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 diluent 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® pipettor and tips

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

    3.10. 10 mL calibrated 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

5. METHOD

    5.1. Preparation for calibration and/or measurement

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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 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 to ensure that they are at proper levels. 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. Microbiology analysis require 20-µm. AGP tests require 100-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 SOM (Standard Operating Method) required for test (located under the SOP tab on the top menu bar).

5.1.3.1.3. If SOM 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.

5.1.3.2. To complete the calibration verification,

5.1.3.2.1. The calibration should be verified monthly.

5.1.3.2.2. To set up the calibration, put 10 mL of Isoton into a vial. Then, add ~3-6 drops of calibration beads. The calibration beads should be 10 µm in diameter.

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

5.1.3.2.4. Press 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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5.1.3.2.4.1.1. 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.

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

5.1.3.2.6. Analyst will be prompted to save run once the graph is closed out.

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

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

5.1.4.1. To set up calibration, put around 10 mL of Isoton into a vial. Then, add ~3-6 drops of calibration beads. The calibration beads should be 10 µm.

5.1.4.2. Press 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.

5.1.4.3. In the software, select calibration aperture.

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

5.1.4.5. Press "Start".

5.1.4.6. 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).

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

5.1.5. Preparing for calibration or measurement

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

5.1.5.2. After running the "change aperture tube wizard", run "instrument start up" (in the software, go to the top menu and select run > instrument start up. All setting should be selected except "auto set current and gains").

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

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5.1.6. 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.

5.2. Background counts

5.2.1. After initial “instrument set up”, upload the appropriate SOP/SOM for the current test. If there isn’t a correct SOP/SOM use the SOP wizard or SOM wizard. See manual for more details.

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

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

5.2.2.2. The dispenser should be set to nine milliliters. The dispenser should be calibrated quarterly per SOP TA-06.32.

5.2.3. Go to run menu and hit start.

5.2.4. Enter file ID (background), sample ID (date), enter operator = initials, enter run # = 1 and hit ok.

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

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

5.2.7. Trouble shooting note: If the mean count is greater than 400, ensure that the vial used for the background counts is clean and fill with fresh Isoton. Check the tip of the isoton dispenser to make sure there is not build-up of extra salt molecules, 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 more than 3 runs, choose the best 3 and delete the others from the folder.

5.2.8. These files should automatically save into the folder “AGP/multisizer/Multisizer Counts”. They will then need to be moved into the background folder (located within AGP/Multisizer/2022 counts/background).

5.2.9. 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 background counts from “AGP/Multisizer/2022 counts/background”. Press “upload to LIMS” and save data into AGP/AGP counts 22/background. Save the file as the date (i.e. 04-07-2020).

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

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

5.3. Analyzing samples

5.3.1. Prepare coulter counter according to 5.1.

5.3.2. Upload SOM/SOP required for this test.

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

5.3.4. Enter sample information including: File ID = flask code, sample ID = LIMS sample number or LAB BLANK, operator = initials, enter run # = 1 and hit ok.

5.3.5. Note: the run number should only be changed on the first run of sample set.

5.3.6. The instrument should complete 3 runs and give an average. These will automatically save to “AGP/Multisizer/Multisizer Counts”.

5.3.7. 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.

5.3.8. 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 background counts from “AGP/Multisizer/2022 counts/background”. Press upload to LIMS and save data into “AGP/AGP counts 23”.

5.3.8.1. Before counts are accepted, the 3 runs for a flask must portray numbers within 10% of each other based on the middle number. If the upper or lower number is out of range, a recount must be performed for the flask. You may use any combination of 3 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.

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6. DATA ARCHIVAL

6.1. To correct raw data entries, place a single line through the incorrect or illegible entry, write the corrected entry near the error, date and initial the correction. Do not overwrite entries.

6.1.1. The corrected entry is written as near as possible to the error or in the comment box on the appropriate bench sheet.

6.1.2. If the correction requires an explanation, write a comment and reference the error with a letter. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space.

6.1.3. Use sequential alphabetical annotation to identify errors and corresponding comments (use A for first error and use A for corresponding explanatory comment; use B for second error, etc.).

6.2. Use a black, waterproof pen for all bench sheets and logbooks.

6.3. FDEP Laboratory Information Management System (LIMS)

7. QUALITY CONTROL

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

7.2. It is required that the analyst reviews the Multisizer 4e Particle Analyzer User's Manual before attempting to use the counter. The use of this SOP is acknowledgement that the manual has been reviewed. The manual is in the Toxicity Lab (Rm B246).

7.3. The calibration beads are purchased from Beckman-Coulter. Be aware of the expiration date on the beads. Do not use if expired.

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

7.5. If a problem occurs during the sample processing or testing, the following steps are performed: 1) Lab supervisor is notified. 2) Client is notified (client makes decision for additional testing). 3) Section administrator is notified. 4) A non-conformance report (NCR) is submitted describing the problem and the corrective action.

7.5.1. To create NCR: open LIMS and go to Laboratory/Administrative/Non-Conformance Report. Refer to https://floridadep.gov/dear/quality-assurance/content/dep-sops for sample collection and preservation, and the Laboratory Quality Manual for corrective action and handling out-of-control data.

8. REFERENCES

8.1. Multisizer 4e Particle Analyzer User's Manual

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APPENDIX OF CHANGES

03/12/2021 Updated to reflect universal language and changed text to be the same throughout.

04/05/2022 Updated pathways and checked for grammar.

04/03/2023 Updated for clarity.

03/13/2024 Checked for readability and added additional troubleshooting steps.

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