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 Meterss

SOP Number: EQ-01-09

Date Revised: 03-31-20

SOP Number	EQ-01-09
Title	Calibration and Maintenance of pH Meters
Revisions Made	• Section 10: Rinse refillable pH electrodes with de-ionized water to remove salt crystals build up, which can be visible around the fill cap or by the protective cap placed over the electrode bulb. • Section 10: Remove air bubbles visible in the pH bulb or by the junction by gently shaking the pH electrode to displace the air bubble. • Section 10: Do not expose any pH or electrodes to high temperatures (>150 °F/65 °C) unless the probe is designed for it. • Section 10: Always rinse the probe with de-ionized water to clean of contaminants before, during and after use. • Section 12: A two-point calibration is required, using two standard pH buffers for pH meters 530 and 530-P.\ • Section 12: A three-point calibration is required for the ExStik pH meter. • Section 12.3: Perform a three-point calibration with ExStik pH meter • Minor editorial changes.

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Title	Calibration and Maintenance of pH Meters
Scope	This protocol describes the method for the operation, two-point calibration, and maintenance of the pH meters used in the laboratory.
Application	pH meters 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:	04/06/2020
Controlled copy number:	0
Date SOP withdrawn:	

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1. Definitions	Abbreviations/definitions are provided in the text.
2. Health and Safety	Follow procedures specified in SOP MB-01, Laboratory Biosafety. The Study Director and/or lead analyst should consult the Safety Data Sheet for specific hazards associated with media and reagents.
3. Personnel Qualifications and Training	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. Instrument Calibration	As noted in section 12.
5. Sample Handling and Storage	1. Measure the pH of media and reagents at room temperature (20ºC to 25ºC) 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 12.5). 2. The buffers used for calibration and reagents (e.g.,1N HCl,1N NaOH, KCl solution) are stored at room temperature in a secondary containment unit. 3. Store electrodes for up to one week (and extended storage) in 25 mL of pH 7 or pH 4 buffer. Do not store electrodes in distilled water. 4. For longer storage periods of electrodes used for pH meter units 530 and 530-P, 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. 5. The ExStik pH meter electrode has a sponge in the electrode protective cap, when longer storage of the ExStik pH meter is required, keep the sponge soaked in pH 4 solution to preserve electrode.
6. Quality Control	For quality control purposes, the required information is documented on the appropriate form (see section 14).
7. Interferences	1. When calibrating the pH meters and taking the pH of a sample, rinse the electrodes thoroughly with de-ionized water and blot (do not wipe) dry with Kimwipes before proceeding to the next solution.
8. Non-conforming Data	1. Management of non-conforming data is consistent with SOP ADM-07, Non-Conformance Reports. 2. To pass calibration, for the 530 and 530-P pH meters, the slope must be 100 ± 5%. If slope is out of range, several corrective

	actions may be necessary to ensure pH meters are working properly. See section 12.6 for troubleshooting and corrective actions. 3. If a pH meter is not functioning as required, consult the technical manual to determine the problem and perform the appropriate corrective action.
9. Data Management	Data will be archived consistent with SOP ADM-03, Records and Archives.
10. Cautions	1. Do not stir the sample while the instrument is reading as this may provide inaccurate readings. 2. Discard used buffer solutions daily. Do not reuse. 3. For 530 and 530-P pH meters: a. 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; record this activity on the pH Meter Calibration Log Form (see section 14). b. Always remove the wetting cap and the fill hole plug during calibration and measurements. Always replace the fill hole plug in between use. c. Rinse refillable pH electrodes with de-ionized water to remove salt crystals build up, which can be visible around the fill cap or by the protective cap placed over the electrode bulb. d. Remove air bubbles visible in the pH bulb or by the junction by gently shaking the pH electrode to displace the air bubble. 4. Do not leave any electrode in organic solvents (for all pH meters) as the tip and body may be damaged. Only store electrodes in pH 7.0 buffer. 5. Do not expose any pH or electrodes to high temperatures (>150 °F/65 °C) unless the probe is designed for it. 6. Always rinse the probe with de-ionized water to clean of contaminants before, during and after use.
11. Special Apparatus and	1. The list of pH meters and electrodes is maintained as an attachment to the SOP. See section 14.

Materials	2. Calibration Buffers: pH 4, pH 7 and pH 10. 3. Fill solution: 3M KCl solution. 4. Storage Solutions: 3M KCl solution for capping or pH 4, pH 7 and pH 10 for buffer immersion. 5. pH Electrode Cleaning Solution. 6. Ceramic replacement junction. 7. 1N Sodium Hydroxide Solution (NaOH). 8. 1N Hydrochloric acid (HCl).
12. Procedure and Analysis	1. Calibrate the pH meters at least once on the day of use as outlined in sections 12.1 through 12.3. 2. A two-point calibration is required, using two standard pH buffers for pH meters 530 and 530-P. 3. A three-point calibration is required for the ExStik pH meter. 4. The standard buffers used are pH 4, pH 7, and pH 10. Dispense a small amount of the required buffers into a smaller container or tube for calibration. 5. Use pH buffers for calibration one time only, do not reuse. After calibration, discard the buffers as per manufacturer's recommendations. 6. For pH meter units 530 and 530-P, use two standard pH buffers to determine the pH of the medium or reagent. a. 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. b. 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. 7. Record the expiration dates of the buffers on the pH Meter Calibration Record Form (see section 14). Also, record the date the buffers are changed under Notes on the pH Meter Calibration Record Form. 8. For pH meter units 530 and 530-P, 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 opening. Bring to level if needed. Record under Notes any adjustments made with the fill solution on the pH Meter Calibration Check Record Form (see section 14).
12.1 Two-point	a. Perform a two-point calibration with calibration buffers 7

calibration pH Meter Model M 530 – (Used for liquid samples)	plus 4 or 10 (whichever is nearest to the expected sample value) b. 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. c. To calibrate, rinse the electrode tip with de-ionized water and blot dry (do not wipe) with Kimwipes, then place the tip of the electrode into the pH 7 buffer and press "Cal" one time so that "pH 7" and "Cal 1" appear. d. 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. e. Rinse the tip of the electrode with de-ionized water and blot dry (do not wipe) with Kimwipes. f. Place the tip of the electrode in the second calibration buffer. If pH 4 buffer is used, press "Cal" one time so that "pH 4" and "Cal 3" appear. If pH 10 buffer is used, press "Cal" two times so that "pH 10" and "Cal 3" appear. g. The pH meter will automatically read the endpoint when the reading 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. h. If the slope is less than 95% or greater than 105%, refer to the troubleshooting section (see section 12.6) of the Corning pH meter 530 and instructions manual (see section 15). i. Rinse the electrode with de-ionized water and blot dry (do not wipe) with Kimwipes. Confirm the calibration by rechecking the pH of the pH 7 buffer a second time (see 12.4 c). Record the pH value and temperature on the pH Meter

	Calibration Check Record Form.
12.2 Two-point Automatic calibration 'Autocal' pH meter M 530-P (Used for liquid samples)	a. Perform a two-point calibration with calibration buffers 7 plus 4 or 10 (whichever is nearest to the expected sample value). For this pH meter, the specified Buffer set is 9. b. Ensure the pH meter MODE is set to display the nominal pH value for the recognized buffer and not the electrode voltage (mV). c. Start the calibration with <CAL>. The number of the specified buffer set is displayed (Buffer set 9) d. The first buffer (Buffer 1) of the buffer set is requested. e. Immerse the electrode tip in the first buffer solution (pH 7). f. Press the <OK> key. The AR display indicator flashes. The nominal pH value for the recognized buffer is displayed. g. As soon as a stable value is recognized, the next buffer is requested (Buffer 2). h. Continue the two-point calibration by thoroughly rinsing the electrode with deionized water. i. Immerse the pH electrode in the second buffer solution (either pH 4 or pH 10). j. Press the <OK> key. The AR display indicator flashes. The nominal pH value for the recognized buffer is displayed. k. As soon as a stable value is recognized, the value of the zero point (ASY) is displayed for 10 seconds, then the value of the slope (SLO) is displayed for 10 seconds. l. After this, the meter automatically switches to the measuring mode. m. Record the pH value and slope on the pH Meter Calibration Check Record Form. n. If the slope is less than 95% or greater than 105%, refer to the troubleshooting section (see section 12.6) of the Corning pH meter 530-P and instructions manual (see section 15). o. Rinse the electrode with de-ionized water and blot dry (do not wipe) with Kimwipes. Confirm the calibration by reading the pH of the pH 7 buffer a second time (see 12.4 c). Record the pH value and temperature on the pH Meter

	Calibration Check Record Form.
12.3 Three-Point calibration of ExStik pH meter (Used for solid samples)	a. Perform a three-point calibration with calibration buffers 7, 4 and 10. b. To calibrate, rinse the electrode tip with de-ionized water and blot dry (do not wipe) with Kimwipes, then place the tip of the electrode into the pH 7 buffer solution and momentarily press the CAL key. Always calibrate the pH 7 buffer first. c. The ExStik automatically recognizes the solution and calibrates itself to that value. When the calibration for pH buffer 7 is complete, the ExStik automatically displays END and returns to normal operation mode. Record the pH value on the pH Meter Calibration Check Record Form. d. Rinse the tip of the electrode with de-ionized water and blot dry (do not wipe) with Kimwipes. e. Proceed to the second calibration buffers (e.g., pH 4 or 10). When the calibration for the second calibration buffer is complete, the ExStik automatically displays END and returns to normal operation mode. Record the pH value on the pH Meter Calibration Check Record Form. f. Rinse the tip of the electrode with de-ionized water and blot dry (do not wipe) with Kimwipes. g. Proceed to the third calibration buffer (e.g., pH 4 or 10). When the calibration for the third calibration buffer is complete, the ExStik automatically displays END and returns to normal operation mode. Record the pH value on the pH Meter Calibration Check Record Form. h. During calibration, the pH reading flashes on the main display. i. When calibration is complete, for each point the ExStik automatically displays END and returns to normal operation mode. j. Record all calibration values on the pH Meter Calibration Check Record Form. k. The appropriate circled indicator 4, 7, or 10 will appear on the ExStik's LCD when a calibration point has been completed. The calibration data is stored until a new

	calibration is performed. Note: if the solution is off more than one pH unit from the pH standards of 4, 7, or 10, the ExStik will assume an error and abort the calibration. CAL and END will be displayed.
12.4 Liquid Sample pH reading (pH meter Models 530 and 530-P)	a. For all media and reagents other than those listed in Attachment 2, measure the pH of the liquid sample prior to autoclaving and verify after autoclaving. See Attachment 2 (section 14) for the list of media and reagents that do not require a pH verification for each batch. b. Measure the pH of the liquid sample at room temperature unless otherwise specified on the Media/Reagent preparation sheet. c. Place the tip of the rinsed and dried electrode in the sample and press "read" (530) or "OK" (530-P) to start the measurement. The decimal point will flash while the electrode is reading. d. 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. e. 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). f. 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. g. Record the adjusted pH of the solution on the Media/Reagent preparation sheet. h. Measure the pH of the sample after autoclaving. Let sample adjust to room temperature prior to pH verification. Follow steps 12.4 c-d. i. The temperature (in ºC) will display at the same time as the pH. Record the temperature at which the pH of the solution was measured on the Media/Reagent preparation sheet. j. Note: Several liquid media do not require a pH verification

		prior to and post autoclaving for each prepared batch. This information is provided on the media and reagents preparation sheets and in Attachment 2. Verify the pH of each new lot of dehydrated media after autoclaving once per lot.
12.5	Solid media pH reading with ExStik meter	a. For all solid media (e.g. agars), other than those listed in Attachment 2, measure the pH of the agar prior to autoclaving and verify after autoclaving. Note: Refer to Attachment 2 (section 14) for list of media that do not require pH verification for each batch. b. Place the electrode on solidified medium, the main display and bar graph indicate the pH reading while the lower display reads temperature. Readings flash until they have stabilized. c. The bar graph is center zero, i.e. at pH 7 there is no display. As the pH rises, the bar moves from the center to the right. d. If the pH drops, the bar moves from the center to the left. e. 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). f. 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. g. Record the adjusted pH of the solution on the Media/Reagent preparation sheet. h. Measure the pH of the sample after autoclaving. Let sample adjust to room temperature prior to pH verification. Follow steps 12.5 c-e.
12.6	Troubleshooting and corrective actions for pH meters 530 and 530-P	a. If slope is out of range (< 95% or > 105%), conduct the following corrective actions in sequential order. If the first corrective action resolves the problem, it is not necessary to continue with subsequent corrective actions. b. First corrective action: change the standard pH buffers and recalibrate the instrument. c. Second corrective action: change the 3M KCl fill solution in the electrode or bring up to volume and re-calibrate the

	instrument. d. Third corrective action: clean the electrode with the pH Electrode Cleaning Solution (follow manufacturer's instructions). See section 12.8 for Electrode Cleaning and Maintenance. Record all troubleshooting activities under the notes section of the pH Meter Calibration Record Form (see section 14). e. If the problem persists or cannot be determined or corrected, call the manufacturer.
12.7 Troubleshooting and corrective actions for ExStik pH meter	a. If the batteries are weak, the "BAT" indicator appears on the LCD screen of the unit. Press the ON/OFF key to turn the ExStik on or off. The auto power off feature shuts the ExStik off automatically after 10 minutes of inactivity to preserve battery life. b. If the meter will not calibrate or displays a -1, reset the meter and attempt to re-calibrate as follows: i. Turn off the meter. ii. Remove the battery cartridge from the top of the meter. iii. Press the ON/OFF button for 10 seconds to bleed off all charges within the meter. iv. Re-insert the batteries and power ON the meter. v. Attempt to re-calibrate the meter. c. If the unit appears to be locked (display frozen) it is possible that the DATA HOLD mode has been inadvertently accessed by pressing the MODE button. Simply press the Mode button again or turn the meter off and restart if the display appears frozen. d. If the meter does not latch up and no button presses revive it, remove the batteries, push the ON button for 10 seconds and then reinsert the batteries. e. If needed, consult the user guide.
12.8 Electrode Cleaning and Maintenance for pH meter units 530 and 530-P	a. Clean the electrode when the slope falls below 95% or is greater than 105% for 530 and 530-P pH meters b. Test the flow of the junction by letting the electrode hang in the air for 1-2 hr. A proper flowing junction will have KCl

	salt crystals forming on it. If none appear, review the following suggestions for cleaning. c. Soak the tip of electrode in the pH Electrode Cleaning Solution for 1-2 hr. Rinse the electrode with de-ionized water and soak in pH 7 buffer until stable. 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 buffer until stable. e. If cleaning does not improve junction flow, replace the junction. To replace, pull out the clogged junction, rinse the electrode barrel with de-ionized water, and replace with a clean junction. Then refill the electrode with 3M KCl fill solution up to the fill hole plug (see section 15). f. After cleaning and maintenance activities, check the calibration of the pH meter and record on the pH Meter Calibration Form. g. Record cleaning and maintenance activities under notes on the pH Meter Calibration Record Form (see section 14).
12.9 Electrode Replacement for ExStik pH meter	a. For EXStik pH meter, the electrode has limited life based on frequency of use and care among other factors. b. To change the electrode, unscrew and completely remove the electrode retaining collar. c. Gently rock the electrode from side to side, pulling it away from the meter, until it disconnects. d. To attach an electrode, carefully plug the electrode into the meter socket (note that the electrode connector is keyed, ensuring proper connection). e. Secure the electrode in place by tightly turning the collar in place (a rubber gasket seals the electrode with the meter).
13. Data Analysis/ Calculations	Not Applicable
14. Forms and Data Sheets	Test Sheets. Test sheets are stored separately from the SOP under the following file names: Attachment 1: Inventory of pH meters and electrodes Attachment 2: List of media and reagents for pH

	verification per lot of dehydrated media pH Meter Calibration Record Form EQ-01-08_F1.docx
15. References	1. Corning Electrodes Information sheet for pH combination 3-in-1 electrodes. 2. Corning Labware & Equipment- Electrodes: How to Maintain the Premium Performance of Your Corning Electrodes. 3. Instruction manual for Corning pH meter M 530. 4. Instruction manual for Corning pH meter M 530-P 5. ExStik waterproof pH meter user guide: http://www.globalw.com/downloads/WQ/ph100.pdf 6. Corning Electrodes Information pamphlet for flat surface electrodes.