Standard Operating Procedure for:
YSI Model 50B/AutoEZ DO Meter Calibration and Probe Maintenance

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LABORATORY
BIOLOGY PROGRAM
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1. EQUIPMENT AND SUPPLIES

    1.1. Calibration

        1.1.1. YSI 50B DO Meter with probe (S/N 96D49137)

        1.1.2. BOD AutoEZ Reader w/YSI probes (S/N A0089)

        1.1.3. BOD bottles and tray

        1.1.4. Computer with BOD AutoEZ program – see SOP BB-33 for program setup

    1.2. Maintenance

        1.2.1. New membrane cap

        1.2.2. Sanding disc

2. REAGENTS AND STANDARDS

    2.1. Deionized water

    2.2. Electrolyte solution

    2.3. 14% Ammonium Hydroxide

        2.3.1. Add 14 mL of concentrated ammonium hydroxide to 86 mL of DI water.

3. YSI MODEL 50B DO METER CALIBRATION

    3.1. Turn on the YSI 50B meter by turning the switch to the **°C** position and allow the temperature to stabilize for at least 15 minutes. Older probes take longer to stabilize at each setting. Make sure the probe and membrane is dry and place in a BOD bottle containing at least 1 inch of water.

    3.2. Turn the switch to the **mg/L** position. Allow the system to stabilize for at least 30 minutes. Older probes require a longer warm-up period. If the probe is still fluctuating, allow more time to stabilize.

    3.3. Turn the switch to **mg/L CAL** and press the [CAL] button. Turn the switch back to the **mg/L** position. The display will show "CAL" and then will display the reading. If the reading drifts more than ±0.02 mg/L, allow more stabilization time and repeat the calibration.

    3.4. Compare the reading to a DO Saturation table and record measurements in the BOD calibration log BOD-Calibrations YYYY.xls" (where YYYY is the current calendar year) located in *X:\Labs\Lab5\Biology\Bench Common\BOD*. If the reading differs by more than 0.30 mg/L from the measurement given in the DO saturation table, check the probe and recalibrate.

4. BOD AUTOEZ DO METER CALIBRATION

    4.1. Prepare 4 BOD bottles with air saturated water. For best results, lightly aerate a flask of DI water for approximately 30 minutes. Allow the water to sit for several minutes

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before pouring into the BOD bottles. Use the YSI 50B Meter to measure the DO of each bottle and record in the BOD Calibration Log. The readings should be within 1 mg/L of the DO Saturation table value. If not, re-aerate the water and repeat the process. The BOD Calibration Log will calculate the average of the four readings. The average result should be used as the Calibration Value for calibrating the AutoEZ

4.2. Place the bottles in row 1 of a tray (see Figure 1) and load the tray into the AutoEZ reader. The tray label should be on the left side, when facing the front of the analyzer (power button side). Make sure the trays and bottles are pushed all the way to the back of the analyzer.

4.3. In the BOD AutoEZ program, select **Read/Manual Controller** from the menu. Move the probes to the appropriate row, over the BOD bottles.

4.4. Click **Park Current Row**. Then click **Raise/Lower**. Wait for the probes to raise, and then click **Calibrate**. In the Calibration window, enter the Calibration Value from step 4.1. After the first reading, click **Read D.O.s**. A message will appear that says "Please move to desired row." Click **OK**. The probes will raise. Click **Calibrate** again. In the Calibration window, enter the Calibration Value from step 4.1. After the DO readings appear, click **Read D.O.s** and record the DO readings and standard deviation in the BOD Calibration Log. Each reading should not differ by more than 0.3 mg/L from the Calibration Value. The standard deviation should not be greater than 0.1. If the readings fail either criterion, check the probes and recalibrate. If the recalibration also fails, the probes should be checked. They may need a new membrane cap or cleaning. Do not use the probe for analysis until it passes a calibration check. If an asterisk appears next to any of the DO readings, select read DO again. If the asterisk remains after rereading, then check the probes. See section 5. Probe Maintenance.

5. PROBE MAINTENANCE

5.1. The DO probes should be checked quarterly, or if meter fails to calibrate, or readings are erratic or unstable.

5.2. Manual meter: turn off the meter. AutoEZ: Using the **manual mode,** select "Probe maintenance". The AutoEZ will move the probes all the way out and down. Remove each probe from the holder.

5.3. Unscrew the membrane cap. Be careful not to touch any of the exposed surfaces on the electrode. Rinse the electrode head with a gentle stream of DI water. Fill the membrane cap with electrolyte solution. Make sure there are no bubbles in the solution and screw the cap back onto the probe. Do not tighten the cap too much, because it can overstretch or tear the membrane. Rinse any excess electrolyte solution. Examine the outside of the membrane, if any bubbles are visible, remove the membrane cap and repeat this step (See Figure 2).

5.4. If the membrane is stretched, wrinkled, or damaged, replace it with a new membrane. The lifetime of the membrane is approximately 2-4 weeks depending on use.

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5.5. Before installing the cap membrane, check if the gold cathode is tarnished. If it is, clean it by gently rubbing it in a twisting motion on the 400-grit wet/dry sanding disc until all deposits are removed (See Figure 3).

5.6. If the silver anode is dirty, clean it by soaking for 2-3 minutes in a 14% ammonium hydroxide solution. Alternatively, soak the probe overnight in a 3% ammonium hydroxide solution. The anode can also be cleaned with the sanding disc after the ammonium soak but only if necessary, to avoid reducing the life of the anode and probe (See Figure 3). After cleaning, rinse the probe with DI water.

5.7. Turn on the manual meter to the temperature setting and follow steps 3.1 and 3.2. Leave it on overnight. In the AutoEZ program in **manual mode**, select "Row 1". Wait for the probes to move back to the first row and select "Park probes". Leave the AutoEZ on overnight to stabilize.

Check the calibration of the probes the next day. See sections 3 and 4.

6. DATA ARCHIVAL

6.1. Record any maintenance or problems with the probe or meter in the *BOD Instrument Maintenance Log*.

7. REFERENCES

7.1. YSI Model 50B Instruction Manual

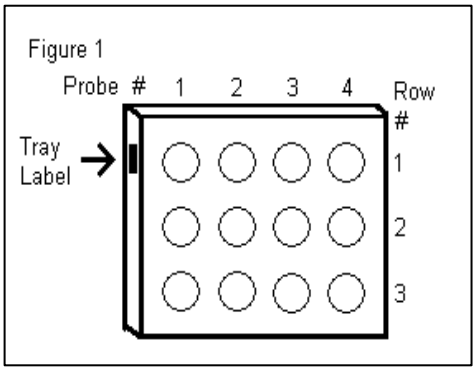
7.2. AutoEZ User's Manual

7.3. YSI 5905/5010 BOD Probe Instruction Manual

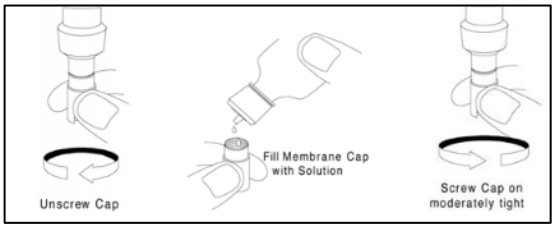
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8. TABLES, DIAGRAMS

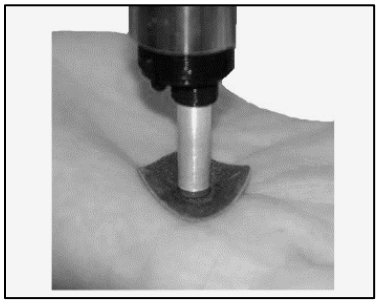
8.1. Figure 1. Tray Diagram



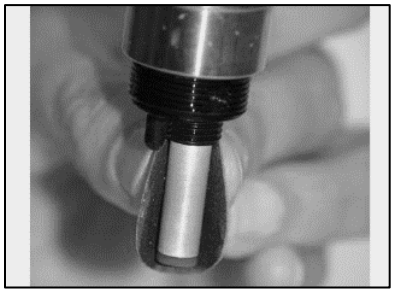
8.2. Figure 2. Installing a cap membrane



8.3 Figure 3. Texturing the Cathode



8.4 Figure 4. Cleaning the Anode



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APPENDIX OF CHANGES

07/08/2009	Updated SOP style. Updated SOP to the current procedure of using YSI readings to calibrate Auto EZ probes.
10/28/2010	Added probe maintenance section.
01/14/2015	Formatted for 508 compliance
02/24/2015	updated to reflect new file location.
05/25/2016	Edited YSI Model 50B Calibration; BOD AutoEZ Calibration; and Probe Maintenance sections
12/29/2016	Made clarifications in AutoEZ calibration section and added figure of tray positions.
12/19/2017	Amended comment about aeration step in section 4.0 and fixed hyper link in section 3.4
04/26/2019	Updated link to BOD folder. Edited probe maintenance section to include specific instructions for the AutoEZ.
04/28/2020	Updated the name and location of data logs. Reorganized procedure instruction in Section 5. Added graphics to section 8.

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