Calibration and Use of the Coulter Counter Model Multisizer 4e

1. SCOPE AND APPLICATION

    1.1. This method is based on the Multisizer 4e Particle Analyzer user manual. It is applicable to the Multisizer 4e particle analyzer. It can be used to determine the quantity, mean volume, and size of particles in aqueous solutions.

2. SUMMARY OF METHOD

    2.1. The Multisizer 4e is powered on. “Change aperture tube wizard” and “Instrument start up” are run. Background counts are completed. Samples are counted and saved into the AGP Multisizer file. These counts are then uploaded to LIMS. The Multisizer calibration is verified monthly using certified calibration beads.

3. EQUIPMENT AND SUPPLIES

    3.1. 1 L electrolyte jar

    3.2. 1 L waste jar

    3.3. 30 mL Dilu-vials—1 for each step of daily Coulter® Counter prep and 1 for each sample flask

    3.4. Multisizer 4e (SN BA23044)

    3.5. Coulter® calibration cup or a 200-mL beaker

    3.6. Coulter® paddle stirrer

    3.7. Computer with access to LIMS and Multisizer software

    3.8. 1 mL Eppendorf® pipette and tips

    3.9. 1 L Lab Industries® repipet jar with 10 mL adjustable repipettor

    3.10. 10 mL graduated cylinder

    3.11. Small brush for cleaning Coulter® Counter aperture

4. REAGENTS AND STANDARDS

    4.1. Isoton II® electrolyte solution (Isoton)

    4.2. 10-µm or 20-µm Latex calibration beads (cytoFLEX Ready to use Daily QC Fluorsphere)

5. METHOD

    5.1. Preparing the instrument for use

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5.1.1. Turn on the Multisizer 4e by pressing the switch located in the rear panel of the instrument. Turn the computer on and open software. When the software opens, check the box that says, “Connect instrument”.

5.1.2. Check the waste and electrolyte jars. Empty the waste or fill the electrolyte jar as needed.

5.1.3. Ensure that the correct aperture size is installed for the current test. AGP tests require 100-µm (this is usually what is in the machine). Microbiology analysis requires 20-µm.

5.1.3.1. To install a new aperture (Skip to 5.1.4 if correct aperture is installed.)

5.1.3.1.1. Clean the new aperture by soaking it in hot water for 5 minutes. Rinse with DI water and clean with aperture cleaning brushes.

5.1.3.1.2. Open SOP (Standard Operating Procedure) required for test (located under the SOP tab on the top menu bar).

5.1.3.1.3. If SOP selected requires a different aperture, a box will appear asking if you want to change the aperture. Click yes.

5.1.3.1.4. “Change aperture tube wizard” will open.

5.1.3.1.5. Follow the steps listed, hitting ‘next’ after each step.

5.1.3.1.6. Unlock the aperture with the knob located above the aperture. Gently pull the current aperture out of the opening. Slowly insert the new aperture into the opening. Lock the aperture into place.

5.1.3.1.7. Continue through all the steps listed. Complete the step by clicking the box to the right of each step.

5.1.3.1.8. For AGP: set the current to 600 µa and set the gain to 4 for the 100 µm aperture. It can also be auto set to 1600 µa and a gain of 2.

5.1.4. To complete the calibration verification (monthly):

5.1.4.1. The calibration should be verified monthly.

5.1.4.2. To set up the calibration, put 10 mL of Isoton into the vial labeled “cal”. Shake bead bottle and add 3-6 drops of 10 µm calibration beads and swirl or tap vial to mix.

5.1.4.3. Place vial on the platform and submerge the aperture.

5.1.4.4. Press preview, run>preview, to ensure that the concentration percentage stays between 5-10%. If it is too low or too high adjust the number of drops added.

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5.1.4.4.1. Alternative method: Calibration can be verified during the "change aperture tube wizard" process. Once the calibration step is reached, remove the flush cup and place a vial prepped with calibration beads.

5.1.4.5. Press verify aperture calibration. Once the calibration is verified, it should say "the calibration is ok." Save this run in AGP/Multisizer/calibration.

5.1.4.6. Analysts will be prompted to save run once the graph is closed out.

5.1.4.7. Calibration should be completed when calibration verification is not acceptable.

5.1.4.8. Must flush a few times with Isoton when finishedto flush out calibration beads.

5.1.5. Calibration (Skip if calibration verification is ok.)

5.1.5.1. To set up calibration, put around 10 mL of Isoton into the vial called "cal". Shake bead bottle and add 3-6 drops of 10 µm calibration beads, shake or tap to mix.

5.1.5.2. Press preview, run>preview, to ensure that the concentration percentage stays between 5-10%. If it is too low or too high, adjust the number of drops added.

5.1.5.3. In the software, select calibration aperture.

5.1.5.4. Enter the calibration beads information (located on the sheet inside of the box).

5.1.5.5. Press "Start".

5.1.5.6. Once the calibration is done, a window will pop up. Press the "find peak" button. Once it is adjusted to the peak, press "accept new Kd". If this does not show up, it will have an SOP button; press that and follow the screen instructions.

5.1.5.7. Save the graph in the calibration folder in "AGP/Multisizer/Multisizer/calibration verification".

5.1.5.8. Must flush a few times with Isoton when finished to flush out calibration beads.

5.1.6. Instrument Start Up

5.1.6.1. Place a vial of clean Isoton on the platform and submerge the aperture.

5.1.6.2. After running the "change aperture tube wizard" (if needed), run "instrument start up" (in the software, go to the top menu and

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select run > instrument start up. All setting should be selected except “auto set current and gains”).

5.1.6.3. The software will then automatically prepare the machine for counts.

5.1.7. Select the correct platform needed (Beaker or Accuvetter STs). To switch the platform, when it is in the down position, pull the platform out and rotate. Generally, we only use the Accuvetter STs base.

5.2. Background counts

5.2.1. Background counts are required to be run anytime the Multisizer is being used for counts (AGP, R. sub for C. dubia food). Background counts are also run weekly when the Multisizer is not in use to keep the instrument functioning properly. This is not a method requirement, but it helps to keep the background counts within an acceptable range, preventing salt buildup.

5.2.2. After initial “instrument set up”, upload the appropriate SOP for the current test. If there isn’t a correct SOP use the SOP wizard or SOM wizard as needed. See manual for more details. We generally use SOPs. The current SOPs used are AGP2026.sop and R. sub Counts 2026.sop.

5.2.3. Place a clean sample vial containing Isoton on the sample platform and raise the platform to immerse the tip of the aperture tube.

5.2.3.1. Ensure that the Isoton is taken from the Isoton dispenser and not the Isoton II® box.

5.2.3.2. The dispenser should be set to nine milliliters. The dispenser should be verified quarterly per SOP TA-06.32.

5.2.4. Go to run menu and hit start.

5.2.5. Enter file ID (background or BKGD), sample ID (date), enter operator (initials), enter run # (1) and hit ok.

5.2.6. Ensure that the graph is set to have x-axis as volume. This can be changed by going to graph > x axis > volume.

5.2.7. The average of the three counts on fresh Isoton should be less than 400 and ideally less than 200.

5.2.8. When troubleshooting, if the mean count is greater than 400, ensure that the vial used for the background counts is clean and fill with fresh Isoton. Rerun the “Instrument Start Up” and see if this helps lower the background count. If not, check the tip of the isotone dispenser to make sure there is not build-up of salt; if present, rinse with autoclaved DI. The system can also be drained and refilled to ensure fresh Isoton is in the system. The aperture can be rinsed with autoclaved DI water or cleaned with hot water. If you have

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more than 3 background runs, choose the best set of 3 and delete the others from the folder.

5.2.9. These files should automatically save into the folder "AGP/Multisizer/Multisizer Counts". Move into the background folder (located within AGP/Multisizer/"YYYY" counts/background).

5.2.10. If the background is needed for AGP counts:

5.2.10.1. Open LIMS>AGP LN manager>select appropriate run ID> press "collect counts from instrument". Select the day and user. Press "open raw data file" and select the correct background counts from "AGP/Multisizer/2025 counts/background". Press "upload to LIMS" and save data into AGP/AGP counts 25/background. Save the file as BKGD and the date (MM.DD.YYYY).

5.2.10.1.1. All raw counts stay within AGP/Multisizer folders. All saved files are placed in the AGP/AGP counts YYYY folders.

5.2.11. The coulter counter is now ready to be used for a sample run.

5.3. Analyzing samples

5.3.1. Upload SOP required for the test (AGP or R. sub counts).

5.3.2. Place vial on the platform and submerge the aperture. Select run > start.

5.3.3. Enter sample information. File ID (flask code, like CA or DD), sample ID(LIMS sample number, LAB BLANK, SALT BLANK, LIMS sample number-DUP or -SUP), operator (initials), enter run # (1) and hit ok.

5.3.4. Note: the run number should only be changed on the first run of the sample set. Do not change it between samples as this will interfere with the way LIMS pulls the data. It can be reset between runs that get entered into LIMS separately (Background, Pre-mix, Inoc, sample run).

5.3.5. The instrument should complete 3 runs per sample and give an average. These will automatically save to "AGP/Multisizer/Multisizer Counts".

5.3.6. During the run, the concentration meter bar on the left side should remain below 10%. If it exceeds 10%, stop the run and dilute the sample appropriately.

5.3.7. Once the counts are completed, Open LIMS>AGP LN manager>select appropriate run ID> press "collect counts from instrument". Select the correct day and user. Press "open raw data file" and select the correct counts from "AGP/Multisizer/Multisizer Counts". Press upload to LIMS and save data into "AGP/AGP counts 'YY'".

5.3.7.1. Before counts are accepted, the 3 counts for a flask must portray numbers within 10% of the middle number. If the upper or lower number is out of range, a recount must be performed for the flask.

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You may use any combination of counts from the completed runs to reach this criterion (the numbers do not have to consecutively be within 10%).

5.4. Shutting down

5.4.1. Flush the system with fresh Isoton.

5.4.2. Place a full vial of Isoton solution on the platform and keep the aperture submerged if the instrument is being used again within the next week.

5.4.3. If the instrument is not being used for the next 2 weeks, drain the system by hitting the "empty" on the bottom bar and store it in Isoton cleaning solution.

5.4.4. Switch off the Multisizer and log off the computer.

6. DATA ARCHIVAL

6.1. FDEP Laboratory Information Management System (LIMS)

7. QUALITY CONTROL

7.1. Complete a calibration verification on the Multisizer 4e monthly.

7.2. The analyst must review the Multisizer 4e Particle Analyzer User's Manual before using the counter. The manual is in the Toxicity Lab (Rm B246).

7.3. The calibration beads are purchased from Beckman-Coulter. Do not use if expired.

7.4. Contaminated materials could include an electrolyte solution, rinse water, calibration bead solution, dirty vials and other glassware.

8. REFERENCES

8.1. DEP SOP TA-06.32, Calibration and Verification of Mechanical and Disposable Pipettes in the Bioassay/AGP Lab

8.2. Multisizer 4e Particle Analyzer User's Manual

APPENDIX OF CHANGES

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