APPENDIX 7 PH MEASURING

1. General information

PH probe is a unit, measuring the solution acidity or alkalinity degree. It is measured on scale of 0 to 14. The term pH is derived from "p", the mathematical symbol for the negative logarithm, and "H", the chemical symbol of Hydrogen. The formal definition of pH is the negative logarithm of the Hydrogen ion activity.

2. pH Electrode

For pH measurement the milk analyzer needs a combination electrode, compatible with most pH electrodes that have BNC connectors and zero potential (the pH where the mill volt output of the electrode equals 0) near 7 pH.

2.1. Electrode part

The electrode is the most important part of the pH measurement. The electrode glass membrane is fragile and must be handled with care. To protect the glass membrane and to maintain activation, a protective rubber cap containing a suitable storage solution covers the glass membrane.

2.2. Electrode care & Electrode maintenance

pH Electrodes are susceptible to dirt and contamination and need to be clean regularly depending on the extent and condition of use. At no time should one touch or rub the glass bulb as this causes the build-up of electrostatic charge.

2.3. Storage

For best results, always keep the pH bulb wet. An optimal storage solution for combination electrode is pH 4 buffer with 225 grams of KCl per liter. Table salt, NaCl, can be used if KCl is not really available. Other pH buffers or tap water are also acceptable storage media, but avoid storage in de-ionized water. The protective rubber cap filled with the buffer solution provides ideal storage for long periods.

2.4. After Use

After measurement is completed, follow the sequence below for storage.
- Wash the electrode and reference junction in de-ionized water.
- Close the refilling hole by returning its rubber sleeve or stopper cap. (Necessary for only refillable electrode).
- Store the electrode as mentioned above (see section Storage).

2.5. Electrolyte Replacement (for refillable electrode only).

The reference electrolyte needs to be refilled when the electrode has been used for a long period, or when the internal electrolyte has dried up. To accomplish this, follow the procedure described below.

- Remove the protective rubber cap or sleeve;
- Remove the protective rubber sleeve to expose the filling port of the electrode;
- Remove the old reference electrolyte with a syringe;
- Fill the new reference electrolyte.

2.6. New electrolyte preparation:

- Open the KCl container;
- Add in de-ionized water until it reaches the level of 20 ml;
- Close the container and shake it to dissolve the KCl;
- Add in fresh electrolyte until it reaches the level of the refilling port. The reference electrolyte used should be 3M(Mol) KCl;
- Replace the rubber sleeve.

2.7. Re-use the electrode.

- Rinse the liquid junction with de-ionized water.

If these steps fail to restore normal electrode response, you may attempt to rejuvenate it (See: Electrode Rejuvenation).

2.8. Electrode cleaning

Electrodes which are mechanically intact can often be restored to normal performance by one or combination of the following procedures.

- Salt deposits:

Dissolve the deposit by immersing the electrode in tap water for ten to fifteen minutes. Then thoroughly rinse with de-ionized water. Wash the electrode pH bulb in a little detergent and water. Rinse electrode tip in with de-ionized water.

- Oil/Grease films:

Wash electrode pH bulb in a little detergent and water. Rinse electrode tip with de-ionized water.

- Clogged Reference Junction:

pH electrodes have junction, which allows the internal fill solution of the measuring electrode to leak out into the solution being measured. The

junction can become clogged by contamination in the solution. If a clogged junction is suspected it is best to clear the junction.
Heat up the diluted KCl solution to 60-80°C. Place the sensing part of the pH electrode into the heated KCl solution for approximately 10 minutes. Allow the electrode to cool while immersed in some unheated KCl solution.

- Protein Deposits
Prepare 1% pepsin solution in 0.1 M HCI. Allow the electrode to stand in this solution for five to ten minutes. Rinse the electrode with de-ionized water.

2.9. Electrode activation

Generally, if the procedure of storage and maintenance had been closely followed, the electrode can
be used immediately. However, should the electrode response become sluggish, it may be possible that the bulb has dehydrated.
The bulb can be dehydrated by immersing the electrode in an ideal storage solution (e.g. buffer pH 4 solution) for 1-2 hours. If this fails, the electrode may require re-activation. If the above procedure does not reactivate the electrode to acceptable status, try rejuvenation the electrode by following the procedure outlined below.

2.10. Rejuvenation Procedure

Dip and stir the electrode in freon or alcohol for 5 minutes.
Leave the electrode in tap water for 15 minutes.
Dip and stir the electrode in concentrated acid (HCI, H_2S_4) for 5 minutes.
Leave the electrode in tap water for 15 minutes.
Dip and stir in strong base (NaOH) for 5 minutes.
Leave the electrode in tap water for 15 minutes.
Test with standard calibration solution.
Finally, test with standard calibration buffer solution to see if the electrode yields acceptable results. You may repeat again for better response (maximum 3 times). If the response does not improve, then the electrode has completed its useful life. Replace with a new electrode.

2.11. Electrode Lifespan

pH electrodes have a finite lifespan due to their inherent properties. How long a pH electrode will last will depend on how it is cared and the solution it is used to measure. Even if an electrode is not used it still ages. Electrode demise can usually be characterized by a sluggish response, erratic readings or a reading, which will not change. When this occurs an electrode can no longer be calibrated. pH electrodes are fragile and have a limited lifespan. How long an electrode will last is determined by how well is maintained and the pH application. The harsher the system, the shorter the lifespan. For this

reason it is always a good idea to have a back-up electrode on hand to avoid any system down time.

3. Buffer Solutions

Buffers are solutions that have constant pH values and the ability to resist changes in that pH level. They are used to calibrate pH measurement system.

PH buffer solution description (Pharmacopoeia standard)

Use only this types standard buffers for calibration!

Description	pH 7.00±0,01/20ºC	pH 4.00±0,01/20ºC
Composition	Potassium dihydrogen phosphate, Di-sodium hydrogen phosphate	Borax, Sodium hydroxide solution
Temperature parameters	10ºC - 7.06 25ºC - 6.99 20ºC - 7.00 30ºC - 6.98 40ºC - 6.95 50ºC - 6.91	10ºC - 4.00 25ºC - 4.00 20ºC - 4.00 30ºC - 4.00 40ºC - 4.00 50ºC - 4.05

4. pH Electrode Calibration

pH Electrodes are like batteries; they run down with time and use. As an electrode ages, its glass changes resistance. For this reason, electrodes need to be calibrated on a regular basis. Calibration in pH buffer solution corrects for this change.
Calibration is an important part of electrode maintenance. This assures not only that the electrode is behaving properly but that the system is operating correctly.
Usually pH meters require calibration at 3 specific pH values. One calibration is usually performed at pH 7, second and third are typically performed at pH 4 and pH 10.

It is best to select a buffer as close as possible to the actual pH value of the sample to be measured. Use standard calibration buffers that the temperature and the sample solution are the same.
Use the operation manual for the corresponding pH meter.

For Sensorex pH electrodes, originally supplied with Lactoscan read the following information:

Temperature compensations
The output of pH electrodes varies with temperature in manner, predicted by theory. When needed, Sensorex can supply electrode holders with build-in automatic temperature compensators. The need of automatic compensation depends on the temperature variation, the pH value being measured. At pH of about 7 there is no error due to temperature and, of course, at a constant temperature there is no error. As shown in the following table, the pH error due to temperature is a function of both the temperature and the pH value being measured. At a pH of about 7 there is no error due to temperature and, of course, at a constant temperature there is no error. The more the temperature changes from the ambient calibration temperature and the more the pH departs from 7 the greater is the pH error.

pH temperature error table

°C	pH										
	2	3	4	5	6	7	8	9	10	11	12
5	.30	.24	.18	.12	.06	0	.06	.12	.18	.24	.30
15	.15	.12	.09	.06	.03	0	.03	.06	.09	.12	.15
25	0	0	0	0	0	0	0	0	0		0
35	.15	.12	.09	.06	.03	0	.03	.06	.09	.12	.15
45	.30	.24	.18	.12	.06	0	.06	.12	.18	.24	.30
55	.45	.36	.27	.18	.09	0	.09	.18	.27	.36	.45
65	.60	.48	.36	.24	.12	0	.12	.24	.36	.48	.60
75	.75	.60	.45	.30	.15	0	.15	.30	.45	.60	.75
85	.90	.72	.54	.36	.18	0	.18	.36	.54	.72	.90

0 pH Error Range Less than .1 pH Error Range

5. PH helpful hints

For greatest accuracy in pH measurement, follow these guidelines:
Use the same technique to measure samples, which was used for calibration.
Be consistent with stirring rates, times and conditions.
Calibrate with buffers, which are close in temperature to that of the sample.
Calibrate the pH electrode regularly, e.g. once an hour for accuracy to within 0.01 pH, or once a day for accuracy to within 0.1 pH.
Use fresh buffers for calibrations. Avoid contamination of the stock buffer solution and do not use it beyond the expiry date.
Keep all connections dry.

Immerse the electrode far enough into the solution to insure the reference junction is below the surface.
Allow adequate time for the electrode to stabilize in standards and samples before taking a reading.
Clean the electrode periodically. Allow more time for aged electrodes.
Do not use the pH electrode in solutions of fluoride ion at low pH. This will etch the glass membrane.
Sulphide vapors can permeate the electrode wick and contaminate the reference element. Minimize contact in such environments and change the reference electrolyte frequently.

6. PH measuring.

Measuring pH is an additional feature of the analyzer and is optional.
Remove the protective rubber cap of the pH electrode. Take care to handle it appropriate in order not to be damaged. Use de-ionized or distilled water to rinse the electrode before usage. Fill in the sample holder with milk, put it in the recess of the analyzer and dip the pH electrode into the milk sample, ensuring complete dip of the electrode in the sample. Stir gently for homogenization of the sample.

Measuring can be done in two modes:

Off line by starting the menu **pH & Co Meter | Measuring**, when the analyzer works only as a pH meter.

On line automatic pH measuring, when measuring the rest of the sample's parameters.
After starting the menu **pH & Co Meter** the following message appears on the display:

pH Calibration
pH Measuring
pH En/Disable
pH U Display
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pH Test
Co Meter Calibr
Co Meter Test
Co Meter En/Dis

Exit

7. pH Calibration.

Serve for pH meter's calibration. For this purpose 2 sample buffers are used, shown on the display as **Low buffer** (for example 3.00 pH) and **High buffer** (for example 7.00 pH). Follow the procedure:
Start the **Calibration** menu.
Put the probe in the **Low buffer**.
Using the analyzer's buttons enter the exact buffer value. The following is shown on the display:

pH Calibr
Put Izopot buff
Buf=xx.xxx

The operator has to enter the buffer's value, when the probe is in its isopotential point.
After that the display shows:

pH Calibr
Put Izopot buff
Buf=xx.xxx
V=x.xxxV Set

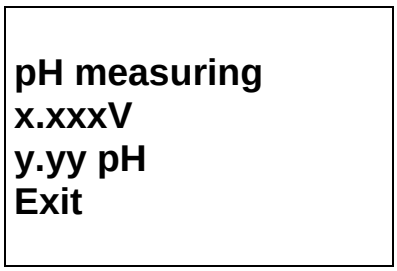
Where **x.xxxxV** is the measured in the probe voltage. Press the button **Set** when the readings stop moving. Repeat the procedure with the **Next buffer**. The following message appears on the display:

pH Calibr OK

Which shows that the calibration procedure was completed successfully. The calibrated device is ready for making measurements.
The device automatically passes in mode pH measuring.

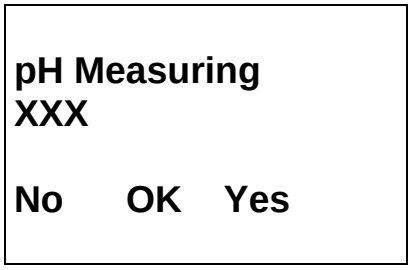
8. pH Measuring.

Entering this menu means that you start measurement of pH in off line, i.e. the analyzer works only as a pH meter. The operator has to put the probe in the sample. The following message is shown on the display:



x.xxx – measured in the probe voltage
y.yyy – measured pH of the sample
By pressing the button **Exit**, the operator may enter the program and to pass to upper menu.

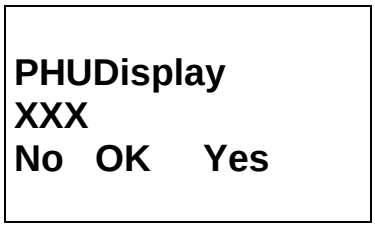
9. pH En/Disable.
Enables or disables the pH measurement during the normal work of the analyzer - On line. After staring it, the following message appears on the display:



Where **XXX** is the current state of the working mode. By pressing the buttons under each of the inscriptions it may be changed. **Yes** – means that during the analyzers normal work the pH will be measured together with the rest of the parameters. **No** – means that the pH measurement will not take place.

10. pH U Display.

Enables or Disables displaying the measured voltage of the pH probe during pH measurement. After starting it the display shows the following:



Where **XXX** is the current state of the displayed mode. By pressing the buttons below the corresponding inscription it may be changed. **Yes** – means that the voltage of the probe will be shown. **No** – it will not be shown. It is valid for the two measuring modes.

11. pH test

Tests the measuring system in production conditions.