



# **US Environmental Protection Agency Office of Pesticide Programs**

**Office of Pesticide Programs  
Microbiology Laboratory  
Environmental Science Center, Ft. Meade, MD**

**Standard Operating Procedure for  
Calibration and Maintenance of pH Meters**

**SOP Number: EQ-01-07**

**Date Revised: 11-25-14**

SOP Number	EQ-01-07
Title	Calibration and Maintenance of pH Meters
Scope	This protocol describes the method for the operation, two point calibration, and maintenance of the Corning pH meter 430 and the Corning pH meter 530.
Application	Corning pH meters 430 and 530 are used to determine the pH of samples, such as media and reagents used in the laboratory. The pH of media and reagents is a critical attribute of such solutions for the proper performance of media and reagents.

	Approval	Date
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Date SOP issued:	_____
Controlled copy number:	_____
Date SOP withdrawn:	_____

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<b>1. Definitions</b>	Abbreviations/definitions are provided in the text.
<b>2. Health and Safety</b>	Follow procedures specified in SOP MB-01, Laboratory Biosafety. The Study Director and/or lead analyst should consult the Material Safety Data Sheet for specific hazards associated with media and reagents.
<b>3. Personnel Qualifications and Training</b>	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
<b>4. Instrument Calibration</b>	As noted below in section 12.
<b>5. Sample Handling and Storage</b>	<ol style="list-style-type: none"> <li>1. Measure the pH of media and reagents at room temperature (20°C to 25°C) unless otherwise specified on the Media/Reagent preparation sheet. For agar based media, the pH is to be taken on a solidified sample (see section 12.4).</li> <li>2. The buffers and reagents (1N HCl and 1N NaOH) are stored adjacent to the pH meter at room temperature in a secondary containment unit.</li> <li>3. Store electrodes for up to one week in 25 mL of pH 7.0 or pH 4.0 buffer. Never store the electrode in distilled water.</li> <li>4. For longer storage periods, remove the bulb protector, fill the wetting cap with the 3M KCl fill solution and push the wetting cap onto the tip of the electrode.</li> </ol>
<b>6. Quality Control</b>	For quality control purposes, the required information is documented on the appropriate form (see section 14).
<b>7. Interferences</b>	<ol style="list-style-type: none"> <li>1. Rinse the pH meter electrode thoroughly with de-ionized water and blot dry with Kimwipes before proceeding to the next solution.</li> </ol>
<b>8. Non-conforming Data</b>	<ol style="list-style-type: none"> <li>1. Management of non-conforming data is consistent with SOP ADM-07, Non-Conformance Reports.</li> <li>2. To pass calibration the slope must be slope 100% ± 5%. If slope is out of range, the first corrective action is to change the standard pH buffers and recalibrate. The second step is to change the 3M KCl fill solution in the electrode or bring up to volume, then re-calibrate. The final corrective action is to clean the electrode with the pH Electrode Cleaning Solution (follow manufacturer's instructions).</li> <li>3. Record all corrective action procedures conducted under the notes section of the pH Meter Calibration Record Form (see section 14).</li> <li>4. If the pH meter is not functioning as required, consult the technical manual to determine the problem and perform corrective action. Notify</li> </ol>

	media prep staff, and if the problem cannot be determined or corrected, call for service.
<b>9. Data Management</b>	Data will be archived consistent with SOP ADM-03, Records and Archives.
<b>10. Cautions</b>	<ol style="list-style-type: none"> <li>1. Do not stir the sample while the instrument is reading, as this may provide inaccurate readings.</li> <li>2. Do not allow the 3M KCL fill solution to run dry. Add 3M KCl fill solution whenever the level falls more than 25 mm below the fill hole. Replace the 3M KCl fill solution at least once a month.</li> <li>3. Always remove the wetting cap and the fill hole plug during calibration and measurements. Replace the fill hole plug in between use.</li> <li>4. Do not leave the electrode in organic solvents as the tip and body may be damaged.</li> <li>5. Discard used buffer solutions daily. Do not reuse.</li> </ol>
<b>11. Special Apparatus and Materials</b>	<ol style="list-style-type: none"> <li>1. Corning pH Meter #1: Model 430 (Serial No. 005166)</li> <li>2. Corning pH Meter #3: Model 530 (Serial No. 301887)</li> <li>3. Corning pH Meter #4: Model 530 (serial No. 304953)</li> <li>4. Electrodes:</li> <li>5. Three '3 in1' Combo w/RJ (Epoxy body) (Corning, now Nova Analytics, Catalog No. 76436). Electrode performance specifications (Manufacturer's claim): the pH range 0.0 -14.0 and the temperature range 0 - 100°C. The electrodes are identified as #5, 10, and 12. These electrodes are used for pH measurement of liquids.</li> <li>6. Two Flat-Surface (Epoxy Body) with Replaceable Junction Electrode (Corning Catalog No. 476286). Electrode performance specifications (Manufacturer's claim): the pH range is 0.0-14.0 and the temperature range is 0-100°C. The electrodes are identified as # 3 and # 4. These electrodes are used for pH measurement of solid media.</li> <li>7. One Semi-Micro Glass Body with Refillable Junction Combo Electrode (Corning Catalog No. 476156). Electrode performance specifications (Manufacturer's claim): the pH range is 0.0-14.0 and the temperature range is 0-100°C. Electrode is identified as #9. The electrode is used for measuring pH of small aliquots of media (<math>\leq 1</math> mL).</li> <li>8. Calibration Buffers: pH 4.0, pH 7.0 and pH 10.0.</li> <li>9. Fill solution: 3M KCl solution.</li> </ol>

	<ol style="list-style-type: none"> <li>10. Storage Solutions: 3M KCl solution for capping OR pH 4.0 or pH 7.0 buffer for buffer immersion.</li> <li>11. pH Electrode Cleaning Solution.</li> <li>12. Ceramic replacement junction.</li> <li>13. 1N Sodium Hydroxide Solution (NaOH).</li> <li>14. 1N Hydrochloric acid (HCl).</li> </ol>
<p><b>12. Procedure and Analysis</b></p>	<ol style="list-style-type: none"> <li>1. Calibrate the pH meter at least once on the day of use as outlined in section 12.1 and 12.2. A two point calibration is required.</li> <li>2. The standard buffers used are pH 4.0, pH 7.0, and pH 10.0. Dispense a small amount of the required buffers into a smaller container for calibration.</li> <li>3. Use two standard pH buffers for calibration.</li> <li>4. Use pH buffers for calibration one time only. After calibration buffers are discarded as per manufacturer's recommendations.</li> <li>5. Use two standard pH buffers to determine the pH of the medium or reagent. If the final pH of the sample falls between pH 7.0 and 10.0, use the pH 7.0 and 10.0 buffers for calibration. If the final pH of the sample falls between pH 4.0 and 7.0, use the pH 4.0 and 7.0 buffers for calibration.</li> <li>6. The expiration dates of the buffers are recorded on the upper part of the pH Meter Calibration Record Form (see section 14). Record the date the buffers are changed under Notes on the form.</li> <li>7. Check the level of the 3M KCl fill solution every day when in use to ensure that it is within 25 mm of the filling hole. Bring to level if needed. Record under Notes any adjustments made with the fill solution on the pH Meter Calibration Check Record Form.</li> </ol>
<p><b>12.1 Two-point calibration pH Meter Model 430</b></p>	<ol style="list-style-type: none"> <li>a. Rinse the probe tip with de-ionized water and blot dry with Kimwipes, then place the tip of the electrode into the pH 7.0 buffer and press "Cal."</li> <li>b. The pH meter will automatically read the endpoint when the reading is stable. The appropriate buffer symbol will appear on the display. Record the pH value on the pH Meter Calibration Check Record Form (see section 14).</li> <li>c. Rinse the tip of the electrode with de-ionized water and blot dry with Kimwipes.</li> <li>d. Place the tip of the electrode in the second calibration buffer (either</li> </ol>

	<p>pH 4.0 buffer or pH 10.0 buffer) and press “Cal”.</p> <ul style="list-style-type: none"> <li>e. The pH meter will automatically read the endpoint when the reading is stable and the appropriate buffer symbol will appear on the display.</li> <li>f. The pH meter will briefly display the electrode slope value. Record the pH and slope values on the pH Meter Calibration Check Record Form. If the slope is less than 95% or greater than 105%, refer to the troubleshooting section of the Corning pH meter 430 instructions manual (see section 15).</li> <li>g. Rinse the electrode with de-ionized water and blot dry with Kimwipes. Confirm the calibration by reading the pH of the pH 7.0 buffer a second time. Record the pH value on the pH Meter Calibration Check Record Form.</li> <li>h. Press “Mode” one time to switch to temperature mode. Record the temperature value on the pH Meter Calibration Check Record Form.</li> <li>i. Press “Mode” two more times to return to pH mode.</li> </ul>
<p><b>12.2 Two-point calibration pH Meter Model 530</b></p>	<ul style="list-style-type: none"> <li>a. Prior to use, verify that the pH meter is set at Auto endpoint as indicated by a letter “A” in the upper left hand corner of the display. If the letter “A” is not indicated, press the “Auto” button until it is displayed. After the endpoint is reached, brackets will appear around the A (e.g. [A]). The temperature and slope will appear at the bottom of the display. When selecting the buffer for calibration, the selected buffer pH will also appear at the bottom of the display, but once the calibration begins, the pH of the buffer will disappear.</li> <li>b. To calibrate, rinse the probe tip with de-ionized water and blot dry with Kimwipes, then place the tip of the electrode into the pH 7.0 buffer and press “Cal” one time so that “pH 7” and “Cal 1” appear.</li> <li>c. The pH meter will automatically read the endpoint when the reading is stable. The appropriate buffer symbol will appear on the display. Record the pH value on the pH Meter Calibration Check Record Form.</li> <li>d. Rinse the tip of the electrode with de-ionized water and blot dry with Kimwipes.</li> <li>e. Place the tip of the electrode in the second calibration buffer. If pH 4.0 buffer is used, press “Cal” one time so that “pH 4” and “Cal 3” appear. If pH 10.0 buffer is used, press “Cal” two times so that “pH 10” and “Cal 3” appear.</li> <li>f. The pH meter will automatically read the endpoint when the reading</li> </ul>

	<p>is stable and the appropriate buffer symbol will appear on the display. The display will show the electrode slope value. Record the pH value and slope on the pH Meter Calibration Check Record Form.</p> <p>g. If the slope is less than 95% or greater than 105%, refer to the troubleshooting section of the Corning pH meter 530 instructions manual (see section 15).</p> <p>h. Rinse the electrode with de-ionized water and blot dry with Kimwipes. Confirm the calibration by reading the pH of the pH 7.0 buffer a second time. Record the pH value and temperature on the pH Meter Calibration Check Record Form.</p>
<p><b>12.3 Liquid Sample pH reading</b></p>	<p>a. Measure the pH of the liquid sample prior to autoclaving and verified after autoclaving.</p> <p>b. Measure the pH of the liquid sample at room temperature unless otherwise specified on the Media/Reagent preparation sheet.</p> <p>c. Place the tip of the rinsed and dried electrode in the sample and press read to start the measurement. The decimal point will flash while the electrode is reading.</p> <p>Note: Do not stir the sample while the instrument is reading. Add acid or base if any pH adjustments are necessary and mix sample thoroughly by stirring. Once sample is mixed stop stirring the sample prior to re-reading the pH. Repeat this process until the desired pH has been reached.</p> <p>d. When the pH reading is stable, record the initial pH of the solution on the Media/Reagent preparation sheet (see SOP MB-10, Media and Reagents Used in Efficacy Testing).</p> <p>e. If the pH of the liquid falls outside of the desired range, adjust the pH of the tempered sample using the specified acid and base solutions and record the amount used on the Media/Reagent preparation sheet.</p> <p>f. Record the adjusted pH of the solution on the Media/Reagent preparation sheet.</p> <p>g. Measure the pH of the sample after autoclaving. Let sample tube adjust to room temperature prior to pH verification. Follow steps 12.2 c-d.</p> <p>h. Record the temperature of the sample.</p> <p>i. For pH model 430, press “Mode” one time to switch to temperature mode. The display will show the temperature in °C. Record the temperature at which the pH of the solution was measured on the</p>



	<p>Media/Reagent preparation sheet. Press “Mode” two more times to return to pH mode.</p> <p>j. For pH model 530, the temperature will display at the same time as the pH. The display will show the temperature in °C. Record the temperature at which the pH of the solution was measured on the Media/Reagent preparation sheet.</p>
<p><b>12.4 Solid sample pH reading (media containing agar)</b></p>	<p>a. Measure the pH of the agar sample prior to autoclaving and verified after autoclaving.</p> <p>b. Measure the pH of the agar sample as a solid.</p> <p>c. Place an aliquot (approximately 5-10 mL) into a petri dish or small weigh boat and let solidify; keep the remainder of the media between 45-50°C (in case the media needs to be adjusted). After the media is solidified, use the Flat-Surface (Epoxy Body) with Replaceable Junction Electrode to measure the pH of the media (see section 15).</p> <p>d. Place the electrode on the surface of the solidified media and press “Read” to start the measurement. The decimal point will be flashing whenever the electrode is reading.</p> <p>e. When the pH reading is stable, record the pH of the media on the Media/Reagent preparation sheet (see SOP MB-10, Media and Reagents Used in Efficacy Testing).</p> <p>f. If the pH of the agar falls outside of the desired range as specified on the media/reagent preparation sheet, adjust the pH of the tempered liquid agar (prior to autoclaving) using the specified acid and base solutions. Discard any media that cannot be adjusted.</p> <p>g. Repeat steps 12.4 a-f as needed. Record the adjusted pH of the solution on the Media/Reagent preparation sheet.</p> <p>h. The pH of some media (e.g. Trypticase Soy Agar) is tested after autoclaving. Follow the instructions on the media/reagent preparation sheet.</p> <p>i. Place an aliquot (approximately 5-10 mL) of the medium into a petri dish or small weigh boat, let solidify; then take the pH of the solidified agar. Record this reading on the Media/Reagent preparation sheet.</p> <p>j. The Flat-Surface electrode is incapable of reading the temperature of the sample and will always read 25°C. Record N/A on the temperature section of the Media/Reagent preparation sheet.</p>
<p><b>12.5 Electrode</b></p>	<p>a. Clean the electrode when the slope falls below 95% or is greater than</p>

<p><b>Cleaning and Maintenance</b></p>	<p>105%.</p> <ul style="list-style-type: none"> <li>b. Test the flow of the junction by letting the electrode hang in the air for 1-2 hrs. A proper flowing junction will have KCl salt crystals forming on it. If none appear, review the following suggestions for cleaning.</li> <li>c. Soak the tip of electrode in the pH Electrode Cleaning Solution for 1-2 hrs. Rinse the electrode with de-ionized water and soak in pH 7.0 buffer until stable.</li> <li>d. For oil contamination: Carefully clean the tip of the electrode using a cotton swab soaked with alcohol or acetone. Rinse the electrode with de-ionized water and soak in pH 7.0 buffer until stable.</li> <li>e. If cleaning does not improve junction flow, the junction will be replaced. To replace, simply pull out the clogged junction, rinse the electrode barrel with de-ionized water, and replace with a clean junction. Then refill electrode with the 3M KCl fill solution up to the fill hole plug (see section 15).</li> <li>f. After cleaning and maintenance activities, check the calibration of the pH meter and record on the pH Meter Calibration Form.</li> <li>g. Record cleaning and maintenance activities under notes on the pH Meter Calibration Record Form.</li> </ul>
<p><b>13. Data Analysis/ Calculations</b></p>	<p>Not Applicable</p>
<p><b>14. Forms and Data Sheets</b></p>	<p>Test Sheets. Test sheets are stored separately from the SOP under the following file names:</p> <p>pH Meter Calibration Record Form EQ-01-07_F1.docx</p>
<p><b>15. References</b></p>	<ol style="list-style-type: none"> <li>1. Corning Electrodes Information sheet for pH combination 3-in-1 electrodes.</li> <li>2. Corning Labware &amp; Equipment- Electrodes: How to Maintain the Premium Performance of Your Corning Electrodes.</li> <li>3. Instruction manual for Corning pH meter 430.</li> <li>4. Instruction manual for Corning pH meter 530.</li> <li>5. Corning Electrodes Information pamphlet for flat surface electrodes.</li> </ol>