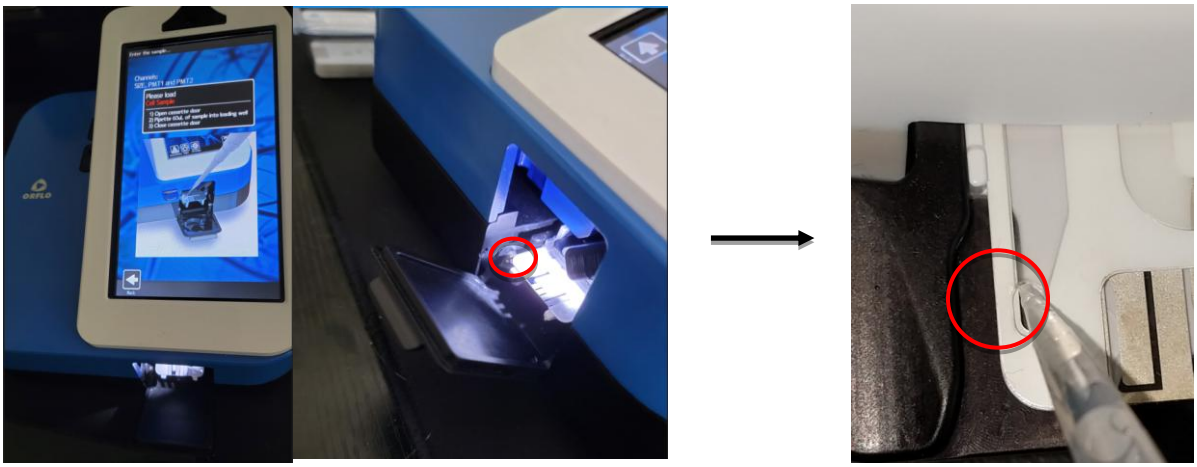
1. Mix beads by slowly (~once every second) inverting the bottle 30x initially (5x between runs, if repeats are being performed)
2. While mixing the beads, turn Moxi V unit on and select "Cell Counts (Size + Viability)" App



3. Open the door fully, insert an unused cassette, close the door, and wait for the laser cassette alignment to complete.

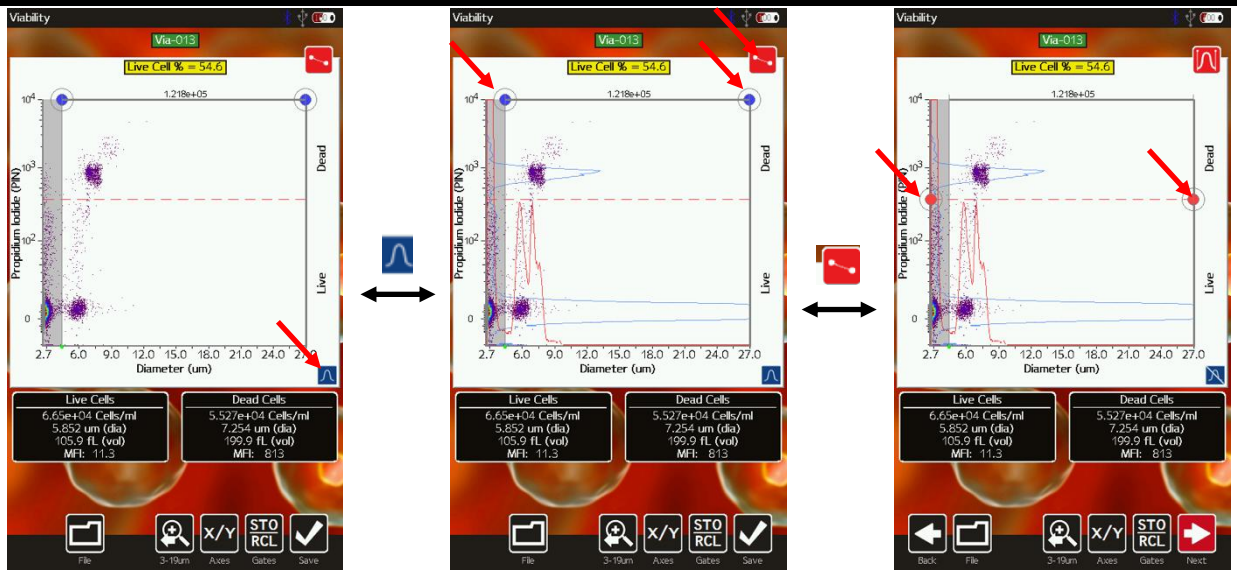


4. When the alignment completes (and prompted to "Enter the Sample" at the top left black bar), open the door to the first stop (45° angle) and pipette 60µL of the check bead sample into the loading well in one fluid motion.

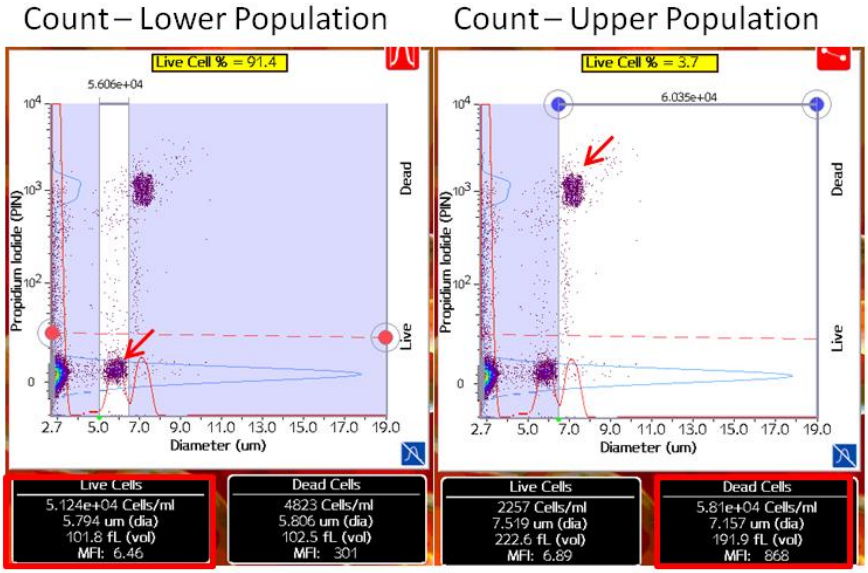


5. Immediately close the door (try not to delay longer than a second or two or the beads will begin to settle). Once the door is closed, the test will automatically run.

RESULTS ANALYSIS



1. The results will be displayed in a scatterplot similar to the results shown above. The data will be analyzed by adjusting both the x-axis and y-axis gates manually.
    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. These histogram overlays will be used for proper gating.
    b. Adjust the size gates using the blue vertical markers (see image above/middle).
    c. Toggle the red button in the upper right of the scatterplot to enable fluorescent gating by touching the red gate icon (top right of scatter plot in the image above/right).
2. Follow the guidelines below for properly isolating and quantifying the two populations of beads present:



a. Dark bead (non-fluorescent) population (image above/left):
   i. Adjust the x-axis size gates such that they are just to the left and right of the first histogram peak (**left gate ~5µm, right gate ~6.5µm**)
   ii. Adjust the y-axis fluorescence gate so that it is directly above the first blue histogram peak; if this was previously done for the light bead population, leave the y-axis gates the same to ensure accurate results
   iii. Record all information displayed in the "Live Cell" box at the bottom of the screen (image above/left; highlighted in red)
   iv. Store the x and y-axis gating information by pressing the "Gates" button and choosing one of the numbers on the top row in green. These gates can now be recalled for future validation tests by pressing the "Gates" button and clicking the corresponding number on the bottom row in red.

b. Light bead (fluorescent) population (image above/right):
   i. Adjust the x-axis size gates such that they are just to the left of the second histogram peak and all the way to the far right side of the plot (**left gate ~6.5µm, right gate ~19µm**)
   ii. Adjust the y-axis fluorescence gate such that it is directly above the first blue histogram peak; if this was previously done for the dark bead population, leave the y-axis gates the same to ensure accurate results
   iii. Record all information displayed in the "Dead Cell" box at the bottom of the screen (image above/right; highlighted in red)
   iv. Store the x and y-axis gating information by pressing the "Gates" button and choosing one of the numbers on the top row in green. These gates can now be recalled for future validation tests by pressing the "Gates" button and clicking the corresponding number on the bottom row in red.

3. Compare 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.

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 mixing of the bottle after sonicating/vortexing is key to ensuring single-bead suspension.
4. The plastic beads can stick to the sidewalls of plastic containers. It is critical to ultrasonicate the beads, prior to first use, to disrupt that adhesion and ensure all beads are in solution.
5. If the beads freeze, they will irreversibly aggregate resulting in lower measured concentrations. Any bead vial that has a noticeable solid mass in it (feel it while mixing) or has been confirmed to have frozen (fully or partially) should be discarded.
6. Temperature of the bead solution or environment can slightly affect reported bead diameter. The values are inversely correlated (temperatures > 22.5˚C will progressively lower the reported bead volume/diameter). Bead solution and environment should be within 20-25˚C.

7. Microbial contamination of the bead solution can also affect the reported diameter. Avoid contamination by handling the beads aseptically whenever possible (e.g., using a clean pipette to aliquot the solution). Store the beads at 2-8˚C when not in use.
8. Improper mixing can affect the reported concentration. Sonicating does not evenly disperse the beads; it only detaches from the walls of the bottle. Slow inversion mixing of the vial after sonicating is key to ensuring a homogenous 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.
	2.2	Updated ORFLO contact information
	2.3	Updated overall protocol to improve test explanations and to be more comprehensive and useable for end users