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. The keyboard 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. Diluent jar (1 L), 2 each (one per instrument)

3.2. Waste jar (1 L), 2 each (one per instrument)

3.3. Dilu-vials, 30 mL

3.4. PC with access to LIMS

3.5. Black Box® Serial-Parallel Converter II

3.6. Coulter® Counter Model Z2 (Ser.#s AB114302 and/or AF47542) with data terminal

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

3.8. Coulter® paddle stirrer

3.9. Eppendorf® pipettor and tips (1 mL)

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

3.11. Graduated cylinder (10 mL calibrated)

4. REAGENTS AND STANDARDS

4.1. Isoton II® electrolyte solution

4.2. Coulter® detergent (Isoterge)

4.3. Latex calibration beads (10-µm)

5. METHOD

5.1. Preparation for calibration and/or measurement

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5.1.1. This procedure presumes 1) the system is filled with diluent, 2) the correct aperture tube is connected, 3) the diluent jar is full, 4) the waste jar is empty, and 5) the instrument is powered on.

5.1.2. Place 25 mL of Isoton II 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 Isoton on the sample platform and gently raise the sample platform to immerse the bottom 3.5 cm of the aperture tube. To move the platform, place one or more fingers under it and press and hold the lock release (front center edge of platform) with your thumb. 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. The platform should be raised to its upper limit without striking the aperture tube.

5.1.3. Ensure that the aperture can be clearly seen in the aperture monitor. When the aperture monitor is properly focused, the aperture can be 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). Use the small brush to remove any debris that blocks the aperture during flushing, purging, or counting. The brush is located beneath the sample platform. Use a gentle sweeping motion to remove debris.

5.1.4. Turn on the PC, log on to the network, and open LIMS. In LIMS select **Laboratory/Biology/AGP Manager**. Ensure that the Black Box® is plugged into the electrical outlet (on the surge protector behind the Coulter Counter). No component including the Black Box® should be unplugged. This is just a check.

5.1.5. Prime the aperture. Press [Functions] on the Z2 data terminal. 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. Use the left/right arrow keys (on the Z2 data terminal) 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.

5.1.7. Lower the sample platform and remove the vial. Replace the removed vial with a vial containing fresh Isoton II. Raise the platform until it stops.

5.1.8. Press [Output] on the Z2 data terminal.

5.2. Coulter Model Z2 Calibration

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. Insert the paddle stirrer into the paddle stirrer holder. Hold the stirrer near the opposite end from the

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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. Turn the stirrer to slow speed (~120 RPM). The stirrer control is located on the top right of the front of the Model Z sample stand. Turn the knob clockwise to increase speed, counter-clockwise to decrease speed.

5.2.4. Ensure that the PC is on, LIMS is open, and the AGP program is selected. Open a Run ID to count. The Run ID selected is not important. The counts collected will neither be saved to disk nor uploaded to LIMS.

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 between 0.3 and 0.7 go to STEP 4.2.10**. 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 II) to the calibration beaker.

5.2.9. Repeat Step 4.2.7 and 4.2.8 as needed until the concentration is within the stated limits, then go to Step 4.2.10.

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. 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

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terminal. This stores the **Kd** factor. The Z2 calibration book is located in the Toxicity Lab (B246).

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

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.

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

5.3. Operation Setup

5.3.1. 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. The appropriate 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. Screen *S2: Setup- Enter analysis data* is displayed. Ensure that the aperture **Kd** is correct. Set *Metered volume* to 0.5.

5.3.3. 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*.

5.3.4. 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.

5.4. Background Counting

5.4.1. Place a clean sample vial containing Isoton II on the sample platform and raise the platform to immerse the tip of the aperture tube. Ensure that the Isoton II is taken from the Isoton dispenser and not the Isoton II box. The dispenser should be set to nine milliliters. The dispenser should be calibrated. Refer to SOP MB-03.3.

5.4.2. Open the Run ID for the background count. Press the **Collect Counts from Instrument** icon. This assumes that one has already logged into LIMS and has opened the AGP utility. Using the filters may enable one to find the appropriate Run ID more quickly. The appropriate Run ID is also on the top right of the count sheet. The **Collect Counts from Instrument** icon is a shortcut found on the desktop of the AGP computers.

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5.4.3. 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 into the appropriate spaces. Enter the correct count day and choose analyst name from the analyst drop-down box. Select *Start Data Collection* on the computer screen. This information is on the count sheet. The vial code and flask code are at the top center of the count sheet. The number to be counted is on the right of the page. Usually the number will be 21, but occasionally, the number will be less than 21. You may have to click and hold on the top of the **Collecting Data** window and move it over in order to see the counts to record on the count sheet.

5.4.4. Press [Output] then [Start] on the Z2 data terminal. 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 count and MCV from this screen will automatically download to the computer. Repeat this Step until three analyses are recorded. Save data per comment. Upload to LIMS, then exit program. The counts and MCV should be displayed on the computer screen beside the appropriate flask and vial codes after the Coulter has finished counting. The average of the three counts on fresh Isoton II should be less than 100 and ideally less than 50. If the mean count is greater than 100, fill a new vial with fresh Isoton II and repeat Steps 4.1 and 4.2. Ensure that the vial used for the background counts is clean. If you are keeping a paper copy, record the count and MCV from this screen. Save the data: \\FLDEP1\LDS_Biology\Bench Common\ToxCommon\AGP\AGP Counts (last two digits of year)/Background. Name the file the date of the count (ex. 03-14-09).5.4.5. Lower the sample platform and remove the background vial.

5.5. Counting a Sample

5.5.1. 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. 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.

5.5.2. Open the Run ID for the count sheet. Press the **Collect Counts from Instrument** icon. This assumes that one has already logged into LIMS and has opened the AGP utility. Using the filters may enable one to find the appropriate Run ID more quickly. The appropriate Run ID is also on the top right of the count sheet. The **Collect Counts from Instrument** icon is a shortcut found on the desktop of the AGP computers.

5.5.3. Select *Auto Setup*. Enter the appropriate flask code, vial code, and number of flasks to be counted into the appropriate spaces. Enter the correct count day and chose analyst name from the analyst box. Select *Start Data Collection.* This information is on the count sheet. The vial code and flask code are at the top center of the count sheet. The number to be counted is on the right of the page. Usually the number will be 21, but occasionally, the number will be

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less than 21. You may have to click and hold on the top of the **Collecting Data** box and move it over in order to see the count and MCV data. To scroll down, stop data collection, press page down, and then start data collection.

5.5.4. 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 download to the computer. Raw counts are recorded on count sheets. **For counts above 100 but less than 1000**, if one (high or low count) count differs by more than 20% from the in between count, a fourth count is done. **For counts above 1000** a fourth count is initiated if one count differs by more than 10% from the mid-range count. 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 code and flask code being counted, as well as on the Count Record Sheet. Then select "start collecting data." This can be done while the Coulter is counting.

5.5.5. If a sample has a high count (>10,000), flush the Counter with Isoton (before placing next vial on the platform). If debris and/or air bubbles are in the aperture tube, perform the flush aperture procedure per Step 4.5.6 below. After being flushed with Isoton II® solution, the aperture tube can be rinsed with DI water from a squirt bottle with the waste being caught in a waste cup or beaker.

5.5.6. 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 II.

5.5.7. If air bubbles or particles are behind the aperture orifice, flush the aperture. Press [Functions] and use the left/right arrow keys (on the Z2 data terminal) to select *Flush Aperture*. Press [Start]. After the *Flush Complete* message appears, press [Output] to return to counting mode. The message *Flush Complete* will display. This process flushes particles or air bubbles away from the rear of the aperture.

5.5.8. After counting a flask, select *Stop* on the **Collecting Data** window. Select *Save to Disk* and save the file under \\FLDEP1\LDS_Biology\Bench Common\ToxCommon\AGP\ **Biology/Bench Common/ToxCommon/AGP/AGP Counts < the year>/<set up date>/<count day>.** Label the file <*Run ID*>. Save the file.5.5.9. Upload the data to LIMS by selecting **Upload to LIMS**.

5.5.9. Repeat Steps 4.5.1 through 4.5.9 until all racks are counted.

5.5.10. After the last count of the day has been completed, remove the last count 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

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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.

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 and initial the correction. Do not overwrite entries.

6.1.1. The corrected entry is written as near its appropriate space as possible or else 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. Initial and date the explanation.

6.2. 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.).

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

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

7. QUALITY CONTROL

7.1. Contaminated materials including electrolyte solution, rinse water, calibration bead solution, dirty vials and other glassware.

7.2. The toxicology section has two Coulter® Z2 particle counters. Both are calibrated per above procedures. It is **required** that the user review the Coulter® Series Z™ Users Manual before attempting to use the counter. The use of this SOP is **acknowledgement** that the manual has been reviewed. The manual is located in the Toxicity Lab (B246).

7.3. Calibrate the Z2 monthly.

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

8. REFERENCES

8.1. Coulter® Z Series User Manual

8.2. Calibration of Disposable Dispensing 10mL, 5mL, and 1mL (TC) Pipettes, DEP SOP MB-03.3

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.

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