Calibration and Use of Fisherbrand™ accumet™ AB315 pH/mV Meter

1. SCOPE AND APPLICATION

1.1. This procedure is based on the *Thermo Fisher Scientific accumet™ AB315 pH/mV Meter User Manual.* It is used to calibrate the meter prior to measuring pH, as well as measuring pH in aqueous solutions.

2. SUMMARY OF METHOD

2.1. The pH benchtop meter is set-up and calibrated. After the initial set-up, daily calibration checks (verifications) are performed and recorded in the LIMS logbook. A three-point verification is used in the morning, and a one-point verification is performed in the afternoon to ensure the probe readings have not drifted. To perform the verifications, the meter is turned on and the desired "MODE" is selected. The probe is placed in the aqueous standard solution and swirled briefly before allowing it to stabilize. The probe is rinsed with DI water between each reading.

2.2. For both the morning and afternoon verifications, if any standard has drifted by ±0.1 units, the meter is recalibrated.

2.3. Volume 1, Module 7 of TNI Standards (Section 1.7.1.6(e)) and WET methods (Section 4.11) state that equipment used for routine support measurements of chemical and physical parameters (i.e. pH) shall be calibrated per manufacturer's instructions. The Operations Manual for the accumet AB315 pH/mV meter advises that for best results, periodic calibration is recommended. The meter is calibrated as needed.

2.4. Interferences: Temperature, probe not rinsed thoroughly, not waiting for the meter to stabilize, contaminated standards.

2.5. Definitions:

2.5.1. ATC: Automatic temperature compensation

2.5.2. NIST: National Institute of Standards and Technology

3. EQUIPMENT AND SUPPLIES

3.1. Fisherbrand™ accumet™ AB315 pH/mV meter

3.2. pH electrode with internal ATC probe

3.3. 30 mL disposable plastic cups

3.4. Thermometer calibrated per SOP TA-06.13 (can be electronic or liquid filled, mercury-filled thermometers are not used in this lab).

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3.5. Rinse water cup

3.6. Kimwipes®

3.7. *Sit Down Parameter Station Calibration Check* Logbook

3.8. *Bioassay Bench Sheets* Logbooks (where applicable)

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

4. REAGENTS AND STANDARDS

4.1. Deionized (DI) Water with 500 mL wash bottle

4.2. Standard buffer solutions, pH 4 or 5, 7, 9 or 10, and 13 (Fisher Scientific certified pH calibration solutions)

4.3. Second-source buffer, pH 7 (Orion® pH 7.0 buffer solution, or another brand different from that of the morning verification.)

5. INITIAL SETUP AND CALIBRATION

5.1. Initial Setup

5.1.1. Unboxing and Assembly

5.1.1.1. Unbox the AB315 pH benchtop meter with electrode holder, electrode, power supply, and appropriate wall plate. Connect the wall plate to the power supply. Connect the assembled power adapter to a power outlet and meter input.

5.1.1.1.1. The input end of the power adapter has two prongs that allow the power adapter to be locked into the meter. The two prongs must be properly aligned when connecting to ensure it is fully connected.

5.1.1.2. Connect the electrode into the “BNC” port on the back of the meter. The temperature probe has a separate input that must be inserted into the “ATC” port.

5.1.1.3. To connect the optional electrode holder, turn the meter over and identify the side of the meter that the holder will be installed on. Remove the screw between the circles on that side of the meter. Align the base plate of the holder with the circles on the meter. Replace the screw and insert the stand with the electrode holder into the metal post on the base plate.

5.1.2. Powering On and Display Settings

5.1.2.1. Turn on the meter. The power button is at the bottom on the right-hand side.

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5.1.2.2. Press the “Menu” key to access the Setup Menu.

5.1.2.3. Pressing the ► and ◄ keys scroll through the various setup items in a cyclical fashion.

5.1.2.4. Press the ▲ and ▼ buttons to change settings. Once a setting is changed, press the “Enter” key to save the change.

5.1.2.5. Press the “Read” key at any time to exit the Setup Menu and enter the main measure mode.

5.1.2.6. Please see the manual for detailed information on all 18 available features and settings accessible in the Setup Menu. The following are the necessary items that must be set up before using the meter for Tox Lab use.

5.1.3. Temperature Calibration

5.1.3.1. Place the pH electrode in a 150mL beaker filled with DI water.

5.1.3.2. Press the ► or ◄ keys to scroll through the Menu list until the “t.CAL” item is shown.

5.1.3.3. The temperature value will blink, indicating the reading from the ATC probe is being measured. Once the reading is stable, the value will stop blinking and the checkmark icon will be shown.

5.1.3.4. Place a NIST certified thermometer into the same beaker as the electrode and use the ▲ and ▼ keys to adjust the temperature reading on the meter to match the reading on the NIST thermometer.

5.1.3.5. Press the “Enter” key to save the change.

5.1.4. Measure Mode

5.1.4.1. Press the ► or ◄ keys to scroll through the Menu list until the “mOdE” item is shown.

5.1.4.2. Use the ▲ and ▼ keys to set the measure mode to “PH”.

5.1.4.3. Press the “Enter” key to save the change.

5.1.5. Calibration Buffer Set

5.1.5.1. Press the ► or ◄ keys to scroll through the Menu list until the “bUFFf” item is shown.

5.1.5.2. Use the ▲ and ▼ keys to set the pH buffer set to “USA”.

5.1.5.3. Press the “Enter” key to save the change.

5.1.6. Read Type

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5.1.6.1. Press the ► or ◄ keys to scroll through the Menu list until the "rEAd" item is shown.

5.1.6.2. Use the ▲ and ▼ keys to set the read type to "AUtO".

5.1.6.3. Press the "Enter" key to save the change.

5.1.7. Temperature Units

5.1.7.1. Press the ► or ◄ keys to scroll through the Menu list until the "tEmP" item is shown.

5.1.7.2. Use the ▲ and ▼ keys to set the units to "°C".

5.1.7.3. Press the "Enter" key to save the change.

5.2. Meter Calibration (pH)

5.2.1. Press the "Cal" key. The screen will display "lStPt" to signify "1st point".

5.2.2. Pour the pH 4 buffer solution into a 30-mL plastic cup.

5.2.3. Rinse the electrode with DI water and place into the pH 4 buffer.

5.2.4. Wait for the pH value to stabilize.

5.2.4.1. While the reading is stabilizing, the stopwatch icon is shown and the reading flashes. When the reading is stable, the checkmark icon is shown, and the reading is solid.

5.2.5. Once the reading is stable, if the value matches the true value of the buffer (value on the bottle), press the "Enter" key. If the reading does not match the true value, use the ▲ and ▼ keys to adjust the reading to match the value on the bottle and press "Enter".

5.2.6. The screen will now display "2ndPt".

5.2.7. Pour the pH 7 buffer solution into a 30-mL plastic cup.

5.2.8. Rinse the electrode with DI water and place into the pH 7 buffer.

5.2.9. Once the reading is stable, if the value matches the true value of the buffer (value on the bottle), press the "Enter" key. If the reading does not match the true value, use the ▲ and ▼ keys to adjust the reading to match the value on the bottle and press "Enter".

5.2.10. The screen will now display "3rdPt".

5.2.11. Pour the pH 10 buffer solution into a 30-mL plastic cup.

5.2.12. Rinse the electrode with DI water and place it into the pH 10 buffer.

5.2.13. Once the reading is stable, if the value matches the true value of the buffer (value on the bottle), press the "Enter" key. If the reading does not match

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the true value, use the ▲ and ▼ keys to adjust the reading to match the value on the bottle and press "Enter".

5.2.14. The screen will display "SAUEd" and then "4thPt". (The meter can save up to 5 calibration points.) Press the "Cal" key to save and end the calibration. No 4th and 5th calibration points will be saved.

5.2.15. The screen will display "SLOPE" with the slope (%) in the bottom right corner. The meter will then display "CAL d0nE" and the meter proceeds to the main measure mode.

5.2.16. After calibration, immerse the pH electrode in the secondary pH 7 buffer solution to verify the calibration.

5.2.16.1. If the pH of the secondary pH 7 buffer is within ±0.1 units and slope (%) is between 92.0% and 102.0%, record values in the *Sit Down Parameter Station Calibration Check* logbook and the LIMS/Laboratory/Technical/Laboratory Logbook/TOX_pH_METER.

5.2.16.1.1. The slope must be entered as a comment when recording the secondary pH value.

5.2.16.2. If the pH is ±0.1 units from the nominal value of the buffer solution, perform the following troubleshooting techniques in the order listed:

5.2.16.2.1. Discard buffer aliquots used during calibration and pour fresh aliquots; discard, rinse, and refill DI rinse vessel; then, repeat the calibration procedure, **or**

5.2.16.2.2. Open new buffers to use for calibration and recalibrate, **or**

5.2.16.2.3. Perform the care and maintenance activities given in Section 7 below and recalibrate, **or**

5.2.16.2.4. If these actions do not result in a passing calibration verification, contact the toxicology manager. The probe may need to be replaced.

5.2.16.3. If the slope is outside a range of 92.0% to 102.0% (but the secondary 7 pH buffer is within the ±0.1 pH units):

5.2.16.3.1. Discard buffer aliquots used during calibration and pour fresh aliquots; discard, rinse, and refill DI rinse vessel; then, repeat the calibration procedure, **or**

5.2.16.3.2. Perform the care and maintenance activities given in Section 7 below and recalibrate.

5.2.16.3.3. If the resulting slope after performing these activities is still outside a range of 92.0% to 102.0% (but the pH buffer 7 is still within ±0.1 pH units), the meter and electrode can

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still be used, but calibrations (with passing verifications) must be performed daily.

5.2.16.3.4. If the slope after calibration continues to drift, a new electrode should be purchased and installed.

5.2.16.4. If the slope is outside a range of 82.0% to 114.0%, the meter must be taken out of service until the electrode has been replaced and a successful calibration including a "good" slope value can be completed.

5.2.17. Remove the probe from the solution and rinse with DI water. If necessary, blot (do not rub) the electrode with a Kimwipe®.

5.2.18. Morning pH verification is coupled with a brief temperature calibration verification. Immerse the temperature probe in the DI rinse cup along with a certified thermometer. Record the pH meter and thermometer readings in the *Sit Down Parameter Station Calibration Check* logbook and LIMS/TOX_THERMOMETER. ±0.2°C of variation is allowed between the two readings. If the difference between readings is greater than ±0.2°C, refer to Section 5.1.3 of this SOP.

5.2.19. End of Day Verification

5.2.19.1. After the final pH measurement has been made each day the pH electrode is immersed in a second-source pH buffer 7 solution. The reading is recorded in the *Sit Down Parameter Station Calibration Check* logbook and LIMS/TOX_pH_METER.

5.2.19.1.1. The End-of-day reading should be within ±0.1 units of the nominal value of the buffer solution.

6. SAMPLE MEASUREMENT

6.1. Measuring pH

6.1.1. After calibration, be sure the meter is in pH measurement mode. (Pressing the "Read" button will toggle between the Setup Menu and measurement mode.)

6.1.2. Immerse the electrode into the sample and stir.

6.1.2.1. Be sure that the glass bulb of the pH electrode is completely immersed in the sample.

6.1.3. Press the "Read" key and wait for the reading to stabilize. (The probe can be moved up and down within the sample cup to increase the speed of stabilization, if needed.) When the reading stabilizes, the checkmark icon will appear, and the measurement is locked on the display until the "Read" key is pressed again.

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6.1.4. Record the measurements appropriately.

7. ELECTRODE CARE AND MAINTENANCE

7.1. The manual, *pH/Temperature Combination Gel-Filled Epoxy Body Electrodes for Beckman, Corning, Denver, Mettler, Pinnacle, Thermo Scientific and WTW meters,* is specific to the electrode and provided the following information.

7.2. For short term storage, immerse the electrode in pH 7 or 4 buffer solution.

7.3. For longer term shortage (e.g. overnight), place the soaker bottle, filled with pH solution or pH 4 over the probe tip.

7.4. Cleaning the Glass Membrane

7.4.1. If beads of water form on the bulb when rinsed with DI water, the bulb can be washed with an acid (like 0.1N HCl) or acetone.

7.5. Unblocking the Junction

7.5.1. Soak the electrode in warm water (approximately 60°C) for 5-10 minutes.

7.5.2. Then, place the electrode in a warm, saturated KCl solution (approximately 60°C), and allow it to cool to room temperature.

7.6. Reconditioning the Sensing Membrane

7.6.1. If the meter becomes sluggish, reconditioning may be necessary.

7.6.2. Immerse the electrode tip in 0.1M HCl for 15 seconds; rinse with DI water and immerse into 0.1M KOH for 15 seconds. Repeat this step several times.

7.6.3. If performance is still inadequate, replace the electrode.

7.6.4. Note there are additional poor performance remedies in the electrode manual. However, the required solution preparations use chemicals not readily available in the lab that are cost ineffective to supply.

8. DATA ARCHIVAL

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

8.2. 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.

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

8.2.2. 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.

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8.2.3. 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.).

8.3. FDEP Laboratory Information Management System (LIMS)

9. QUALITY CONTROL

9.1. A three-point calibration is performed on the meter. The low and high buffer solutions bracket the expected pH values of the solution being measured.

9.1.1. If a measured sample pH is outside of the bracketed range, the meter should be recalibrated to include the measured pH.

9.2. The second-source pH buffer should read <±0.1 pH units of the exact pH value at the measured temperature. If the reading is outside of this range, repeat the calibration procedure. If the analyst believes the problem is with the buffers, new buffers can be poured and used. If the probe is the cause, the probe can be changed and the meter recalibrated.

9.3. A pH 7 buffer is used to perform an end-of-day calibration check to show that the meter did not fall out of calibration and that there was no drift. It is done after the last measurement of the day and before the meter is shutdown. The measurement should be <±0.1 pH units of the exact pH value at the measured temperature.

9.4. For each lot number of pH buffer solution, a certificate of analysis must be obtained. They are saved at \\floridadep\data\DEAR\Labs\Lab5\Biology\BenchCommon\ToxCommon\Certificates of Analysis. The certificate of analysis label can be removed from the side of the container or obtained from the www.Fishersci.com website.

9.5. The pH electrode should be stabilized (at thermal equilibrium) before a measurement is recorded. Swirling helps hasten probe acclimation. It can require up to 2 minutes for the probe to stabilize; however, buffers are kept at room temperature (~25˚C), and most samples are warmed to ~25˚C, so thermal equilibrium usually occurs within seconds.

9.6. If the electrode needs to be cleaned with a Kimwipe® between measurements, it must be blotted, never rubbed. An electrical charge could form and cause interference with measurements.

10. SAFETY/HAZARDOUS WASTE MANAGEMENT

10.1. Proper laboratory protection—including lab hood or ventilation system, lab coat, gloves, and safety glasses—is required when working with chemicals and unprocessed samples.

10.2. Refer to the Laboratory Quality Manual for sample disposal.

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10.3. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management.

10.4. Read MSDS information for reagent handling and disposal.

11. REFERENCES

11.1. Fisherbrand accument AB315 pH/mV Meter User Manual

11.2. Fisher Scientific Website

11.3. FDEP Laboratory Quality Manual

11.4. FDEP Laboratory Health and Safety Manual

11.5. DEP SOP TA-06.13, *Thermometer Accuracy Check and Use*

11.6. MSDS Information Sheets

APPENDIX OF SIGNIFICANT CHANGES

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