Calibration and Use of the Coulter® Counter Model Z2

1. SCOPE AND APPLICATION

   1.1. This method is based in the Coulter® Z Series user manual. It is applicable to the Coulter® Model Z2 particle counter. It can be used to determine the quantity and mean volume of particles (meeting the size requirements of the aperture as described in the user manual) in aqueous solutions.

2. SUMMARY OF METHOD

   2.1. The Z2 is turned on. The aperture tube is flushed and rinsed with the appropriate solutions. Certified calibration particles are used to calibrate the Z2 monthly. The Z2 data terminal is used to set up the Z2 for counting samples. Background counts are taken. Samples are counted. Data is collected via the FDEP AGP Manager Program in LIMS. After use, the Z2 aperture tube is rinsed and flushed. The instrument is powered off.

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. Computer with access to the Laboratory Information Management System (LIMS)

   3.5. Black Box® Serial-Parallel Converter II

   3.6. Coulter® Counter Model Z2 (Ser.# AB114302) with data terminal

   3.7. Coulter® Calibration cup or a 200-mL beaker

   3.8. Coulter® paddle stirrer

   3.9. 1 mL Eppendorf® pipettor and tips

   3.10. 1 L Lab Industries® repipet jar with 10 mL adjustable repipettor

   3.11. 10 mL Calibrated graduated cylinder

   3.12. Small brush for cleaning Coulter® Counter aperture

4. REAGENTS AND STANDARDS

   4.1. Isoton II® electrolyte solution (Isoton)

   4.2. Coulter® detergent (Isoterge)

   4.3. 10-µm Latex calibration beads

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5. METHOD

5.1. Preparation for calibration and/or measurement

5.1.1. Before proceeding, ensure that 1) the Model Z2 system is filled with diluent, 2) the correct aperture tube is connected, 3) the diluent jar is full, 4) the waste jar is empty, 5) the instrument is powered on and 6) the Black Box® is plugged into the electrical outlet (on the surge protector behind the Coulter Counter)

5.1.2. Place 25 mL of Isoton into a 30-mL vial. Remove the storage vial containing Isoterge from the sample platform and place it out of the way. Place the vial of fresh Isoton on the sample platform and gently raise the sample platform to immerse the bottom 3.5 cm of the aperture tube. The platform should be raised to its upper limit without striking the aperture tube.

5.1.2.1. To move the platform, place one or more fingers under it and press and hold the lock release (front center edge of platform). Raise or lower the platform to the desired level. Release the lock button before removing your fingers from beneath the platform. The platform is raised until the tip of the aperture tube is close to, but not touching, the bottom of the vial.

5.1.3. Ensure that the aperture can be clearly seen in the aperture monitor. When the aperture monitor is properly focused, the aperture is seen as a small circle on a bright disk. To obtain a clear image, adjust the [Focus] knob located to the right of the sample platform.

5.1.3.1. If there is any debris that blocks the aperture during flushing, purging, or counting, use the small brush to remove it. Use a gentle sweeping motion to remove debris.

5.1.4. Flush the aperture. On the Z2 data terminal, use the left/right arrow keys to select *Flush aperture*. Press [Start]. The message *Flush Complete* will display. This process flushes particles or air bubbles away from the rear of the aperture.

5.1.5. Prime the aperture. On the Z2 data terminal, press [Functions]. Use the left/right arrow keys to select *Prime aperture*. Press [Start] once and then a second time. *Prime aperture* pumps the detergent from the tubing and replaces it with diluent. The message *Aperture Primed* will display when the priming is complete.

5.1.6. Flush the aperture once more with fresh Isoton. Leave the aperture in the Isoton until ready for the next step.

5.1.7. For Calibration, go to Step 5.2. For Background, review Step 5.3., then go to Step 5.4. For Sample Analysis, review Step 5.3, then go to Step 5.5.

5.2. Coulter Model Z2 Calibration

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5.2.1. Place 75 ± 10 mL of Isoton II into the Coulter® calibration beaker. Dispense 20 drops of the 10-µm calibration beads into the beaker.

5.2.2. Lower the sample platform and remove the vial of Isoton. Insert the paddle stirrer into the paddle stirrer holder. Hold the stirrer near the opposite end from the paddle. Carefully insert the end into the claw of the holder. Push upward. DO NOT PUSH SIDEWAYS. The end will travel approximately 1.5 cm into the holder.

5.2.3. Place the Coulter® calibration beaker on the sample platform. Raise the platform. Do not allow the stirrer to touch the bottom or the sides of the beaker.

5.2.3.1. The stirrer depth can be adjusted using the knob located on the top left of the Model Z sample stand.

5.2.4. Turn the stirrer to slow speed (~120 RPM).

5.2.4.1. The stirrer control is located on the top right of the Model Z sample stand. Turn the knob clockwise to increase speed, counterclockwise to decrease speed.

5.2.5. On the Z2 data terminal press [CAL]. Screen *C1:* displays. Use the cursor (left/right) key to select *fl* (equals cubic micron) in the *Select Units* field. Use the cursor (up/down) to highlight the *Calibrator Size* field. Use the Z2 data terminal keypad to enter the calibration bead size. (Enter the nominal size from the certificate that is enclosed in the bead box.) Use *fl* not *µm*. After the bead size is entered, the *measure aperture* field will automatically change to *yes*.

5.2.6. Press [Start] on the Z2 data terminal. Screen *C4: Calibration* will display. When the messages have displayed, Screen *C3: Cal- Instrument Settings* will display. The message area displays several messages including: Flushing (if just powered up), measuring aperture flow rate, measuring aperture resistance.

5.2.7. Press [Start] on the Z2 data terminal: Screen *C6: Calibrate* will display. The calibration will begin. On the Z2 data terminal screen, a vertical bar labeled SAT (saturation) monitors particle concentration. The black line should be ≥ 0.3 and ≤ 0.7.

5.2.8. If the SAT bar shows a concentration of < 0.3 or > 0.7, press [Stop] on the Z2 data terminal. **If the bar shows ≥ 0.3 and ≤ 0.7 go to STEP 5.2.10**. Screen *C9: Calibration Factor Calibration interrupted* will display. Adjust the sample concentration by adding more calibration beads or diluent as needed. If the bar is < 0.3, add calibration beads to the calibration beaker. If the bar is > 0.7, add diluent (Isoton) to the calibration beaker.

5.2.9. Repeat Step 5.2.7. and 5.2.8. as needed until the concentration is within the stated limits, then go to Step 5.2.10.

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5.2.10. After the calibration has run, Screen *C7: Calibration Factor* will display. Record the **Kd**. Press [Start]. Screen *C6: Calibrate* will display. Repeat this Step until a total of five (5) **Kd** values have been generated. Average the five **Kd** values. **Kd** is the diameter calibration constant.

5.2.11. Record the averaged **Kd** in the Z2 calibration book (located in B246). Press *Cal* on the Z2 data terminal. The *C7* screen status message will update to *Edit Kd if required.* Use the keypad to enter the averaged **Kd**. Press [Start] on the Z2 data terminal. This stores the **Kd** factor.

5.2.12. Lower the sample platform. Remove the calibration beaker. Remove the paddle stirrer. Grasp the holder and the stirrer (one with each hand). Pull down on the stirrer. DO NOT PULL SIDEWAYS. Rinse the calibration beaker and the stirrer in DI water. Allow them to dry and then return them to storage.

5.2.13. Fill a vial with DI water. Gently rinse the aperture tube by raising and lowering the vial over the tip of the aperture tube. Repeat with fresh DI water until the aperture has been thoroughly rinsed.

5.2.14. Fill a clean vial with Isoton. Place the vial on the sample platform. Raise the sample platform to immerse the tip of the aperture tube in the Isoton and **flush** the aperture.

5.2.15. Ensure that the operation settings in Step 5.3. are correct, then go to Step 5.4.-Background Counting.

5.3. Operation Setup

5.3.1. After the initial set-up, the following operation settings should already be selected, and no changes should be needed. The only unit that should need changing on a regular basis will be the **Kd** after each calibration

5.3.2. Press [Setup] on the Z2 data terminal until Screen *S1: Setup- Enter size data* is displayed. Ensure that the aperture tube, **Kd**, and units (fl) are correct. Use the Z2 keypad to enter 350 for **Tu** and 14 for **Tl**. Move cursor to *count mode*, select *between*. Aperture = 100, **Kd** = calibration factor entered, units = fl.

5.3.3. Press [Setup] on the Z2 data terminal. Screen *S2: Setup- Enter analysis data* is displayed. Ensure that the aperture **Kd** is correct. Set *Metered volume* to 0.5.

5.3.4. Press [Output] on the Z2 data terminal. Screen *A1: Analysis- output format* is displayed. For *Result type*, select *Count.* For *Dilution factor*, select *10*. For *Switch units* select *fl*.

5.3.5. Press [Output] on the Z2 data terminal until Screen *A10: Printout configuration* is displayed. For *Mode,* select *Automatic*. For *Form*, select *Long.* For *Graph*, select *Yes.* For *Table,* select *N/A.* Go to background counting for the button to press after the N/A for table selection.

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5.4. Background Counting

5.4.1. Ensure that the PC is on, LIMS is open, and the AGP program is selected. Select a run ID to use for counting.

5.4.1.1. Selecting the “Use date filter?” box will reduce the number of run IDs to choose from.

5.4.1.2. If the Model Z2 was just calibrated, the run ID selected is not important. The counts collected will not be uploaded to LIMS.

5.4.1.3. If samples are to be read, select the run ID the samples belong to.

5.4.2. In AGP Manager, press the **Collect Counts from Instrument** icon.

5.4.3. In the Run window, select the “Daily Background” radial.

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

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

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

5.4.4.2.1. Before daily use, the calibration of the dispenser can be checked with a 10 mL calibrated graduated cylinder. The dispenser can be adjusted as necessary so that 9 mL is dispensed with each pump.

5.4.5. Select *Auto Setup*. Enter *B* for the flask code, enter *B* for the vial code, and enter one *<1>* for the number of flasks to be counted. Click OK. Enter the correct count day and choose analyst name from the analyst drop-down box. Select *Start Data Collection* on the computer screen.

5.4.6. On the Z2 data terminal, press [Output] then [Start]. Screen *A2: analysis* will display on the terminal. The message *Counting* will be displayed on the terminal. When the analysis is completed, Screen *A4 Analysis result* will display on the terminal. The counts and MCVs should be displayed on the computer screen beside the appropriate flask and vial codes. Repeat this step until three results are recorded.

5.4.6.1. The average of the three counts on fresh Isoton should be less than 100 and ideally less than 50. If the mean count is greater than 100, ensure that the vial used for the background counts is clean and fill with fresh Isoton. Repeat Step 5.4.6.

5.4.6.2. If samples will be counted, record the counts and MCVs onto the Background printout.

5.4.7. Save data.

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5.4.7.1. If Z2 just calibrated, click the "Save to disk" icon and save the Background counts to X:\Labs\Lab5\Biology\Bench Common\ToxCommon\AGP\AGP Counts [last two digits of year]\Background. Save as the date in MM-DD-YY format and label it "after calibration." Close Run window.

5.4.7.2. If samples are to be read, save the Background counts as in the above step, but do not add "after calibration" to the file name. Also, click on the "Upload to LIMS" icon. Close Run window.

5.4.8. Lower the sample platform and remove the background vial.

5.4.9. Flush the aperture with fresh Isoton.

5.5. Counting Samples

5.5.1. Ensure that the PC is on, LIMS is open, and the AGP program is selected. Select the correct run ID to use for counting.

5.5.1.1. Selecting the "Use date filter?" box will reduce the number of run IDs to choose from.

5.5.2. Press the **Collect Counts from Instrument** icon.

5.5.3. In the Run window, select the "Sample Data" radial.

5.5.4. Select *Auto Setup*. Enter the appropriate flask code, vial code, and number of flasks to be counted into the appropriate spaces. Click OK. Enter the correct count day and choose analyst name from the analyst box. Select *Start Data Collection.*

5.5.4.1. Fill in the flask and vial information on the daily count sheet.

5.5.5. Place a sample vial containing 10 mL of diluted sample on the sample platform and raise the platform to immerse the tip of the aperture tube. On the Z2 data terminal, press [Output].

5.5.5.1. If [Output] is pressed twice, a graphical display will appear on the data screen. If the graph is displayed, press [Output] again to return to the count screen.

5.5.6. Press [Start] on the Z2 data terminal. Screen *A2: analysis* will display. The message *Counting* will be displayed on the terminal. When the analysis is completed, screen *A4 Analysis result* will display. The cell count and MCV from this screen will automatically appear in the Run window.

5.5.6.1. **For counts above 100 but less than 1000**, if one count differs by more than 20% from the mid-range count, a fourth count is done.

5.5.6.2. **For counts above 1000,** a fourth count is initiated if one count differs by more than 10% from the mid-range count.

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5.5.6.3. For counts where the message **high concentration** appears, the sample is diluted as necessary and recounted. When a dilution is necessary, select "stop" on the "collecting data" box, and enter the dilution factor into the correct box next to the vial and flask code being counted, as well as on the Count Record Sheet. Then select "start collecting data."

5.5.7. If a sample has a high count (>10,000), flush the Counter with Isoton before the next vial is counted.

5.5.7.1. If debris and/or air bubbles are in the aperture tube, flush the aperture with Isoton.

5.5.8. Between counts, if the aperture is to be exposed to air for more than a minute, immerse the tip of the aperture tube in fresh Isoton.

5.5.9. After counting a run, select *Stop* on the **Collecting Data** window. Select *Save to Disk* and save to X:\Labs\Lab5\Biology\Bench Common\ToxCommon\AGP\AGP Counts [Last two digits of the year]\[Run start date]. Name the file *<Run ID>*.

5.5.10. Upload the data to LIMS by selecting **Upload to LIMS**.

5.5.11. Repeat Steps 5.5.1. through 5.5.10. until all runs have been counted.

5.5.12. After the last count of the day has been completed, remove the last sample vial from the sample platform. Rinse the aperture tip with a vial of DI water, and then place a vial containing fresh Isoterge detergent on the platform. Immerse the aperture tip in the Isoterge.

5.6. Shutting down the Coulter Model Z2

5.6.1. Ensure that the aperture tip is immersed in a vial of fresh Isoterge.

5.6.2. Flush the aperture. Press [Functions]. Use the left/right arrow key (on the Z2 data terminal) to select *Flush Aperture*. Press [Start].

5.6.3. Prime the aperture. Press [Functions]. Use the left/right arrow key to select *Prime Aperture*. Press [Start] once and then a second time. *Prime aperture* pumps the Isoton II from the tubing and replaces it with Isoterge detergent. The message *Aperture Primed* will display when the priming is complete.

5.6.4. After the *Aperture Primed* message appears on the data terminal, turn the instrument off.

5.6.5. Empty the waste jar into the sink. Return the jar to the holder and replace and tighten the lid.

5.6.6. Fill the diluent jar with fresh Isoton.

5.6.7. Shut down the computer.

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6. DATA ARCHIVAL

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

6.2. 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.

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

6.2.2. 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.

6.2.3. 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.).

7. QUALITY CONTROL

7.1. If a problem occurs during sample processing, a nonconformance report (NCR) is submitted describing the problem and the corrective action.

7.2. Calibrate the Z2 monthly.

7.3. It is **required** that the analyst reviews the Coulter® Series Z™ 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).

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

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

8. REFERENCES

8.1. Coulter® Z Series User Manual

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

8.3. DEP Laboratory Quality Manual https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

8.4. DEP Laboratory Health and Safety Manual https://www.fldepnet.org/system/files/Administrative-Services/governing-documents/Health%20and%20Safety%20Manual%20December%202019.pdf

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APPENDIX OF CHANGES

01/04/2008 Added Scope and Application, Summary of Method and Appendix of Changes.

12/30/2008 Added Interferences and Reference Sections.

08/08/2013 Reformatted SOP.

02/09/2015 Formatted for 508 compliance.

07/10/2015 Updated links.

09/18/2017 Reformatted SOP for clarity; updated file directories and references.

08/23/2018 Changed font to Calibri

08/29/2019 Added links to Laboratory Quality Manual and Laboratory Health and Safety Manual

09/11/2020 Updated equipment section with universal language. Updated data archival section with universal language. Updated links.

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