HISTO-LOGIC®

No reader should utilize materials and/or undertake procedures discussed in HISTO-LOGIC articles unless the reader, by reason of education, training and experience, has a complete understanding of the chemical and physical properties of all materials to be utilized and the function of each material by which any procedure is accomplished.

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pH Simplified

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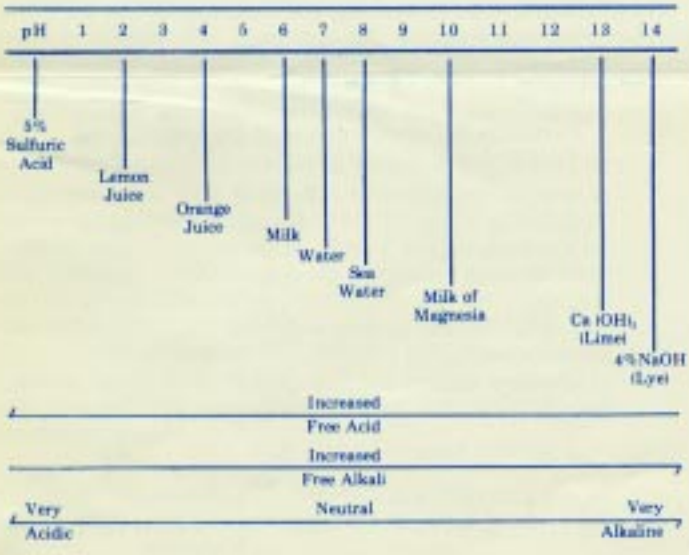
Markson Science, Inc.

Editor's Note: The length of this article will necessitate publication in three parts. Part two will appear in **Histo-Logic**, Vol. IX, No. 3, July, 1979. Part three will appear in Vol. IX, No. 4, October, 1979.

What is pH?
pH is the Unit of Measure Used to Express the Degree of Acidity of a Substance.

The centimeter is a unit measure of length. The gram is a unit measure of weight. Similarly, pH is the unit of measure we use to say how much free or active acid is in a substance. The pH scale goes from 0 to 14. A pH of 0 means a very high acid activity; a pH of 14 means a very low acid activity. In between these two extremes is a pH of 7. This is the pH of pure water. Tables 1 and 2 give some examples of the pH or active acidity of various products and water solutions. Addition of a strong acid, such as sulfuric acid (H_2SO_4), to water makes the resulting solution very high in active acid concentration. This is called an acidic solution. The addition of a strong base or alkali material, such as sodium hydroxide (NaOH), to water makes the resulting solution very low in active acid concentration. This is called a very basic or alkaline solution. Water, which is neither very acidic nor very alkaline, is said to be neutral. The pH scale is a quantitative way of expressing the active acid or alkali concentration of a solution.

Table 1



* 1975 Markson Science, Inc. Reprints available from: Editor, Science Supply News, Markson Science, P.O. Box 767, Del Mar, California 92014.

Table 2
pH of Common Products

Product	pH Range	Product	pH Range
Human Blood	7.4-7.5	Borax	9.2
Tomatoes	4.0-4.4	Peaches	3.4-3.6
Eggs	7.6-8.0	Cottage Cheese	5.0-6.0
Dog Blood	6.9-7.2	Tuna Fish	5.9-6.1
Photo Developer	12.0	Beer	4.0-5.0
Apple	2.9-3.3	Cheese	4.8-6.4
Bananas	4.5-4.7	Wheat Flour	5.5-6.5
Pumpkin	4.8-5.2	Corn	6.0-6.5

Why pH is Important:

The pH or acidity of a solution is important throughout all phases of chemistry and biochemistry.

In the Chemical Industry: The efficient production of nylon, as well as other modern fibers, depends on rigid pH control.

In Biochemistry: The pH of our blood is normally controlled to within a few tenths of a pH unit by our body chemistry. If our blood pH changes as much as half a pH unit, serious illness will result. Proper skin pH is essential for a healthy complexion. The pH of one's stomach directly affects the digestive process.

In Agronomy: The pH of the soil regulates the availability of nutrients for plant growth, as well as the activity of soil bacteria. In alkaline soils (pH 8 and above) the amount of nitrogen, phosphorus, iron and other nutrients in solution become so low that special treatment is necessary to insure proper growth.

In Food Science: The efficient production of food products depends upon careful pH control. The proper curd size, uniformity, and structure of cottage cheese is directly related to the pH at cutting time. Yeast can ferment and leaven a dough only within certain pH limits. Jelly will not gel properly unless the pH is in the 3.5 region.

In the Pulp and Paper Industry: pH control is essential to the proper operation of bleaching plants and wet-end processes. Also, in order to conform with environmental protection regulations, the pH of wastewater from these plants must be controlled.

In Chemical Research and Engineering: Accurate pH measurement is necessary to the study of many chemical processes. The researcher needs to know the pH at which a chemical reaction proceeds at its fastest in order to understand the reaction. The engineer uses the information to develop practical commercial processes.

In Environmental Research and Pollution Control: The pH of a river or lake is important in maintaining a proper ecological balance. The pH of the water directly affects the physiological functions and nutrient utilization by plant and animal life. Extremes in pH can reduce a lake to a lifeless, smelly bog.

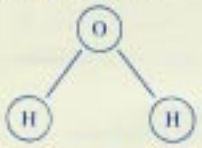
Protecting our waterways requires constant monitoring of industrial effluent. Plating and metal finishing plants tend to produce acidic wastewater, as do mining operations. Chemical plants often have very alkaline wastewater.

pH measurements are used as a guide to the proper neutralization of these plant wastes, as well as to monitor the

final effluent quality. Occasionally, an acidic stream can be combined with an alkaline stream to produce a final stream which is close to neutral. pH measurements assure the proper management of this cost saving technique.

More About pH (For Those Who Really Want to Know):

To understand more about pH, we need to know more about the chemistry of water. A molecule of water (Fig. 1) is composed of one oxygen atom and two hydrogen atoms and looks something like this.



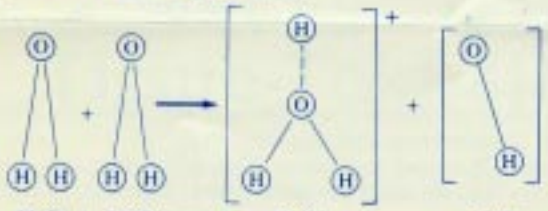
H=Hydrogen

O=Oxygen

Water Molecule (H_2O)

FIGURE 1

In pure water, most of the water molecules remain intact. However, a very small amount of them react with each other (Fig. 2) in the following manner.



$H_2O + H_2O \longrightarrow H_3O^+ + OH^-$

Water Water — Hydronium Ion (Acid) + Hydroxyl Ion (Base)

FIGURE 2

The hydronium ion (H_3O^+) is the chemical unit which accounts for the acidic properties of a solution. The hydroxyl ion is the chemical which accounts for the basic or alkaline properties of a solution. As you can see, when pure water reacts as described in Figure 2, it produces an equal amount of H_3O^+ and OH^-. Thus, it does not have an excess of either ion. It is therefore called a neutral solution.

If a strong acid, such as hydrochloric acid (HCl), is added to water, it reacts with some of the water molecules as follows:

$$HCl + H_2O \longrightarrow H_3O^+ + Cl^-$$

Thus, the addition of HCl to water increases the H_3O^+ or acid concentration of the resulting solution.

If a strong base, such as sodium hydroxide, is added to water, it ionizes as follows:

$$NaOH \longrightarrow Na^+ + OH^-$$

Thus, the addition of NaOH to water increases the OH^- or alkali concentration of the resulting solutions.

Another interesting aspect of water is that the concentration of H_3O^+ and OH^- remain in balance with each other. An increase in the concentration of H_3O^+ causes a proportional decrease in the concentration of OH^-. Accordingly, a table (3) can be constructed which shows the relationship of pH, H_3O^+ concentration, and OH^- concentration.

Table 3

Ion Activity (Moles/litre)

pH	H_3O^+ (Acid)	OH^- (Base)
0	1.0	0.00000000000001
1	0.1	0.0000000000001
|	|	|
13	0.0000000000001	0.1
14	0.00000000000001	1.0

Five Things to Note About Table 3:

1. As the acid (H_3O^+) concentration decreases, the pH increases.
2. As the acid (H_3O^+) concentration decreases, the base (OH^-) concentration increases proportionately.
3. At pH 7 the acid (H_3O^+) and base (OH^-) concentrations are equal. This is called the neutral point.
4. The pH scale represents the number of places the decimal point is moved to the left of one in expressing the acid (H_3O^+) concentration.
5. Each pH unit represents a ten-fold change in H_3O^+ or OH^- concentration. For example, solution at pH 6 is 10 times more concentrated in H_3O^+ ions than a solution at pH 7.

Thus, you can see from Table 3 that the pH scale is a far more concise way of quantitatively expressing the acidity of a solution.

Solution to Problems in Staining Techniques

An Editorial

During a review of numerous textbooks and publications on histologic technique, it became evident that the type of water (tap, distilled, demineralized, or de-ionized) to be used in staining procedures is frequently not mentioned. Too little attention has been devoted to this important aspect of preparing stains and solutions.

The concentration of hydrogen ions plays a significant role in staining reactions. Variations in this factor will alter the pH of a solution, especially if the preparation does not require the addition of an acid, metal, or base.

Table 1 illustrates the range in pH of tap and distilled water obtained during a 90-day period in our laboratories. Control of hydrogen ion concentration is a requirement for more definitive and differential staining reactions.

Dr. Ralph D. Lillie,[1] in his excellent book, suggests that when purchasing dyes, it is wise to specify the color index number (C.I. No.). He further provides the index number for the dye color for most of the methods he presents, and often the source of the dye. Unfortunately, this essential information has been omitted from some other authoritative texts. It is possible for inconsistent and even negative staining reactions to occur if dyes bearing identical names but different color index numbers are interchanged.

Table 1
Variation in Concentration of Hydrogen Ions in Distilled and Tap Water Over a 90-Day Period

Reading	Type of Water	pH
First reading	AD*	5.3
	Tap	6.8
2 weeks later	AD	5.1
	Tap	6.9
4 weeks later	AD	4.9
	Tap	7.2
3 months later	AD	4.4
	Tap	7.3
Over-all Comparative Variation		
	AD	4.4-5.3
	Tap	6.8-7.3

* AD=distilled water

Basic fuchsin of C.I. No. 42510 is required for the preparation of aldehyde fuchsin† to demonstrate Paget cells. Basic fuchsin of C.I. No. 42500 cannot be employed because the possibility of inferior results is markedly increased. In a personal conversation, Dr. Lillie disclosed that basic fuchsin (C.I. No. 42500) is unacceptable for staining acid-fast bacilli. He recommended C.I. No. 42510 instead. Likewise, methyl green (C.I. No. 52590) is the dye of choice in the methyl green-pyronin Y procedure to demonstrate desoxyribose and ribose nucleic acids.

In many instances dyes of the same name having variations in C.I. number, as well as dyes having the same C.I. number, produce variations in staining reactions. It is also useful to know the dye lot or batch number, in the event the technician encounters a poor dye with the proper C.I. number. These problems can be reduced or eliminated with the establishment of a simple 3x5" card file of salient information on various stains and C.I. numbers that by experience have proven best for given procedures (Table 2). This information will assist in insuring that the laboratory reorders the same item. This in turn provides a method for quality control of the staining method and will identify the dye with the technique.

Table 2
Dye Information Required

1. Dye:	Acid Fuchsin
2. Source:	Harleco (Cat. No. 218)
3. Dye content:	65%
4. C.I. No.	42685
5. Lot No.	648
6. Technique:	Van Gieson - Masson

Dye content is also an important aspect of tissue staining since it is possible to obtain a given dye with a wide range of dye contents. For example, acid fuchsin (C.I. No. 42685) ranges from 58-75%. Eosin varies from 90 to 94%, and pararosanilin (C.I. No. 42500) may be obtained in concentrations from 94 to 99%. Such differences are not rare. A study was conducted to reveal the importance of dye content. Acid fuchsin C.I. No. 42685 was selected for the survey. Lots of the dye from five manufacturers and distributors yielded concentrations of 58, 60, 65, 66 and 75%, respectively. The acid fuchsin dyes rated at 75 and 58% concentrations were selected. A one-tenth of 1% aqueous solution was prepared with each. The 75% solution has a pH of 2.9 and the 58% solution a pH of 4.6. The latter dye did not stain tissue sections. The former performed satisfactorily. The conclusion from this elementary study was that the pH is dependent upon dye content and was responsible for unsatisfactory staining when the 58% product was used.

Additional solutions, one-tenth of 1%, were prepared, and 1 ml of glacial acetic acid, ACS, was added to each. The solution with an initial dye content of 75% yielded a pH of 2.65. A pH of 2.75 was observed in the diluted, acidified 58% product. The staining results from both solutions were satisfactory and identical. The relationship of pH and dye content is obvious, important, and cannot be overlooked or disregarded. The variation in dye content and the pH of the staining solution may explain the varied staining results obtained in different laboratories even though identical staining procedures are employed.

Reference:

1. Lillie, R.D.: *Histopathologic Technic and Practical Histochemistry*, Third Edition, McGraw-Hill, New York, p. 36, 1965.

† Dr. Robert W. Mowry recently studied aldehyde fuchsin staining. This excellent article can be found in: *Stain Technology*, Vol. 53: No. 3, 141-153, 1978.

Technique to Prevent Collapse of Eye Globe and Detachment of the Retina During Processing

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The following technique was developed to prevent collapse of eye globes and detachment of the retina during processing. The procedure has not been attempted on human eye globes and therefore no comment will be made in that regard. It has been used extensively on dog and rabbit eyes with good results.

The rabbit eyes were fixed in 10% neutral buffered formalin and the dog eyes were fixed in Zenker's solution.

Technique:

1. After fixation, the eye is opened with a flat razor blade, starting several millimeters from either side of the optic nerve and passing through the cornea, just outside the limbus. The center portion containing the pupil and optic nerve is that portion used for processing.
2. If the pathology does not involve the vitreous humor, remove it by placing the eye in water.
3. Cut strips of paper* the length of the circumference of the eye and twice the thickness of the cut surfaces.
4. Fold the paper strip in half and roll around a pencil.
5. Place one end of the paper against the ciliary process and gently unroll the paper inside the eye. Trim off excess paper. Dip the eye in water to allow paper to soften and form against the inner wall of the eye. (Note: If retina is detached, attempt to tease in place before applying paper.)
6. Process eye in the conventional manner. Remove paper before embedding.

* The most satisfactory paper is bond, and that used for reproduction on xerox machines.

Improved Calcium Demonstration

An Editorial

The Von Kossa silver procedure for demonstrating calcium salts has been used extensively since 1901. The technique is excellent but the results are often variable because of several oversights on the part of many histology technicians. Some of the problems are:

1. Use of unbuffered formalin for fixation.
2. Use of artificial light for silver development.
3. Inadequate exposure of tissue sections to the silver solution.

The problem related to the use of unbuffered formalin is explained in another editorial appearing elsewhere in this issue of **Histo-Logic**. Therefore, no further comment on this subject will be made here.

The second problem relates to the use of artificial light (ultra-violet lamp or 100 watt bulb) instead of direct sunlight for development of the silver nitrate. Direct sunlight produces black silver deposits in a few minutes, while artificial lamps produce brown results after a prolonged period. One need not wait for a sunny day to take advantage of the results achieved by sunlight, since a cloudy day will produce the same results. However, the exposure period may have to be extended.

Inadequate exposure of the tissue to the silver nitrate solution is the third problem. Slides should be placed in the 5% silver nitrate solution in **direct sunlight** for 10-15 minutes. If the day is cloudy, slides should be exposed to the silver nitrate solution for 30 minutes. The Von Kossa method for calcium with appropriate modifications follows.

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Von Kossa Method for Calcium

Fixation:
10% buffered neutral formalin

Microtomy:
Cut sections at 6 microns

Solutions:

5% Silver Nitrate

Silver nitrate	5.0 ml
Distilled water	100.0 ml

5% Sodium Thiosulfate (Hypo)

Sodium thiosulfate	5.0 ml
Distilled water	100.0 ml

Nuclear Fast Red (Kernechtrot)
Dissolve 0.1 gm nuclear fast red in 100 ml of a 5% solution of aluminum sulfate with aid of heat. Cool, filter, add grain of thymol as a preservative.

Staining Procedure:

1. Deparaffinize and hydrate slides to distilled water.
2. Place slides in silver nitrate solution for 15 or 30 minutes (see introduction).
3. Rinse slides in distilled water.
4. Place slides in sodium thiosulfate solution for 2 minutes.
5. Rinse slides well in distilled water.
6. Counterstain slides in nuclear fast red solution for 5 minutes.
7. Rinse slides in distilled water.
8. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene, two changes each.
9. Mount coverslip with resinous media.

Results:

Calcium salts	- black
Nuclei	- red
Cytoplasm	- light pink

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The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Lee G. Luna, Editor, Histo-Logic, P.O. Box 36, Lanham, Maryland 20801. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.