



FOOD SAFETY LAB / MILK QUALITY
IMPROVEMENT PROGRAM

Standard Operating Procedure



Title: **Calibration, Usage, and Maintenance of Beckman Coulter 470 pH Meter**

SOP #: **5.3.6**

Version: **01**

Revision Date: **n/a**

Effective Date: **08/18/2020**

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**Name of Protocol – Calibration, Usage, and
Maintenance of Beckman Coulter 470 pH Meter**

FILE NAME: pH Meter Protocol.docx



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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to set forth standard guidelines for calibration, usage and maintenance of the lab pH meter located in the Media Room (Room 352B).

1.2 Scope

This SOP applies to the Food Safety Lab, and the Milk Quality Improvement Program Lab. This SOP also applies to members of the Worobo Lab, Snyder Lab, and Alcaine Lab who conduct research in the lab and use the Media Room to prepare buffers and reagents.

This SOP applies only to the Beckman Coulter 470 hand held pH meter located in the Media Room (Room 352B)

Definitions

pH: (potential of hydrogen or power of hydrogen) is used to express the acidity or alkalinity of a solution on a scale of 0 to 14, where less than 7 represents acidity, 7 neutrality, and more than 7 alkalinity.

Slope: Slope = mV/pH unit. A perfect pH electrode, at 25 °C, produces a slope of 59.16 mV per pH unit. The difference between this perfect slope reading and the electrode's actual reading is called the slope error. Even new probes have slope error. The manufacturer determines the acceptable range of error. For our pH meter this value is between 85 and 102 % of perfect slope. Slope percentage can differ in other meters.

1.3 Safety

Solutions used to raise or lower pH are dangerous and should be handled with extreme caution: The solutions used for our lab are hydrochloric acid (HCl), 1N and 6N solutions and sodium hydroxide (NaOH), 1N and 5N solutions. These solutions must ALWAYS be prepared using a chemical safety hood with proper PPE.



SECTION 2 MATERIALS

- pH meter
- probe
- Buffered storage solution
- Calibration Buffers, 4.0 (Red), 7.0 (Yellow), 10.0 (blue), BDH,
- 50 ml tubes
- Ultrapure DH₂O in squirt bottle
- Kim wipes
- Users Manual



SECTION 3 PROCEDURES

3.1 General Information

3.1.1 Product specifications:

- Meter: Beckman Coulter pH Meter, Model 470, hand held, battery operated. pH range from -2.00 to 19.99, accuracy ± 0.01 , up to 5 point calibration
- Electrode (Probe): Beckman Coulter, Epoxy Combination pH Electrode, Gel-Filled. Must be stored in buffered solution. To order a replacement Beckman Coulter probe use VWR Catalog Number A57177.

3.1.2 Calibration Requirements: pH Meter must be calibrated every time it is used. A spreadsheet for recording calibration slope readings is located near the meter and must be annotated each time the meter is calibrated. A blank calibration sheet is attached as Appendix A.

3.2 pH Meter Calibration

3.2.1 Calibration points. This pH meter will accept up to five calibration points between 1.68 and 12.45. The manufacturer recommends usage of at least 3 calibration points to insure the greatest accuracy. When you calibrate the meter in the media room, you will use a 3 point calibration at points 4.01, 7.00 and 10.01. The calibration buffers in stock for this meter correspond to those measurements. (See figure 6) This covers the expected pH of most buffers and solutions used in the lab. If you need to pH outside of that range, you will have to order the appropriate standard buffer for calibration.

3.2.2 Calibration Instructions (Refer to Figure 1 on the following page)

- Turn on the meter. The default setting is pH mode. Select "CAL" for calibration mode. The first calibration point will show on the meter as 1.68. **IMPORTANT:** Press the scroll down arrow once to set the first calibration point at 4.01. If you do not, the calibration is faulty.
- Rinse the probe in ultrapure water, blot gently with a Kim Wipe, and place the probe in the 4.01 calibration buffer (red) Never wipe the glass electrode inside the bottom of the probe.,
- Press enter. The CAL icon and pH standard will flash until complete. When the 4.01 calibration is complete the meter will beep 3 times and begin to flash 7.00 for the next calibration point.
- Rinse the probe in ultrapure water, blot, and place the probe in the 7.00 buffer solution (yellow).
- Press enter. The display will flash until the calibration is completed. When the 7.00 calibration is completed, the meter will begin to flash 10.01 for the next calibration point.
- Rinse the probe in ultrapure water, blot, and place in the 10.01 calibration buffer (blue).



- Press enter. When the 10.01 calibration is completed, the meter will beep 3 times and will display the slope, mV offset and number of calibration points.
- Per the user's manual for this equipment, the slope shown on the meter must be between 85% and 102%. (See figure 2) If the slope is within the correct range, **press Enter to accept the calibration**. If the slope is not within the correct range, do not use the meter. Refer to Section 4 Troubleshooting, Table 3.
- Press the pH button to switch from calibration mode to pH mode.

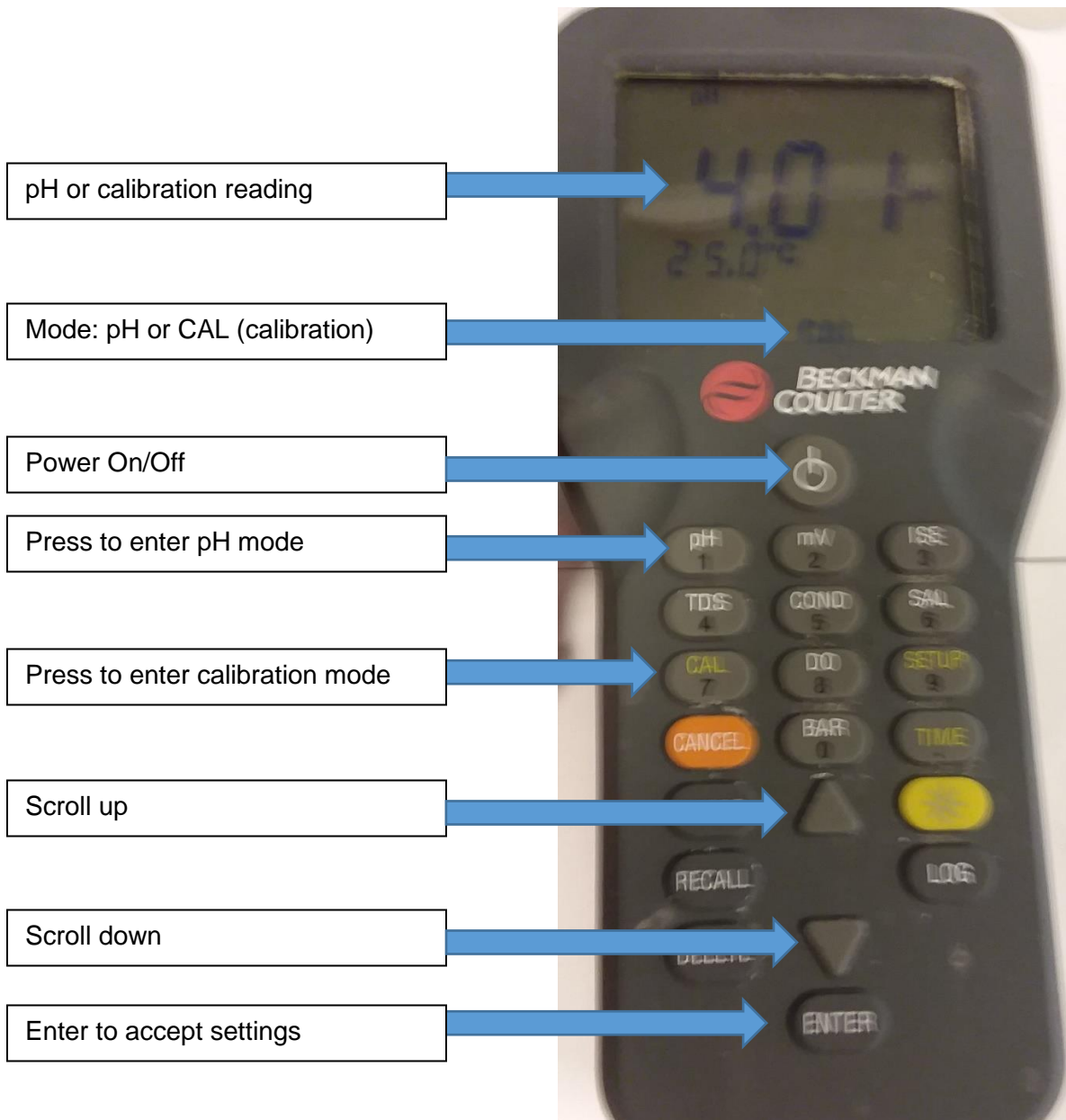


FIGURE 1

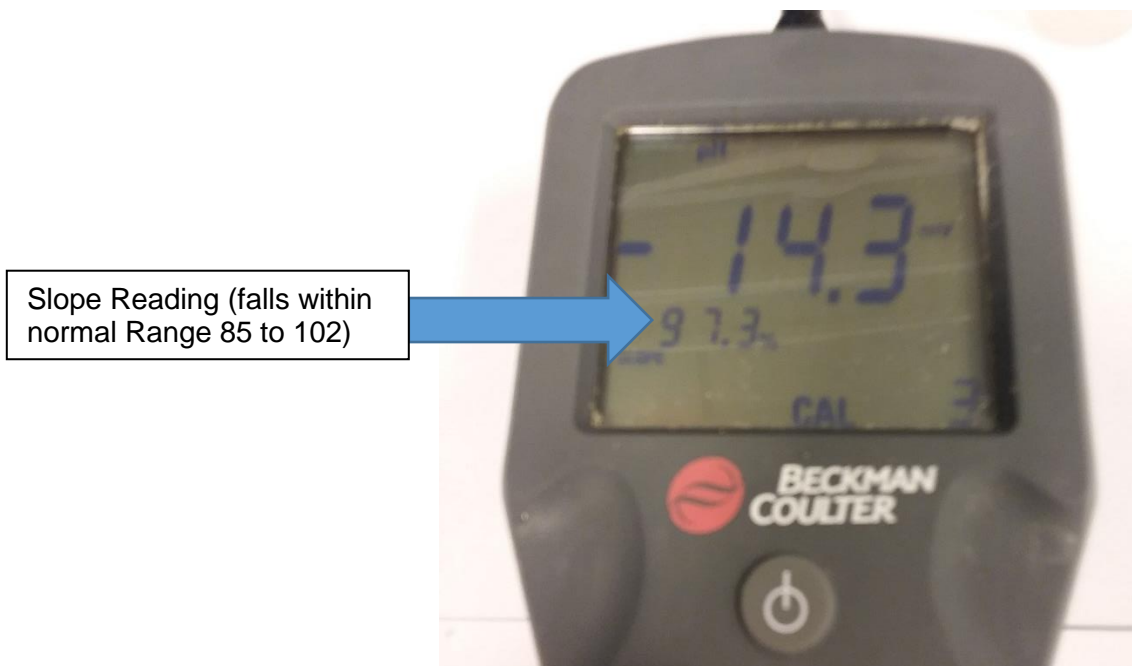


FIGURE 2

3.3 pH Meter Usage

3.3.1 Usage warning: DO NOT use the pH meter probe in the media room for substances contaminated with BSL-1 or BSL-2 organisms. A separate probe is available for use with these materials.

3.3.2 pH measurement.

After calibrating the pH meter, make sure the meter is in pH mode by pressing the “pH” button. Rinse the probe in ultrapure water. If using a stir plate, turn it to a very low setting. Place the probe in the solution so that the glass bulb at the bottom of the probe is completely submerged. The pH values will continue to change until the solution pH is reached. The meter will beep 3 times. Use the appropriate solution to raise or lower the pH if it is not at the desired reading. Add solution with disposable 2 ml pipettes located near the meter. Please use separate pipettes for HCl and NaOH. Make sure that you allow the meter to reach a pH reading and beep three times before adding more solution.

3.3.3 Solutions used to raise or lower pH. (See figure 3)

- The solution usually used in the media room to lower pH is Hydrochloric Acid (HCl). The solution is available in 1N and 6N concentrations. These are usually pre-made by the media room team, but if none is made, you can prepare the solution. The recipes for 1N and 6N are in table 1 below. HCl is dangerous. Read the Material Safety Data Sheet (MSDS) for HCl prior to use. Always prepare the solutions under a chemical safety hood wearing proper PPE. Always add the acid to water, never add water to the acid.



- The solution usually used in the media room to raise pH is Sodium Hydroxide (NaOH). The solution is available in 1N and 5N concentrations. These are usually pre-made by the media room team, but if none is made, you can prepare the solution. The recipes for 1N and 5N are in table 1 below. NaOH is dangerous. Read the Material Safety Data Sheet (MSDS) prior to use. Always prepare the solutions under a chemical safety hood wearing proper PPE. Always add the NaOH to water, never add water to the NaOH.

TABLE 1

SOLUTION	ULTRAPURE DH ₂ O	NaOH or HCl	ALWAYS
1N NaOH	100 ml	4 g Sodium Hydroxide (Solid)	Add the NaOH to the water
5N NaOH	100 ml	20.3 g Sodium Hydroxide (Solid)	Add the NaOH to the water
1N HCl	91.7 ml	8.3 ml Hydrochloric Acid (12N)	Add the HCl to the water
6N HCl	50 ml	50 ml Hydrochloric Acid (12N)	Add the HCl to the water

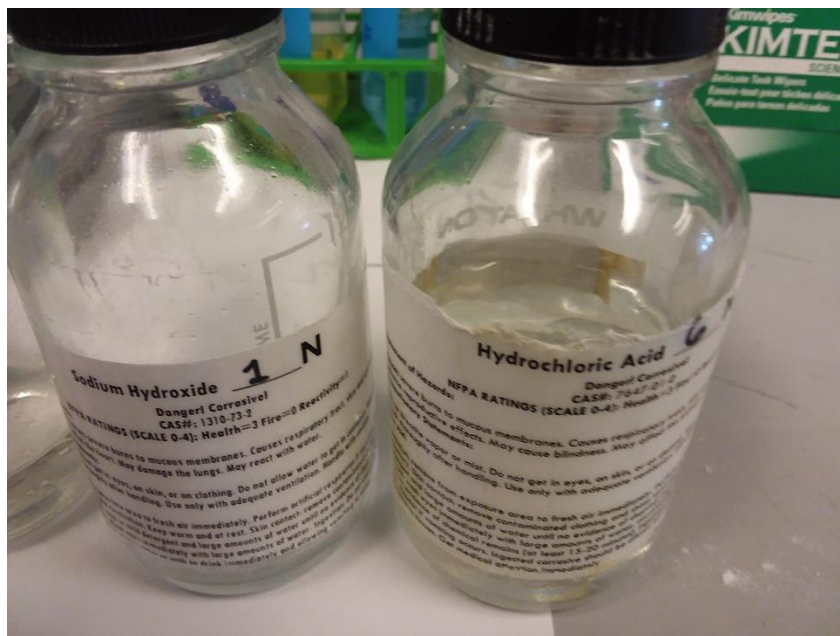


FIGURE 3

3.4 pH Meter, probe, and buffer maintenance

3.4.1 pH meter maintenance. The meter is battery operated. When the batteries are low, a low battery warning will flash on the screen. Replace the batteries immediately when low. To save battery life, the meter turns itself off 20 minutes after last activity. The meter should be wiped clean with a damp cloth when dirty. Do not submerge the meter in water.

3.4.2 Probe maintenance. The probe must be stored in buffer. (See figure 4) Damage will occur if the probe is left out of solution for too long. The probe should be rinsed when transferred from one

solution to another. Otherwise, readings may be incorrect. (See figure 5). Blot gently with a kimwipe to remove excess liquid. NEVER wipe the glass bulb at the bottom of the probe.

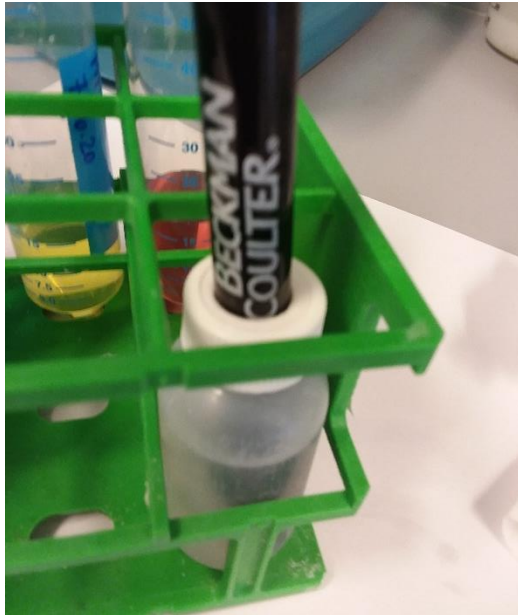


FIGURE 4



FIGURE 5

3.4.3 Buffer maintenance

- Calibration Standard Buffers are stored in the media room in 500 ml bottles. (See figure 6) Located near the meter are 50 ml tubes containing 20-25 ml of each type of buffer. These will be used each time the meter is calibrated. Due to contamination with other solutions, the buffers in the tubes should be changed at least once per week. A label is attached to the rack containing the buffers. Each time the buffers are changed, record the date on the label.
- Calibration Standard Buffers have expiration dates on them. Do not use the buffers if they are expired. Use the following information to order new buffers:

4.01 (Red)	500 ml	VWR Catalog number: BDH5018-500ML
7.00 (Yellow)	500 ml	VWR Catalog number: BDH5046-500ML
10.01 (Blue)	500 ml	VWR Catalog number: BDH5072-500ML

- Probe storage buffer maintenance: The pH Meter probe buffer comes in a case with 4 118ml bottles. These are stored in the media room. The buffer in the probe storage bottle should be replaced from the larger bottles when the solution is low. Before putting clean buffer in the storage bottle, rinse the bottle to remove crusted buffer from the bottle.
- If the storage buffer stock is low, or the buffer has expired, use the following information to reorder: Beckman Coulter Standard Probe Storage Buffer, 118 ml, 4/case, VWR catalog number BKA60217.



FIGURE 6



SECTION 4 TROUBLESHOOTING

4.1 Troubleshooting guide (from user’s manual)

TABLE 2

Problem	Possible Cause	Solution
No Display	Auto off function has turned meter off	Press power button to restart meter
	Meter still has no display	Replace AA batteries
Trouble calibrating. Display does not stop flashing during calibration	Probe sensor is dirty	Clean probe with warm buffer or ultrapure water
	Buffers may be contaminated or expired	Replace buffers and re-calibrate
	Batteries are low	Replace batteries
	Connection between probe and meter is loose	Check the connection at the junction of the meter and probe. Turn clockwise to tighten connection
pH reading is unstable.	Probe sensor is dirty	Clean probe with warm buffer or ultrapure water
	Connection between probe and meter is loose	Check the connection at the junction of the meter and probe. Turn clockwise to tightened connection
	pH is still adjusting due to added acid or base, or the temperature of the solution is changing.	Wait until solution reaches stable temperature. Meter should reach stable pH before adding base or acid
	Probe is damaged	Check for breakage or cloudiness on the probe. Order new probe if damaged.

4.2 Built in meter error codes. This meter has system error codes that will flash when the meter is not operating correctly. The most common codes are listed in Table 3. Other error codes are possible if using a probe other than a standard pH meter probe. To see these, please refer to the user’s manual.



TABLE 3

Code	Cause	User Actions
E03	Electrode off-set error. The mV reading is too high	Clean electrode. If this does not clear the error, replace the pH probe. User can override this error (See manual)
E04	Electrode slope error. The slope reading is below 85 or above 102.	Clean Electrode. Replace buffer solutions. Remove magnetic stir bars from the solution. (These can interfere with signals) Make sure temperature is stable. If the error persists, replace the electrode. User can temporarily override this error. (See user's manual)
E08	Too long to calibrate, signal not stable during calibration	Clean Electrode. Replace buffer solutions. Remove magnetic stir bars from the solution. Make sure temperature is stable. If the error persists, replace the electrode. User cannot override error
E14	Extremely low battery warning	Replace batteries immediately. User cannot override error

4.3 Other common problems with pH meter readings.

- Problem: pH reading will not stabilize.

Cause: User does not wait for signal before adding additional HCl or NaOH. Example: pH is 7.80. Desired pH is 8.00. User adds a drop of 1N Sodium Hydroxide to raise pH. Meter is testing pH but is not finished and has not beeped. User adds several more drops of NaOH to solution. pH continues to fluctuate. When meter finally stops, the pH is above 8.00

Solution: When adding acid or base to lower or raise pH, always wait for the acid or base to reach equilibrium in the solution and for the pH meter to beep that it has finished reading the pH.

- Problem: pH reading of TRIS will not stabilize.

Cause: TRIS solution is losing temperature while pH reading is conducted.

Solution: If the buffer to be tested will be used at room temperature, it should reach a stable room temperature before testing the pH. The pH of most solutions will vary based on the temperature of the solution, but TRIS has a very notable change. In general, the change in pH per degree C for TRIS is 0.028 pH units. (Example: If a TRIS solution at 25° C has a pH of 8.00, the pH at 5° C will be 8.58 and the pH at 37° C will be 7.71).



SECTION 5 REFERENCES

5.1 Beckman Coulter User's Manual. Series 400 and 500 pH and Electrochemistry Meters, May 2008

5.2 New England Biolabs. <https://www.neb.com/tools-and-resources/usage-guidelines/ph-vs-temperature-for-tris-buffer>

5.4 Sigma Aldrich. https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Aldrich/General_Information/1/labwarenotes_v1_6.pdf Labware Notes: Checking pH Electrode Functionality, How to Calculate Slope Percentage.



SECTION 6

METHOD VERSION & CHANGES

VERSION	DATE	EDITOR	COMMENTS
Version 1	7/27/2020	Maureen Gunderson	Original SOP