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SAKURA

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Managing Editor, Theresa Ford
Scientific Editor, Vinnie Della Speranza, MS, HTL(ASCP)HT, MT

40 Years — A Remarkable Milestone

As 2011 draws to a close, most of us are likely distracted with deadlines coming due or projects just beginning. It seems that when one issue of this publication is nearing completion, I am already preparing for the next. I was recently reminded that this year, 2011, marks the 40th year that ***HistoLogic***® has been a voice of the histotechnology community through the generosity of Sakura Finetek USA today, and what was formerly the Lab-Tek Products division of Miles Laboratories, Inc. in years gone by.

HistoLogic was the vision and dream of Lee G. Luna, whose name may be familiar to many of our readers. Perhaps you have his textbooks on your shelf, likely tattered from many years of use at the bench, or some of the 150+ published papers he authored. You may not know that he worked tirelessly for histotechnologists everywhere, committed in his belief that the discipline and its practitioners needed a vehicle with which to communicate and share their knowledge with one another. To that end, Luna founded and became the first scientific editor of ***HistoLogic***, the first issue of which was published in 1971.

To put this in perspective, ***HistoLogic*** was founded before all but two of the state histotechnology societies in the United States and well before the creation of the National Society for Histotechnology (NSH). It was established at a time when there truly was no other means for histotechs to communicate or conferences for them to attend. As a result, Luna's dream, in the form of this bulletin, was realized. He served as this publication's first editor for 21 years. I can only speculate whether this publication contributed to the formation of the NSH, which was incorporated in 1973. I do believe that ***HistoLogic*** brought to histologists of the time a glimpse of what could be in the discipline. The coalition of histology practitioners that is now the NSH was the result of efforts by Luna and many others whose passion became the organization that serves our community of professionals today. Visit **www.nsh.org** to learn more about this organization and what it can do for you.

Over the years, ***HistoLogic*** has been received by more than one million readers from around the globe, largely the result of the generous contributions from so many authors who have offered the wealth of information contained within the ***HistoLogic*** archives (**http://www.sakura-americas.com/histologic/index.html**). I am humbled by the distinguished editors who have preceded me and by the privilege Sakura has entrusted in me to continue the rich legacy that was and is ***HistoLogic***.

I sometimes wonder if Luna would approve of today's version of his creation. I hope that he would.

If you are reading these words, I hope you will consider contributing to this publication's rich history by sharing your work or knowledge with others who read these pages. Many aspiring authors have had their first published article appear here. I'm sure all would tell you how gratifying it can be to see your work in print and to know that the information you share can help other histotechs in their work. If you have news to share but are not quite sure how to go about it, I hope you will contact me. I'm sure I can help you.

How many of you have that very first issue of ***HistoLogic*** still in your library, Vol. I, No.1 dated July 1971? If you do, I'd love to hear from you. Let's keep this rich tradition and valuable resource going strong for many more years to come!

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Optimization of Automated Staining Procedures for Gomori Iron and Schmorl Reaction for Lipofuscin Using the Sakura Tissue-Tek® Prisma® Stainer and Tissue-Tek® Glas™ *g2* Coverslipper

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Abstract

Gomori iron and Schmorl reaction are two special staining procedures routinely performed in the histology laboratory to demonstrate various pigments present in animal tissues being evaluated for preclinical toxicology studies. Gomori iron stain, commonly referred to as the Prussian blue reaction, is used to detect the presence of iron deposits (Fig. 1). Schmorl reaction stain, commonly referred to as the Turnbull blue reaction, is used to indicate sites of reducing activity, specifically lipofuscin. Although both stains are commonly performed manually, ie, without the use of automated tissue stainers, we have developed an optimized method for each stain to be performed using automated equipment that maintains staining quality, decreases the time required by a technician to perform this task, and is economical.

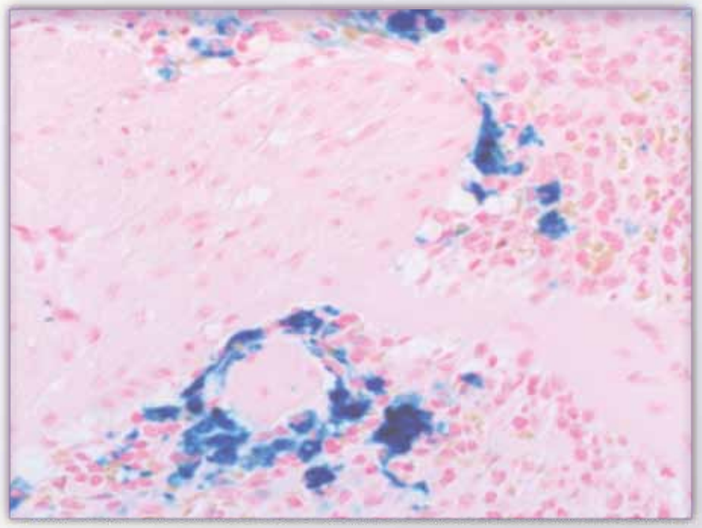

Fig. 1. Gomori iron stain of dog spleen by the automated method. Note the positive Prussian blue reaction of iron pigment. 20X

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Table 1. Automated Gomori Iron Staining Protocol

Step	Reagent Reservoir	Reagent	Time
1	Start	Program start	--:--:--
2	01	Xylene	3 minutes
3	02	Xylene	3 minutes
4	03	Xylene	3 minutes
5	09	100% ethyl alcohol	2 minutes
6	10	100% ethyl alcohol	1 minute
7	11	95% ethyl alcohol	1 minute
8	12	70% ethyl alcohol	1 minute
9	28	Distilled water	1 minute
10	17	20% HCl/ 10% $K_4[Fe(CN)_6]$	20 minutes
11	Wash	Running tap water	2 minutes
12	24	Nuclear fast red	2 minutes
13	Wash	Running tap water	2 minutes
14	12	70% ethyl alcohol	1 minute
15	11	95% ethyl alcohol	2 minutes
16	19	100% ethyl alcohol	4 minutes
17	18	Xylene	4 minutes
18	End	End station	--:--:--
19	Automatic transfer to the Tissue-Tek® Glas™ *g2* coverslipper		

Table 2. Automated Schmorl Reaction Staining Protocol

Step	Reagent Reservoir	Reagent	Time
1	Start	Program start	--:--:--
2	01	Xylene	3 minutes
3	02	Xylene	3 minutes
4	03	Xylene	3 minutes
5	09	100% ethyl alcohol	2 minutes
6	10	100% ethyl alcohol	1 minute
7	11	95% ethyl alcohol	1 minute
8	12	70% ethyl alcohol	1 minute
9	28	Distilled water	1 minute
10	17	1% $FeCl_3$/ 1% $K_3[Fe(CN)_6]$	5 minutes
11	Wash	Running tap water	2 minutes
12	24	Nuclear fast red	5 minutes
13	Wash	Running tap water	2 minutes
14	12	70% ethyl alcohol	1 minute
15	11	95% ethyl alcohol	2 minutes
16	19	100% ethyl alcohol	4 minutes
17	18	Xylene	4 minutes
18	End	End station	--:--:--
19	Automatic transfer to the Tissue-Tek® Glas™ *g2* coverslipper		

Introduction

Gomori iron and Schmorl reaction stains are often requested on animal tissues (most commonly in the spleen and liver) for investigational new drug (IND) studies. We developed automated methods for these staining procedures in order to: (1) produce staining results comparable to the quality of our current manual methods, (2) run simultaneously with our routine hematoxylin and eosin (H&E) staining protocol to ensure no loss in production time of study slides, (3) be economically feasible, and (4) reduce staining variability experienced when manual staining is performed by a variety of technicians. We also evaluated the staining quality and cost (time and materials) of slides produced manually compared to slides produced utilizing the Sakura Tissue-Tek® Prisma® automated slide stainer and the Sakura Tissue Tek® Glas™ *g2* coverslipper (Sakura Finetek USA, Inc., Torrance, CA).

Materials and Methods

Stock samples of dog spleen and liver were fixed in 10% neutral buffered formalin (NBF) for approximately 48 hours. The tissues were processed overnight using a Sakura Tissue-Tek® VIP E300™ processor (Sakura Finetek USA, Inc.), embedded in paraffin blocks, and sectioned at 4 microns on an automated rotary microtome. The slides were dried in a slide dryer at approximately 60°C for 1 hour.

Our Gomori iron staining method utilizes 10% potassium ferrocyanide and is counterstained with 0.1% nuclear fast red (kernechtrot). Our method for the Schmorl reaction stain for lipofuscin combines 1% ferric chloride and 1% potassium ferricyanide into a working solution and is counterstained with 0.1% nuclear fast red (kernechtrot). All reagents were purchased commercially from Poly Scientific R&D Corp (Bayshore, NY) for staining consistency.

Manual Method

For each staining procedure, reagent containers were filled with approximately 500 mL of the appropriate reagent and placed under a ventilated hood. For each manual staining run, 2 slide racks containing 20 slides each were moved from one staining container to the next, with each slide rack remaining in the container for the appropriate time as determined by the specific manual staining protocol. The technician manually moved the slide racks to each container or washing station and provided agitation (as required) by moving the slide racks up and down in the staining container. Slides were coverslipped using the Tissue-Tek® Glas™ *g2* automated coverslipper.

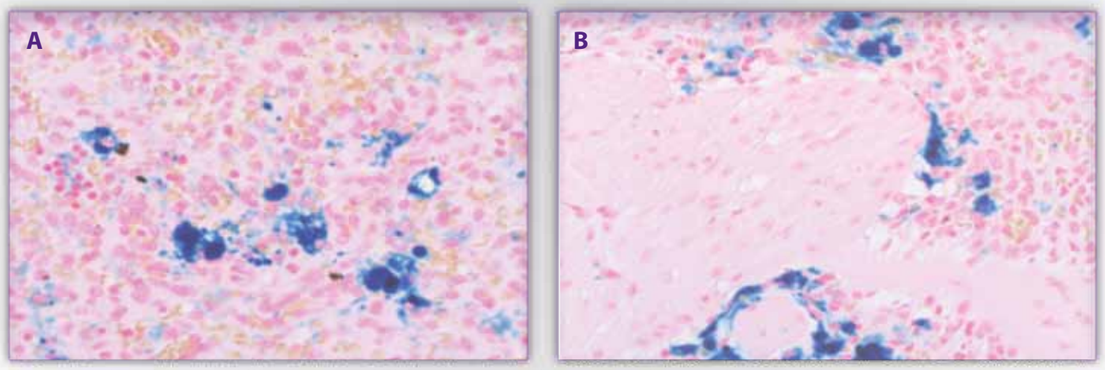

Fig. 2. Comparison of the manual (A) and automated (B) Gomori iron stain of dog spleen indicates no discernible difference in staining quality between the 2 staining methods. Note the positive Prussian blue reaction of iron pigment with both methods. 20X

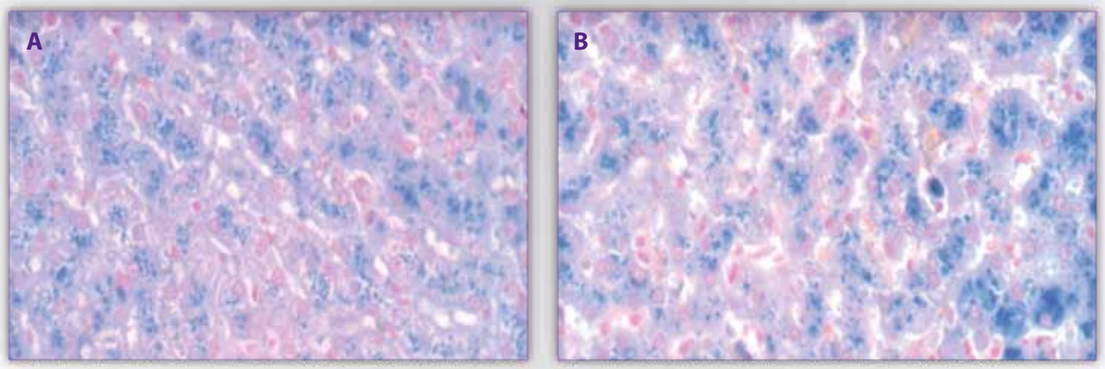

Fig. 3. Comparison of the manual (A) and automated (B) Schmorl reaction stain for lipofuscin in dog liver indicates no discernible difference in staining quality between the 2 staining methods. Note the positive Turnbull blue reaction of the lipofuscin with both methods. 20X

Automated Method

Separate staining protocols (Tables 1 and 2) were programmed into the Tissue-Tek® Prisma® stainer using an H&E compatible standard configuration. The mix was set to agitate the reagents for all stations for both staining protocols. Heating and drying stations on the stainer were not used. Standard reservoirs were filled with approximately 700 mL of each reagent with the exception of the working solutions of potassium ferrocyanide/hydrochloric acid (Gomori iron) and ferric chloride/potassium ferricyanide (Schmorl reaction). Approximately 500 mL of each of the working solutions mentioned above was used to prevent total submersion of the metal hangers on the slide basket adaptors in the solution—it is important to prevent chemical reactions between those reagents and the metallic hangers. Tap water was used following the working solution of potassium ferrocyanide/hydrochloric acid for both the manual and automated Gomori iron staining procedures. The stainer was loaded with 120 slides and the appropriate program selected. Additional slides were loaded and the routine H&E program was selected to run concurrently with either the Gomori iron or Schmorl reaction stains. Slides were coverslipped using the Tissue-Tek® Glas™ *g2* automated coverslipper.

Results

Slide Quality

Two board-certified veterinary pathologists microscopically compared slides stained manually with slides stained using the automated Gomori stain for iron and the Schmorl reaction for lipofuscin. The slides stained via both automated methods were equivalent in staining quality to slides stained manually (Figs. 2 and 3). The results demonstrate that the structures of interest stained appropriately with both automated procedures.

Time Savings

The manual staining method for the Gomori iron and Schmorl reaction requires approximately 2 hours of a technician's time for preparing reagents and doing the numerous steps involved, including filling reagent containers, manually performing the stain, and transferring the slides to and from the coverslipper. That leaves limited time during the 2 hours to do other laboratory tasks. In contrast, the automated procedure utilizes approximately 30 minutes of a technician's time, which includes filling reagent containers, starting the automated program, and removing the slides from the coverslipper. Once the staining program is started on the automated stainer, the technician can walk away from the machine to perform other tasks. Additionally, there is no loss of time in completing routine H&E staining of study slides since the Gomori iron and Schmorl reaction stains can run concurrently with the H&E program, resulting in overall increased laboratory efficiency.

Cost Savings

The cost of some of the reagents (10% potassium ferrocyanide, 1% ferric chloride, and 1% potassium ferricyanide) used in the manual method was greater (per 120 slides) than the cost of reagents in the automated method, which allowed for the greatest cost savings (Tables 3 and 4). All of the staining reagents could be used for at least 120 slides in the automated method, with the stain quality of the last slide comparable to the quality of the first slide. The 10% potassium ferrocyanide utilized in the Gomori iron manual stain and the ferric chloride/potassium ferricyanide working solution used in the manual Schmorl reaction stain were discarded after each manual staining run of 40 slides.

Additional cost savings for both methods can be achieved by preparing the 10% potassium ferrocyanide, 1% ferric chloride, and 1% potassium ferricyanide solutions in-house rather than purchasing the reagents commercially.

The cost savings for the automated staining method versus the manual staining method for the Gomori iron stain and Schmorl reaction stain were $26 and $40, respectively, per 120 slides. In terms of reagent cost, the savings seem minimal; however, the biggest cost savings are realized in technician time as a result of increased productivity, which makes it highly advantageous to use the automated special staining procedures rather than the manual procedures for both stains.

Table 3. Cost Comparison: Manual vs Automated Staining for Gomori Iron

Reagent	Manual (per 120 slides)	Automated (per 120 slides)
10% potassium ferrocyanide	$62	$31
Nuclear fast red	$88	$105
100% ethyl alcohol	$11	$10
95%/70% ethyl alcohol	$13	$6
Xylene	$39	$35
Total costs	**$213**	**$187**

Table 4. Cost Comparison: Manual vs Automated Staining for Schmorl Reaction

Reagent	Manual (per 120 slides)	Automated (per 120 slides)
1% ferric chloride	$68	$34
1% potassium ferricyanide	$23	$12
Nuclear fast red	$88	$105
100% ethyl alcohol	$11	$10
95%/70% ethyl alcohol	$13	$6
Xylene	$39	$35
Total costs	**$242**	**$202**

Summary

Improved efficiency, productivity, and streamlining of tasks are advantageous in today's highly competitive workplace. The automation of these special stains in our laboratory resulted in increased productivity by reducing technician time required to perform each stain. Automation also yielded a reduction in reagent costs. In addition, the automated procedure for the Gomori iron and Schmorl reaction stains on the Sakura Tissue-Tek® Prisma® stainer produced slides that were comparable to those obtained by the manual staining method with improved consistency from batch to batch. Because the stainer can accommodate concurrent runs of the H&E and either of the special stains, production/output of study slides is also increased. As laboratory workloads increase, with an increased emphasis on rapid, reliable, and consistent quality, automated special staining is one more way to efficiently manage workflow.

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Fast Green FCF or Ehrlich's Hematoxylin as Counterstain to Periodic Acid-Schiff Reaction: A Comparative Study

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Abstract

Counterstains have always been employed to provide background contrast during histological and histochemical studies. In this study, two counterstains for the periodic acid-Schiff (PAS) reaction were compared—fast green FCF and Ehrlich's hematoxylin. The fast green FCF counterstain provided a sharper contrast between PAS-positive structures and PAS-negative structures than the Ehrlich's hematoxylin counterstain, but Ehrlich's hematoxylin enhanced the cell nucleus morphology and position. This work offers support for the use of Ehrlich's hematoxylin in the study of cell nucleus morphology.

Introduction

The morphology and histology of some species of teleost fish digestive tract have been studied and reported in the literature.[1-3] The histochemistry of the mucous layer has also been researched in several fish species. The investigations revealed that the mucus-secreting structures of the alimentary canal include intestinal goblet cells, surface epithelia, and some glands.[4-9]

The PAS reaction is a useful indicator of the presence of carbohydrate (mucin and glycogen) in the tissues. The principle of the reaction is that periodic acid will bring about oxidative cleavage of the carbon-to-carbon bond in 1,2-glycol or their amino or alkylamino derivatives to form di-aldehydes. These aldehydes will react with fuchsin-sulfurous acid, which combines with the basic pararosaniline to form a magenta-colored compound.[10]

While most research with the PAS reaction in fish has employed either fast green FCF or Harris hematoxylin as the counterstain, there is little information on the nature of the PAS reaction counterstained with Ehrlich's hematoxylin. Therefore, in this study we investigated the use of Ehrlich's hematoxylin as well as fast green FCF as counterstains to the PAS reaction on sections from the digestive tract of adult African catfish (*Clarias gariepinus*). The result of this study will fill the knowledge gap and help clinicians in diagnosing fish diseases related to carbohydrate absorption and storage.

Materials and Methods

Ten adult African catfish (*C gariepinus*) of both sexes weighing 41.1 g, with an average length of 21.2 cm, were used in this study. The fish were obtained from Chris Farm—a commercial aquaculture in Umuahia town of southeastern Nigeria. The fish were killed by decapitation and the digestive tracts were quickly removed. Samples of esophagus, stomach, and intestine were fixed in 10% formalin saline. Tissues were dehydrated through graded ethanol, cleared in xylene, and embedded in paraffin wax. Two 5 µm sections from all samples were stained with PAS. One slide was counterstained with fast green FCF, while the other was counterstained with Ehrlich's hematoxylin.[11,12] The stained sections were examined with an Olympus microscope, and micrographs were taken using the Scope Image model ADC microscope.

Results

The emphasis in this study was to compare the tinctorial effects of using fast green FCF or Ehrlich's hematoxylin as counterstains to the PAS reaction. In the fast green FCF counterstained slides, the PAS-positive entities were sharply contrasted. These structures include the mucus-producing cells of the esophageal epithelia (Fig. 1), columnar cells of the stomach mucosa (Fig. 3), and intestinal goblet cells (Fig. 5).

In the Ehrlich's hematoxylin counterstained slides, the aforementioned PAS-positive entities were demonstrated but not as sharply contrasted as in the fast green FCF counterstained slides. The Ehrlich's hematoxylin was picked up by the nuclei of esophageal club cells (Fig. 2), stomach oxyntopeptic secretory cells (Fig. 4), and the nuclei of intestinal epithelial cells and lymphocytes that infiltrated the entire digestive tract (Fig. 6).

Conclusion

In this study, counterstaining PAS with Ehrlich's hematoxylin enhanced the nuclear morphology of the cells, while localization of PAS-positive entities was accentuated with the fast green FCF counterstain. As a result, Ehrlich's hematoxylin can best be utilized in light microscopy studies that use nuclear morphology and the presence of PAS-positive entities as tools for histological and pathologic analysis of organs and tissues.

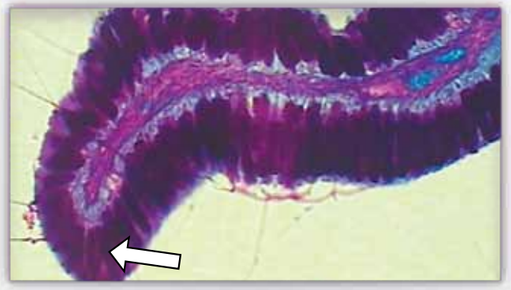

Fig. 1. Section of the esophagus. PAS-positive entities (white arrow). PAS/fast green FCF, 400X

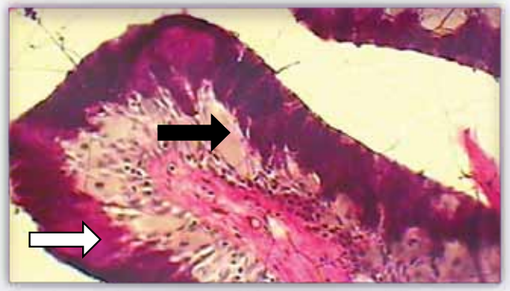

Fig. 2. Section of the esophagus. PAS-positive entities (white arrow); club cells showing clear nuclear morphology (black arrow). PAS/Ehrlich's hematoxylin, 400X

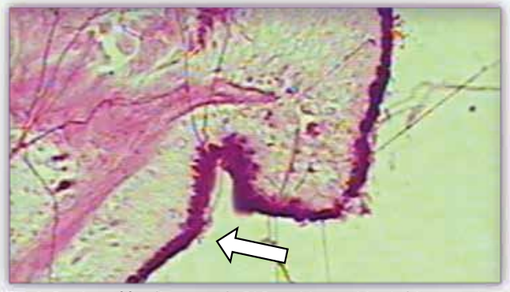

Fig. 3. Section of fundic stomach. PAS-positive entities (white arrow). Note the absence of clear nuclear morphology in the cells of the tunica mucosa. PAS/fast green FCF, 400X

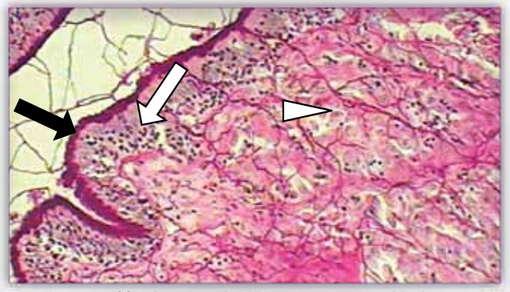

Fig. 4. Section of fundic stomach. PAS-positive entities (black arrow); nucleus of oxyntopeptic cell (arrowhead). Note the clarity of the nuclear morphology of the intraepithelial lymphocytes (white arrow). PAS/Ehrlich's hematoxylin, 400X

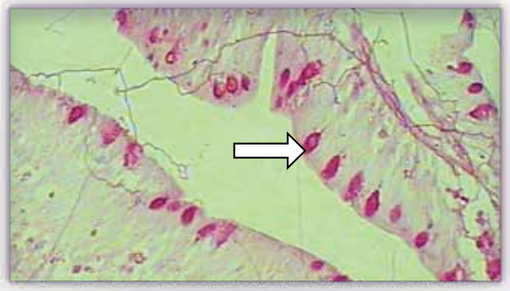

Fig. 5. Section of middle intestine. PAS-positive entity (white arrow). PAS/fast green FCF, 400X

Fig. 6. Section of middle intestine. PAS-positive entities (white arrow); nucleus of epithelia columnar cell (black arrow); nucleus of lymphocyte (arrowhead). PAS/Ehrlich's hematoxylin, 400X

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A Practical Roadmap for Adopting Rapid Tissue Processing (RTP) to Meet the Changing Needs of the Healthcare Workplace (Part 2)

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In adopting RTP, the vision for change must be well communicated to all the people involved and a compelling case for a workplace transformation has to be made to all stakeholders. Finally, the commitment has to be made to purchase a rapid tissue processor. There is still much planning and preparation before a go-live date can be set and there is ample opportunity to set up your organization to be successful. The most general concept to understand at this stage is that the change process needed to transform a traditional linear workflow into a one-piece flow will require a series of phases. Each phase will take some time. Depending on your organization's capacity to be change-hardy, some of the phases will require a considerable length of time. Skipping a phase, or not recognizing and respecting the length of time needed, creates only the illusion of speed. It does not produce a satisfying result; in fact, it can have a devastating impact on your organization's transformation. At worst, it can seriously hamper your progress and, at best, it can slow down your progress toward complete transformation. This next series of recommendations for planning is a compilation of successes experienced by RTP users from multiple sites.

Planning For and Creating Short-Term Wins

Recommendations from experienced RTP users:

- Consider requesting an on-site instrument demonstration. Work with the vendor to design simulated actual work activities that allow staff members to experience hands-on specimen receipt, handling, grossing, fixation, and processing steps. Plan to carry the embedded cassettes throughout the cutting, staining, and labeling process so that pathologists and histologists can clearly visualize the entire process. Having access to microscopes to assess the quality of the stained slides is critical. Building an evaluation form (Fig. 1) that allows everyone to make notes of their impressions of this proposed workflow will give valuable data for the future success of the project.
- It is necessary to consider planning for the on-site demonstration to test the workflow and to assess the end-product results.
- Data, data, and more data must be obtained from pathologist evaluations and approvals. Building a data method and a form (Fig. 2) to capture all the critical information will allow decisions based on facts rather than perceptions.

PINK VS YELLOW/ORANGE				
CASE #	TISSUE	COMMENT PINK SLIDE	COMMENT YELLOW/ ORANGE SLIDE	COMMENT ON DIAGNOSTIC VALUE
	MUSCLE LARGE			
	MUSCLE SMALL Bx			
	SKIN ELLIPSE			
	SKIN PUNCH			
	PROSTATE CORE			
	LUNG LARGE			
	TRANSBRONCH			
	MYOMETRIUM			
	LEIOMYOMA			
	COLON			
	FAT			
	OVARY CYST WALL			
	TURBT			
	KIDNEY CORE Bx			
	KIDNEY			
	KIDNEY			
	SKIN PUNCH			
	FAT SUBCUT			
	LUNG LARGE			
	TRANSBRONCH			
	PLACENTA			
	"MEMBRANES, CORD"			
	BREAST			
	BREAST CORE Bx			
	SKIN PUNCH			
	KIDNEY			
	KIDNEY CORE Bx			
	KIDNEY TUMOR		can you grade tumor?	

Fig. 1. Sample worksheet for comparing conventional processing with rapid tissue processing.

NAME OF YOUR INSTITUTION HERE

Please use one form for each case

Case # and block IDs

Run # _____ # of blocks on run _____Date processed _____

Immersion time per station _____ Time in formalin_____

Describe grossing method (fork, cutting board, thickness)

Time in molecular fix before grossing _____ Time in molecular fix after grossing_____
Time in pre-processing before grossing _____ Time in pre-processing solution _____
Other notes (reprocessed, decal, etc)

Embedding notes

Microtomy notes

Pathologist notes/requests (reprocess, restain, specials, immunos, etc)

Fig. 2. Sample rapid tissue processing report for documenting grossing method, embedding and microtomy notes, and pathologist evaluation.

- Think through what information will be needed for the validation studies and the documentation required. If you can capture some of these parameters and data during the on-site evaluation, you will have a more precise idea for future implementation planning.
- Plan for ancillary testing for immunohistochemistry (IHC), special stains, and any other additional testing procedures your lab routinely performs. This will require selecting some high-volume stains and/or choosing stains that are known to be challenges for consistent, high-quality staining.
- Staff the lab to allow techs to get time off the bench so they can be involved and invested in the on-site demonstration of the instrument. This allows your front-line experts to weigh in on any adjustments or changes to your workflow.

A Failure to Plan Is a Plan for Failure

Any project's success, whether it's a plumbing project in your home or a sweeping transformation project within the workplace, carries significant risk for failure. Stating broadly defined goals is useful, but can become more effective when they are prominently posted and constantly communicated throughout the workplace. Such goals do not have to be complex or use highly technical jargon. In fact, simple goal statements carry more weight when everyone within the team can identify with the goal itself and their own individual role in achieving it. Here are two specific examples of goal statements:

- "Provide same-day surgical pathology service for 80% of all cases within the next 6 months."
- "Improve overall surgical pathology turnaround time (TAT) to less than 6 hours for 90% of dermatologic cases submitted by 10 AM each workday."

The common thread between these 2 simple statements is that they are **S**pecific, **M**easurable, **A**ction-oriented, **R**ealistic, and have a **T**ime frame. This SMART acronym is widely described and implemented in the world of project management.

Employees cannot hit the target unless they can see it. Goal setting affects performance by focusing behavior in the defined direction (ie, task actions that directly contribute to improving TAT). It can energize behavior by motivating people to put forth effort to reach difficult (but not impossible) goals.

Key elements for project success require that you "begin with the end in mind"[1]:

1. Set a target date for start-up (a date by which the lab will complete the transition from a linear work process to RTP).
2. Define the individual discrete work tasks that will be included in the transformation to RTP (Fig. 3). Think about each work task as being its own individual package, within which are steps or milestones to be accomplished.
3. Assign your resources to each discrete work task; resources may include people, materials, or methods.
4. If appropriate to the size of the lab operation, consider using project management software and techniques to build your case and to design your data collection tools. For example, use a "Project Who Does What When Report" or a Gantt chart to demonstrate your timeline (Fig. 4).
5. Involve stakeholders in the planning process for testing, validation, documentation, and data collection and analysis.

EXAMPLES OF DISCRETE WORK TASKS	
Facilities	Microtomy
Staffing	H&E stains and protocols
Tissue processing	Special stains
Vendor technical support	Instrument cleaning and maintenance
Special tissue handling	Block transport
Grossing artifacts	Immunohistochemistry
Special biopsy handling	Workflow issues
Requisitions	Supplies management
Training	Delivery and installation
LIS issues	Long-range planning
Cassette labeler	Glass slide etcher
Surgical Pathology reports flow	

Fig. 3. Examples of discrete work tasks that the implementation team must address throughout the transition.

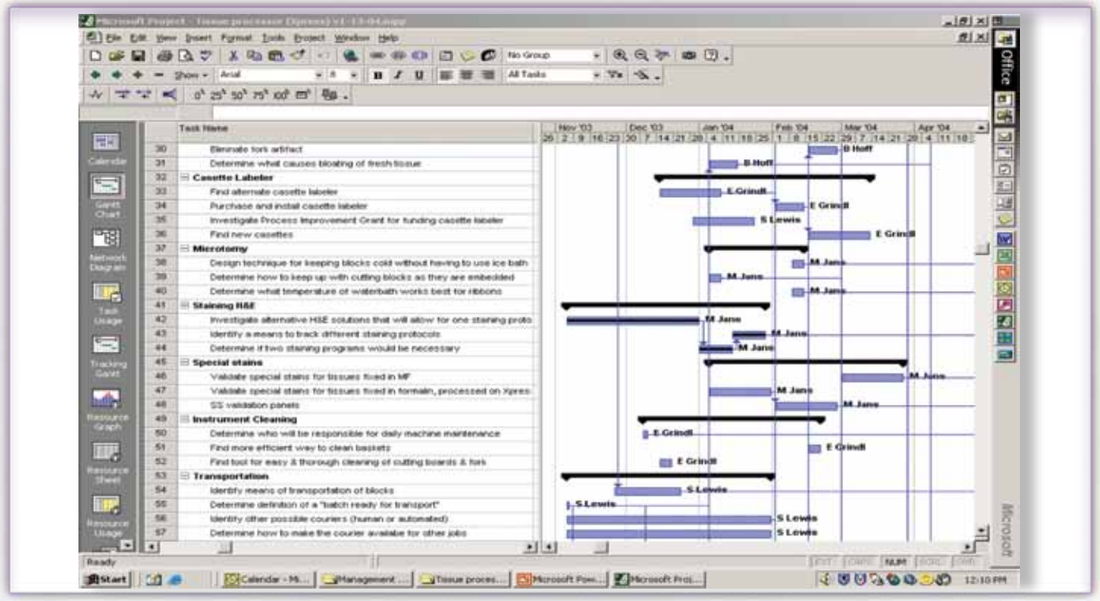

Fig. 4. A sample Gantt chart that illustrates who is responsible for each discrete work task and its time frame for completion.

Devising Opportunities to Realize Success

The transition from linear workflow to RTP takes time and considerable planning to execute smoothly. It is understandable that, while pathologists, managers, and staff members become very focused on the discrete work tasks needed for the conversion, it is also necessary to revisit the big-picture statement. It is important to recognize that all the effort and energy cannot go on indefinitely without some concrete evidence of everyone's contribution to the stated goals presented. There can be a risk of losing momentum if there is no formal recognition and celebration when goals are achieved; people can become overwhelmed and give up. Worse, they may revert to being change-resistant. Either way, hard-fought gains can be quickly lost. Strategies to recognize and reward the entire group must be built into the overall transition plan at the outset.

Defining short-term wins is different from the more global goal of improving patient case TAT. Managers or other change leaders, typically a lead pathologist, can build ways to recognize and celebrate the journey toward RTP as part of project management. Such celebrations can keep morale high while encouraging the process transformation. Often laboratorians devote themselves and their work energy toward getting the workload out, which they do extremely well. What is sometimes omitted, however, is planning for the opportunity to stop and celebrate accomplishments. Here are some specific examples of ways in which a lab staff workgroup can plan for these types of events:

- Conduct a fun event for naming the rapid tissue processor. Have staff members submit ballots to nominate a name for the instrument. Post the nominations and have everyone vote for a favorite. Design a name pin for the instrument, possibly using the institution's logo, and then "pin" the instrument.
- Have a go-live celebratory event, perhaps a potluck meal, where treats are provided.
- Have the pathologists sign a "Congratulations on the New Arrival" card for the staff; decorate the break room with a baby shower-type theme.
- Nominate staff members for any appropriate institutional awards—remember to include not only the team directly involved in all the planning and go-live activities but also the front-line staff members who remained at the bench producing the day-to-day work.

Unforeseen Complications (Also Known as the School of Hard Knocks)

Expect to encounter issues and problems along the road to your transformation that will require additional time, temporary reallocation of staff, and sometimes additional money. Even the most carefully planned projects run into delays, or more often require a course adjustment to the original action or go-live date. The capacity to keep focused on the original goals and to weigh competing priorities or interruptions can allow you to make progress—even though progress may sometimes occur in small or incremental gains. Networking with sites that are already up and running with RTP can become your greatest resource. Table 1 describes various unforeseen issues that were compiled from multiple sites, and includes a brief description of the resolution.

		0:00													
Accession #	Date Accessioned	Time Accessioned	Date Onto Processor	Time Onto Processor	Date to Hot Seat	Time to Hot Seat	Date Signed Out	Time Signed Out	TAT From Accessioning to Processor	TAT From Time Onto Processor to Hot Seat	TAT From Hot Seat to Verified	TAT From Accessioning to Time Signed Out		Number of Cases	AVG TAT From Accessioning to Processor	AVG TAT From Accessioning to Time Signed Out
	5/28/04	10:02	5/28/04	13:40	5/29/04	10:32	6/1/04	13:26	3.63	20.87	74.90	99.40				
	5/28/04	10:20	5/28/04	13:40	5/29/04	9:19	6/4/04	16:09	3.33	19.65	150.83	173.82				
	5/28/04	10:43	5/28/04	12:15	5/28/04	15:58	5/28/04	16:34	1.53	3.72	0.60	5.85				
	5/28/04	10:47	5/28/04	16:00	5/29/04	11:18	6/1/04	13:05	5.22	19.30	73.78	98.30				
	5/28/04	10:49	5/28/04	12:15	5/28/04	15:58	6/2/04	8:00	1.43	3.72	112.03	117.18				
	5/28/04	10:52	5/28/04	12:30	6/1/04	14:10	6/1/04	14:45	1.63	97.67	0.58	99.88				
	5/28/04	10:54	5/28/04	13:30	6/1/04	10:27	6/1/04	14:33	2.60	92.95	4.10	99.65				
	5/28/04	11:04	5/28/04	12:35	5/28/04	15:58	6/1/04	15:30	1.52	3.38	95.53	100.43				
	5/28/04	11:19	5/28/04	13:40	5/29/04	10:32	6/1/04	13:28	2.35	20.87	74.93	98.15				
	5/28/04	11:27	5/28/04	12:30	5/29/04	10:32	6/1/04	13:31	1.05	22.03	74.98	98.07				
	5/28/04	11:29	5/28/04	13:40	5/29/04	10:32	6/1/04	13:32	2.18	20.87	75.00	98.05				
	5/28/04	12:04	5/28/04	13:50	5/29/04	10:44	6/1/04	12:57	1.77	20.90	74.22	96.88				
	5/28/04	12:08	5/28/04	13:50	5/29/04	10:44	6/1/04	12:57	1.70	20.90	74.22	96.82				
	5/28/04	12:45	5/28/04	16:00	5/29/04	11:18	6/1/04	13:06	3.25	19.30	73.80	96.35				
	5/28/04	13:14	5/28/04	16:40	5/29/04	10:44	6/1/04	12:58	3.43	18.07	74.23	95.73				
	5/28/04	13:23	5/28/04	16:40	5/29/04	10:44	6/1/04	17:48	3.28	18.07	79.07	100.42				
	5/28/04	13:25	5/28/04	14:40	5/29/04	10:44	6/1/04	12:59	1.25	20.07	74.25	95.57				
									5.03	9.72	24.11	38.86				
									2.60	4.18	20.53	30.25				
					0:00	19:52				Number of cases:	AVG TAT from Accessioning to Processor	AVG TAT from Accessioning to Time Signed Out				
										75	5:03	9:72				

Fig. 5. Sample turnaround report to illustrate real gains achieved (if any) during implementation of rapid tissue processing.

Launching the Go-live for a Seamless Introduction

All the hard work for the demonstration, purchase, and validation studies are completed. The actual launch for RTP is now imminent. Everyone wants a seamless transition, and now is the time to plan for the communication of critical information to all persons involved in the process change. Standard tools such as simple TAT reports (Fig. 5) can effectively describe a typical workday. These can be designed by accessing workload reports within your laboratory information system or by designing graphs and charts using standard Excel or Access software. Reporting TAT on a regular basis, either daily, weekly, or monthly, gives valuable information that can be used to track specimens by service (eg, neuropathology or cardiology) or by type (eg, dermpath or gastrointestinal clinic). This information can be used for trending; for example, renal pathology specimens requiring fast TAT for assessing transplant rejection status demonstrate a better record with timeliness. Further investigation may show that those clinicians have additional transport staff who can deliver specimens to the lab in a more timely fashion.

Table 1. Issues, Resolutions, and Outcomes

Issue	Proposed resolution	Outcome
Ink used in current cassette labeler washed off the cassettes.	Try different inks. Allow ink to fully dry before immersion into reagents.	Bought a new cassette labeler with reagent-resistant imprint.
Existing cutting (dissection) tools do not support the thin tissue sections needed for successful RTP.	Try re-education and retraining of gross dissection personnel. Find a grossing staff member who has the right technique; have that person instruct and train. Evaluate grossing tools that are designed to produce thin tissue sections.	Use vendor expertise to re-educate and retrain in-house grossing staff. Purchase newer grossing tools.
The rapid tissue processor has a footprint that cannot be fit into the lab space.	Enlist lab staff to redesign existing work space to reconfigure current instruments and workflow. Consider removing existing conventional tissue processors to a remote location.	Moved existing equipment to different locations within the lab. Redesigned an ancillary work space to incorporate RTP into its own work cell.
Existing electrical and plumbing drain configuration do not support the rapid tissue processor.	Use lab space redesign to locate other possible electrical or plumbing support.	Upgrade facilities to meet the needs of RTP.
Changes to workflow and the transfer/handling of work product require that embedding stations or stainers be repositioned.	Use workflow tools, if appropriate to the size and scope of the lab operation, to document the path of workflow (eg, spaghetti diagrams), measuring time intervals when the specimen "waits."	Use this information to determine the best placement of support instruments, such as embedding stations or automated stainers and coverslippers.
Information Technology Systems workflow (eg, worksheets, logs, etc) does not meet the needs of RTP.	Consider redesigning worksheets and logs. Consider whether specimen tracking systems could aid in a seamless work process.	
RTP workflow requires different staffing or work schedules, such as longer hours of operation or on-call availability.	Define the lab operational needs, and then allow staff members to make suggestions for changes in work assignments or schedules.	Adopt temporary changes to work schedules. Collect data that can be analyzed and used to either support the changes or to try an alternative.

CASE # AND BLOCK IDs	TISSUE TYPE	TIME ON FORMALIN	MOL. FIX TOTAL OR BEFORE/ AFTER GROSS	MINUTES IN PREPROCESS SOLUTION	MINUTES PER PROCESSING STATION	# OF BLOCKS RETRACTED/ TOTAL # BLOCKS	TISSUE THICKNESS/ GROSSING TECHNIQUE	OTHER INFO
S03- A1-9	femoral head	24 h	60 m	30	30	0/9	2.5	decal-12 h
S03- B1-10	femoral head	24 h	25 m	30	30	1/10	2.5	decal-18 h
S03- A1-8	liver	2-3 d	15 m	30	30	0/8	2.0 board	
S03- A1-7	placenta	3 d	30 m	30	30	0/7	1.5	
S03- B1-7	fat and skin	0	30 m	30	30	2/7	1.5 fork	
S03- A1-22	mass	3 d	20 m	30	30	1/22	1.5	
	kidney	0	25 m	15	15	17/17	2	reprocess 12 blocks (halved)
	kidney	0	25 m	20	30	19/24	2	
	tonsil	0	30 m	15	15	6/6 min	1.5	cut bad
	gallbladder	0	20 m	15	15	3/3	1.5	very retracted
	placenta	1 h	15 m	15	15	0/4	1.5	aborted run after 18 minutes
	placenta	1 h	15 h	30/15	15	2/4	1.5	
	myoma	0	16 h	0	15	5/5 min	1.5	
	myoma	0	15 m/16 h	30 m/15 m	15	5/7	1.5 board	
	kidney cores				15	0/3		
	gallbladder	overnight	15 m	15	15	3/3	1.5	
	kidney	overnight	20 m	15	15	3/7	1.5	
	kidney	overnight	15 m	15	15	4/9	1.5	
	abd. mass	0	12 h	30	30	0/1	1.5 board	good
	abd. mass	0	15 m	20	15	6/6		
	abd. mass	0	3 d	15	15	4/5	1.5	
	colon	overnight	20 m	15	15	0/10		millipore on biopsies is gone
	colon	overnight	20 m	15	15	0/10	1.5	
	neuro mass	0	16 h	30	15	3/3	1.5	
	neuro mass	10 m	20 m	30	15	2/3	1.5 board	
	neuro mass	0	16 h	30	30	3/4		biopsy was ok
	neuro mass	0	16 h	15	30	1/3	1.5 board	best of lot
	neuro mass	10 m	15 m	15	15	3/5	1.5	

Fig. 6. Sample case data summary sheet provides critical information at a glance for each case.

ACCESSION NUMBER

Please use one form for each case

Time grossing is complete

TIME STAMP WILL GO HERE

Time case is picked up for 1-2 hours of formalin fixation

Time onto Xpress processor

This form should accompany the paper work until the case is delivered to the hot seat. Time stamp in Histology and return to (person's name)

Comments:

ACCESSION NUMBER

Time grossing is complete

TIME STAMP WILL GO HERE

Time case is picked up for 1-2 hours of formalin fixation

Time onto Xpress processor

This form should accompany the paper work until the case is delivered to the hot seat. Time stamp in Histology and return to (person's name)

Comments

Fig. 7. Sample case tracking form provides data for sample turnaround time assessment.

Try to discover the reasons for success, in addition to the reasons for failure. Remember to look back to see what improvements have been sustained over a longer time interval. Think about assessment methods that could measure the effect on patient care and clinician satisfaction with RTP. And remember to plan for, and celebrate, accomplishments that become apparent through analysis of the information.

Efficient Problem Reporting, Immediate Resolution, and Communication to All Stakeholders

Inevitably, there will be problems reported with tissue fixation, cutting, and/or staining artifacts. Laboratorians are skilled problem solvers and are often able to resolve issues quickly and efficiently. However, a critical part of launching any new initiative requires the documentation of problems reported, the steps used to resolve the problems, and documentation of adequate acceptable resolution. Although designing yet another form to track such occurrences may appear onerous, the ability to track and trend problems allows everyone to then use a standard series of steps to solve similar problems. It also promotes confidence from the customer—whether pathologist or clinician—to view the lab staff as reliable, knowledgeable professionals who can deliver a quality work product. Simple documentation forms that give the pertinent details about the problem and are easy for the pathologist or histologist to use can become invaluable sources (Figs. 6 and 7).

Sustaining the New Initiative

There can be much excitement when launching any new initiative or program, and that in itself can carry a significant momentum. Difficulties often arise when the first blush of excitement fades and staff become accustomed to the new workflow. Initial benefits, such as schedule changes and case sign-out, become the norm and people focus once again on just getting through the workload each day. Some strategies for maintaining the forward motion may include:

1. **Continue to share the vision:** revisit the initial goals that were set. Ask whether it is now time to readjust those goals to meet even higher standards of service or patient care. If it is determined that new goals should be set, consider assigning this task to staff members who may now be supportive of going to the next level.
2. **Document successes:** review the milestones achieved and present the results using a timeline covering weeks or months. If appropriate, tell meaningful and vivid stories that relate directly to improvement in patient care, and then formally recognize the individuals who contributed toward a specific success.
3. **Respond quickly to problems** and show progress toward resolutions. Continue to catalog problems reported with specimens undergoing RTP. Consider graphing the reports and posting them daily in a lab work area so that both pathologists and lab staff can readily view them.
4. **Take advantage of opportunities within your lab staff** to build change-hardy work groups—new employees and those who can be promoted will offer the enthusiasm to form a cohesive staff effort to promote new changes.

Conclusion

Link the vision to RTP and support the lab staff and pathologists in achieving those goals. It can be amazing what happens for patient care when the entire lab staff team is on board, fully trained, and dedicated to providing service with RTP.

Reference

1. Covey SR. *The 7 Habits of Highly Effective People.* New York, NY: Simon & Schuster Inc; 1989.

My Life-changing Opportunity to Train Histotechs in Africa (Part 2)

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Throw your dream into space like a kite, and you do not know what it will bring back, a new life, a new friend, a new love, or a new country.

—Anaïs Nin

My preparation to return to Rwanda was easier the second time—I needed only a few booster immunizations and malaria medication. I planned to be in Rwanda for an entire month to help teach the two histotechnology courses at the Kigali Health Institute (KHI). I communicated with the staff at the sponsoring organization, the American Society for Clinical Pathology (ASCP), about what would be expected of me, and I also reached out to another ASCP volunteer who had been to KHI to teach immunology during the previous semester.

I loaded copies of my current teaching materials onto an external drive, packed donated books and teaching materials into my suitcases, and off I went. This trip's flights took me on the familiar route through Brussels and into Kigali, and I again arrived after nightfall. The hotel had sent a car, and I traveled into the city with other guests from the US and other countries, all there for different purposes. My host hotel for this trip was Hotel Serena (Fig. 1), a very nice 4-story hotel catering to American and European clientele. It is more modern than the Hotel des Milles Colines where I stayed on my first trip—air conditioning, wonderful restaurants, spa-type amenities, and friendly, responsive staff. I became friends with several of the staff (Fig. 2), and I still communicate with some of them since my return home.

On the day after arrival, I found my way to the KHI campus, just a few blocks from the hotel. I was to make that trip at least twice a day for the next month—walking downhill to the school, uphill to the hotel. The hotel would provide a car for my trip when it rained or when I had a heavy load, but I rather enjoyed the walk each day. I became friendly with people I saw each day along my route, exchanging greetings with the street cleaners and armed guards at several of the office buildings between the hotel and school. The weather during my month-long stay was generally warm, with high temperatures in the 80s. However, late in my visit the rains came more frequently, with thunderstorms and winds accompanying the rain, and temperatures that cooled to the low 60s.

The Centers for Disease Control and Prevention (CDC) representative in Rwanda, David McAlister, is a laboratorian who has been working in African countries for many years and who acts as a liaison between the ASCP groups and the Ministries of Health in Rwanda. With offices in the American Embassy in Kigali, he is an invaluable resource and guide, and he keeps the ASCP volunteers informed of any possible safety issues while they are in country. He and I met during my first few days in Kigali and several other times during my stay, discussing laboratory and educational issues. His assistant is a young Rwanda native named Eugene, who was also very helpful during my trip.

On my first Monday in Kigali, I made my way to KHI, where I met with Mr Aliyu Attahiru, the head of the Department of Biological Laboratory Sciences. He and I discussed my role while in country, and I was surprised to learn that he wanted me to teach two entire histotechnology courses while I was there! The first would be the histology course for the 2nd year students, a class of 41 young people. After I finished that course, I would then teach the 3rd year course to the 50 students enrolled. All this in one month! Needless to say, I was overwhelmed.

I returned to the hotel that night with my head spinning, thinking of ways to revise my curriculum to include what could be taught in such a short term with the constraints in place at KHI. That night became the pattern for the entire trip—preparing in the evening for the next day's teaching. Since the students have no access to books, and photocopying is very expensive, I would produce handouts for them using the hotel's business services copier. Each day was an adventure in language differences, classroom accommodations, technology complications, and time constraints. People's names in Rwanda are challenging: first names are generally European-like, such as Jean Claude, David, Agnes, Firmin, Gaspar, Clarice, and Eugene, but the last names are very difficult to decipher and pronounce. Names such as Bigirimana, Nshimiyimana, Muhigirwara, and Bizeranye are common; as you can imagine, we Americans generally use their first names only. The students encouraged me to pronounce their last names, and often smiled or kindly laughed when I tried!

One of the language difficulties came while I was teaching embedding techniques. Using clay tissue models that I had made, I demonstrated and discussed how to orient cross-sections of tubular structures such as an appendix. At least that's what I thought I had been teaching. I was surprised to see the results from a worksheet the students completed where they were asked to draw tissues oriented in rectangles that represented a base mold. When asked to show a "cross-section of appendix," most of them drew an "X" in the rectangle. Evidently, my term "cross-section" meant a literal "cross" to them. That night, I searched the French-English dictionary to find an alternative word: transverse. When I explained to the students the next day using "transverse," they understood! Several other language-related misunderstandings occurred, but we managed to work through them all.

The KHI has LCD projectors, which I was able to connect to my laptop. However, the cords were worn and the projected colors were not true; sometimes everything was green, sometimes red. The classrooms had no projector screens, so we used the painted, pitted, and discolored concrete walls, which do NOT help when teaching colors of stains! Although the laboratory department does have a teaching lab, the only histology-related item in the lab is one microtome. In order to teach stains, I developed a sheet that has coplin jar drawings on it, to represent the order of staining procedures. I had the students

Fig. 1. Hotel Serena in Kigali, Rwanda.

share 15 sets of colored pencils to color in the reagents and color changes in the tissue section representations on the drawings. Along with the clay tissue pieces that I had made for embedding practice, these sheets were helpful to students who had no other way of experiencing the technical aspects of histotechnology. Each student did have the opportunity to cut tissue by placing a block in the microtome and turning the flywheel enough to get a ribbon. No one practiced longer than 5 minutes, and that was the extent of their microtomy experience. While those lab experiences are not very worthwhile by our standards, they were more than other KHI students had ever been given!

Students in the laboratory program at KHI are primarily male, with only about 20% female students. Most are in their twenties, and several have some disability that involves their lower extremities. I later learned that the government encourages young, intelligent, disabled people to go into careers where they do not rely on their ability to walk, such as in laboratory work. That helped explain the large number of disabled students in the laboratory program. Only a very few are married and have families; most live in the housing provided at KHI and have very limited finances. The students were wary of me at first, but it was a mutual feeling: I wondered how I could teach these young people who were so different from the students I taught at home. But it didn't take long for us to trust each other, greet each other genuinely, work through our language differences, and actually like each other. At the end of the course with the 2nd year students, I asked to take pictures of groups of the students, and they asked for pictures of themselves with me—one at a time! Those pictures are treasures for me. Some of the students still email me periodically, asking when I'll be back to teach again.

While there, I was so happy to see my old histotech friend Leopold again. I finally had the opportunity to meet his wife, Chantell, and his children, Leopoldine and Arsene, when they traveled 2 hours by bus to meet me at my hotel one weekend. I look forward to getting to know the family even better on future trips.

One day I took a trip with the CDC representative, Eugene (Fig . 3), to Butare to visit the laboratory run by Dr Louis. At the time of my trip in 2008, it had been the only working histology lab in the country, although I did not get to visit it at that time. The road between Kigali and Butare is two lanes and paved, and I'm told it is one of the best highways in the country. It is winding and goes over many hills, providing great views of the countryside, small villages, schools, and farms along the way. Crops range from pineapple to peas and tomatoes. We stopped alongside the road to purchase roasted corn, cooked on a makeshift grill right there on the roadside, and it was tasty!

The Butare lab is large and well equipped with multiheaded microscopes, Leica and Microm microtomes, and Thermo processors and stainers. Although Leopold no longer works in this lab, there are several other young men who are capable of doing histotechnique. However, all is not good: because of the lack of reagents, the tissues received in the laboratory do not get processed, cut, and stained in a timely manner. And once they are stained, the pathologist (who is also a full-time faculty member at the medical school) cannot keep up with the workload. Consequently, surgical specimens may wait more than a year to be grossed, and after being cut and stained, the completed report may take another year or two for completion. My first thought was why should patients have surgery if the tissue sent to the lab is not being processed in order to make a timely diagnosis? One of the issues is the ordering

system and the length of time between placing an order and its receipt. Another is lack of personnel, and still another is lack of maintenance on instrumentation. Laboratory accreditation, similar to our CAP, is only now being introduced to laboratories in Africa. While in Butare, I presented Dr Louis (Fig. 4) with a donated copy of *Histotechnology: A Self Instructional Text*, 2nd edition, by Frieda L. Carson (ASCP Press, Chicago, IL, 1997), which is the most recent histology text the laboratory now owns. I am sure the histotechs there use it often.

The laboratory at the King Faisal Hospital in Kigali now has an operational histology lab! All the instrumentation that was still in crates in 2008 is now being used every day. However, they too have reagent and supply procurement issues, but not as critical as the lab in Butare. Dr Bigirimana has hired a British-trained Kenyan to run the histology laboratory, and he has now trained two young women who are showing great potential to be good histotechs. It was very rewarding to visit this lab and see the improvements in healthcare delivery since I was there last. I also left them with a donated copy of the Carson book, and they were most appreciative.

As in 2008, I was in Africa during the Easter holiday. On Good Friday, Eugene, the young Rwandese man who works with the CDC, offered to be my guide and interpreter for an

Fig. 2. Hotel Serena staff.

outing. I had expressed interest in finding groups of women who make baskets, and he wanted to help me have that experience. Basket weaving has always been a traditional craft in Rwanda, but, after the 1994 genocide, it has become even more meaningful. Groups of women, often widows of men who were killed, along with wives of the killers, come together to weave baskets, now known as "peace baskets." Eugene had heard about a house in a Kigali neighborhood that was used by two of these women's groups, and he took me in his car. (Owning a car in Rwanda is a true status symbol, since most Rwandese do not earn enough money to afford a vehicle.) We arrived at the house and the ladies were happy to see me, knowing that I probably would spend money there. Not only were the women so welcoming, but they also allowed me to take a few weaving stitches (Fig. 5); I realized quite quickly that it is difficult and precise work, and I gave it up after only a few stitches. Some of the women knew a little English, but most spoke only Kinyarwanda or some French, so it was a blessing to have Eugene there to translate for us. It takes approximately 40 hours to weave one 10-inch basket, and the women sit flat on the floor while they work. The craftsmanship is incredibly beautiful and colorful, and very labor-intensive. I did, in fact, purchase many of their baskets. My suitcase was heavier with baskets on my way home, and I could have bought their entire inventory!

After our basket-weaving experience, Eugene treated me to a visit to his home. As a young man, he is one of the truly successful people in Rwanda, and the future of

Fig. 3. Eugene, the CDC representative, in Dr Louis's laboratory in Butare.

Rwanda is more promising because of young people like him. Since he is educated and has a very good job, he can rent a one-bedroom home, he owns a car, and he is very respected. He learned to cook from an aunt, and he prepared a dinner from fresh vegetables and meats that he had purchased earlier in the day from a local market. We dined on stewed potatoes, a green vegetable called doe doe (similar to spinach), beef liver, and rice—all prepared while we visited and I watched. It was an interesting evening, learning more about his family and his life. During the month I spent in Kigali, Eugene and I talked several times by phone and in person. I look forward to a long friendship with this intelligent and progressive young man!

Another non-lab-related activity that I enjoyed was visiting fabric stores. I asked a concierge at the hotel if he knew the locations of any quilting groups or fabric shops. A few days later, he and I walked several miles into the commercial area of the city, and we found the shops where fabrics are sold. This was unlike any fabric shopping experience I've ever had—in one large building, individual shops were tucked into small rooms, with folded fabrics stacked from table height to above my head! Colorful, riotous prints and batiks in 6-, 10- and 12-yard lengths were displayed in the stacks and also hanging from rods around the rooms (Fig. 6). Many of the pieces were made and dyed in African countries, but some were from China and Holland. In all, I brought home 25 pounds of fabric. I have a plan for it, but still have not been brave enough to cut that precious cloth!

In the month I was at the Hotel Serena, I met many interesting people, including hotel employees and other world travelers.

One American hotel guest that I met was in Rwanda to implement organic farming, and I was privileged to accompany him and his group to a farm on one of the rare days that I was not at the school. Since I grew up in a rural Texas farming family, I was very interested to see that aspect of Rwandan life. Most Rwandan farms are small and not generally able to support the family that owns the land. With US aid and guidance, cooperatives of farmers have come together to pool resources. As a result, they have been more successful in producing plentiful crops to sell at market, allowing them to support their families. The farm that I visited grows pomegranates organically. I learned that there are 21 varieties of pomegranates; that farm had at least a few trees of each variety. It was a very interesting day, visiting with the local farmers, watching them work with their crude farm implements without the aid of mechanization of any kind, and being outside in the beautiful countryside.

My last week in Kigali coincided with the week during which the country commemorates the 1994 genocide. The people honor the dead and hope for continued peace. There are

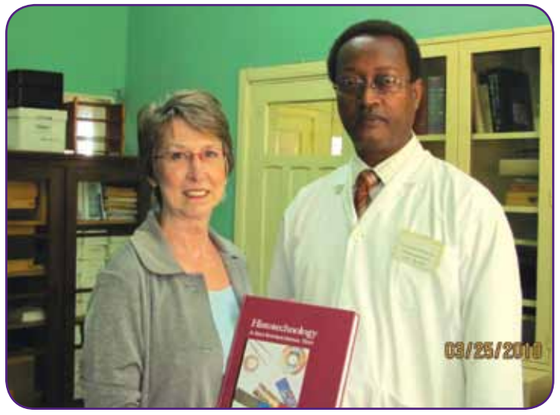

Fig. 4. Author presenting Carson book to Dr Louis.

ongoing rallies at their national stadium, with dignitaries speaking and entertainers performing during the week. One day is a national holiday, and all shops and restaurants are closed to remember the day when the killings began in April 1994. During that week, no festivities are allowed. Schools are closed, work schedules are limited, and people gather with their families to visit gravesites where relatives are buried.

Being away from home for an entire month was difficult. Even though the hotel was nice and comfortable, and their food and service were good, it was not home. However, I could have stayed longer because there is so much work still to be done there. I know I will return to Kigali. KHI needs me to teach and mentor new instructors; hospital labs need me to help upgrade their services and procedures; the basket weavers need me to help them find a distributor for their wares. And, most selfishly, I need to continue some of the work I began, because I feel that part of my heart is still there. My goal is not to "save the world" but to do whatever will help in small ways, with a smile, whether it be a purchase of local goods, teaching a histology technique, or sharing my knowledge and blessings with those who have less.

At the 2010 NSH meeting in Seattle, I was surprised and blessed to have been awarded the Lee G. Luna Foreign Travel Scholarship so I could continue my work in Africa. I hope to use the travel scholarship to return to Rwanda sometime in 2012, where I am expecting to teach at KHI as well as train histotechs for a new hospital histology laboratory in Kigali, which will be the third lab in the country. Again, I am working with the ASCP and CDC so that I can best fill the needs in Rwanda. In 2011, I was fortunate enough to spend a week in Nigeria and another week in Lesotho working with lab educators on curriculum revisions. And I will continue to look forward to other opportunities to travel to Africa for years to come!

Fig. 5. Author receiving a basket-weaving lesson.

Fig. 6. A small sample of the dazzling array of colorful fabrics available in Kigali.

I am grateful to everyone in the histotechnology profession who has believed in me, encouraged me, challenged me, and appreciated me. Each of us is who we are because of the people around us and the experiences we have had. And I am a much better person for having been associated with histotechs and Rwandans!

Back to Basics—A Review of Solution Mathematics

Joelle Weaver, MAOM, HTL(ASCP)

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Abstract

Work at the histology bench involves an ever-increasing use of instrumentation and other technology. These developments have advanced the histology field by decreasing testing turnaround times and increasing the standardization that helps reduce variability and the errors that result.[1-3] However, as we become more reliant on technology, we can become rusty with the basics, such as working with mathematical formulas, which used to be part of our daily duties in the lab. We can usually rely on technology to assist us, but it is still beneficial to review manual calculations and refresh our understanding of the basic underlying mathematical principles involved.

Introduction

Many people may not consider themselves good in math. However, being comfortable working with math doesn't require a special ability, as proposed by the familiar right brain (sequential/logical)-left brain (simultaneous/analogic) theories.[4] Understanding math is primarily about seeing patterns in the world.[5] You don't have to love math, but a review of basic mathematical principles and formulas may make you feel more confident at the histology bench when called upon to perform calculations. To that end, this article reviews some basic concepts, operations with numbers, and generic types of solution-making calculations in a simple and condensed format.

Methods and Examples

Table 1. Review of Basic Definitions

Term	Definition
Solute	Usually the substance in lesser quantity; can be solid or liquid, which is dissolved into the solution or in a solution diluent.
Solvent	Substance usually in greater quantity in a solution into which the solute is dissolved.
Percent	Expresses a quantity of solute to a quantity of solution times 100. It is often written as mass of solute/mass or volume of solution.
Ratio	This expression provides information about the relative parts of the amounts within a whole.
Molarity	Moles of a solute per liter of a solution.
Mole	Weight of a salt in grams equal to its molecular weight.
Normality	Expresses the relationship of molarity to the number of hydrogen or hydroxyl ions produced in solution, expressed as molarity multiplied by the number of dissociable ions.

Working With Already Prepared Solutions

For convenience and stability, manual stain solutions and other reagents in histology may be prepared first as a stock solution. The stock solution will contain many of the essential ingredients of the working solution, or perhaps be a higher concentration of the working solution. Stock solutions are used for increasing stability that extends the shelf life of a solution. This provides a savings on reagent costs, and serves to decrease preparation time at the time of use. The stock solution can be manipulated by adding final ingredients or preparing a more dilute solution from the concentrated one. This new solution that was prepared from the stock solution is called a working solution.

In many manual staining procedures, it is common for working solutions to be prepared from a more concentrated stock solution, whose strength is recorded with the letter "X". The number noted with the "X" denotes how many more times the stock is concentrated than the working solution. Most procedures call for preparing a 1X solution from the more concentrated stock, unless the procedure states otherwise.

Example: Gomori Periodic Acid-Methenamine Silver Method

Stock Methenamine Silver
3% methenamine. . . . . 100 mL
5% silver nitrate. . . . . 5 mL

Add silver nitrate in small amounts to the methenamine silver solution, mixing after each addition. A white precipitate will form that can be cleared with agitation. Stock solution should be clear before use. Filter and store in brown bottle. Solution is stable for several months at 4°C.

Working Methenamine Silver
Stock methenamine silver. . . . . 50 mL
5% sodium borate (Borax). . . . . 5 mL
Filter into chemically clean glassware.[6]

Use this formula to determine how much stock solution you will need

$V_1C_1 = V_2C_2$

V_1 = how much volume of stock solution you will be working with

C_1 = the concentration of the stock solution

V_2 = the volume needed to make the concentration or dilution of the working solution

C_2 = the desired concentration of the working solution

This simple algebraic formula states that the volume of the starting solution (V_1) multiplied by the concentration in the starting solution (C_1) equals the volume of the desired solution (V_2) multiplied by the concentration of the desired solution (C_2).

The $V_1C_1 = V_2C_2$ formula is quite useful for determining the volume of stock solution needed when diluting the stock solution of known concentration to a desired final concentration or volume for the working solution. Note that the same units of measure must be used on each side of the equation and any necessary operations must be performed to both sides of the equation, just as in solving any algebraic equation.

Volume/ Volume dilution example:

How much 100% ethanol will be needed to make 500 mL of an 85% ethanol solution?

V_1 (initial) = unknown

C_1 (initial) = 100%

V_2 (final) = 500 mL

C_2 (final) = 85%

Substitute the known values into the equation.

$V_1C_1 = V_2C_2$

V_1 (100%) = (500 mL) (85%)

V_1 (100%) = 42,500

V_1 = 42,500/100 = 425 mL

425 mL of 100% ethanol will be needed to make 500 mL of an 85% ethanol solution by adding water, quantity sufficient, to the desired 500 mL volume, which is 75 mL of water.

Check your answer: Divide the calculated amount (425 mL) by your desired volume (500 mL), resulting in 0.85, which is 85% when written as a decimal number.

Is a given solution dilute or concentrated?

The determination of concentration rests on the measure of solutes in relation to its diluent in any solution. Concentration could be expressed in:

1. Percent concentrations—weight/volume (w/v), weight/weight (w/w), or volume/volume (v/v)
2. Molarity—moles of solute/liters of solution
3. Relative terms—ratios comparing parts to other parts of a whole

These are different ways of expressing the amounts of solute to solution, and their relationships to each other.

Ratios

The volume-to-volume dilution example on page 45 illustrates the resulting concentration when a given volume of solute is added to a specified volume of diluent. Solute concentrations can also be expressed as a ratio. The ratio will express the relative amount of the solute to the final volume of diluent. For example, in a 1:10 dilution of a sugar-water solution, you would mix one part sugar with nine parts water. Therefore, our sugar solution consists of 1 part sugar + 9 parts water (Fig. 1). A ratio merely gives information about the relative parts of a whole.[5] Ratios can also be written as division problems; in the example above, the ratio of sugar to water is 1 ÷ 9. Division problems can often be expressed as fractions. Remember this distinction, however—fractions are expressions of a *part* to a *whole* and differ from ratios, which are expressions of a *part* to a *part*.[5] When working with ratios, unlike with many other algebraic problems, the measurements are not as crucial since the relative amounts will always be the same no matter the measurements used, but it is ideal if these are kept consistent throughout.

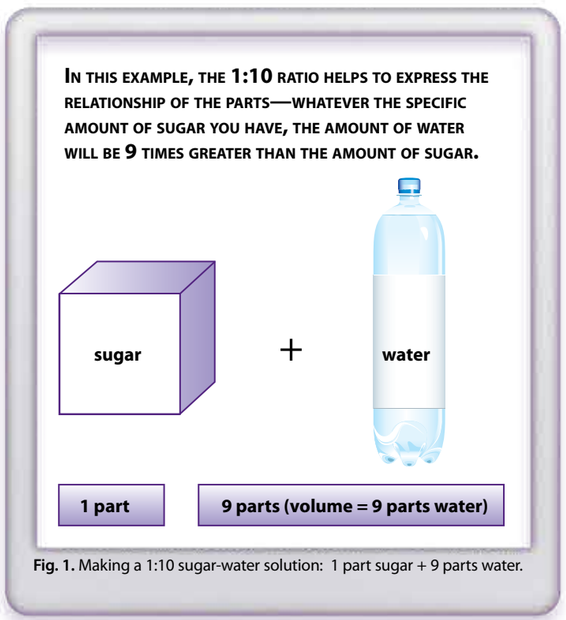

Fig. 1. Making a 1:10 sugar-water solution: 1 part sugar + 9 parts water.

Molarity and Normality

Molar and normal solutions may be encountered in histology, especially when working with buffers and solutions that are needed for enzyme histochemistry and some immunohistochemical techniques. Molar solutions are easy to calculate once the gram molecular weight of the substance is known. Normal solutions are a little more complicated and require the determination of the valence electron configuration.

A mole is the amount of a substance or matter that contains 6.022367 x 10^{23} atoms of the substance (Avogadro's number). A mole is equivalent to 1 gram of the formula weight of that substance if it were dissolved in 1 mL of water.[7] The formula weight (FW) is determined by adding the sum of the atomic weights of all the atoms in the molecule, written as grams.

Molarity calculation

The example below will determine how much solute you will need to prepare a solution of a substance with a particular molarity and volume according to the formula:

Formula weight (moles/liter) x Molarity (grams/mole) x Volume (liters) = Grams of solute needed

How much calcium chloride ($CaCl_2$) will be needed to make 200 mL of a 0.2 M $CaCl_2$ solution?

In this example, you will first need the formula weight of calcium chloride (see Calculating a Formula Weight below). You should also convert the volume to liters for this problem (1 L = 1000 mL).

Formula Weight x Molarity x Volume = Grams of solute

110.9 moles/L x 0.2 g/mole x 0.2 L = $\underline{4.44 \text{ g } CaCl_2}$

Calculating a Formula Weight

1. List the elements in the compound from the balanced chemical equation.
2. Determine the number of atoms of each and multiply the number of atoms x atomic weights.
3. Add together the total masses of the elements.
4. Atomic weights: calcium = 40.1, chloride = 35.4.
5. $CaCl_2$ = 40.1 x 2(35.4) = 110.9.

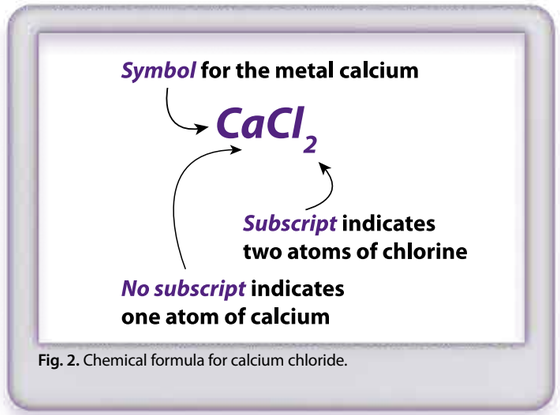

Fig. 2. Chemical formula for calcium chloride.

Normality

The concentration of acids and bases in laboratory solutions is sometimes expressed in units called normality. Normality is an expression of a simple relationship of acid and base ions produced in a solution as it relates to the molarity of the solution. Normality is simply the molarity multiplied by the number of H^+ or OH^- ions produced. For simple polyatomic ions, such as HCl, the normality is equal to the molarity.[7] A polyatomic molecule such as H_2SO_4 (sulfuric acid), however, produces two H^+ ions in solution, so the normality will always be twice the molarity. Note that ***normality is always equal to or greater than the molarity in any solution***.

The equivalent weight (EW) is the amount of solute needed to equal one mole of hydrogen ions, and this value depends on the valence of the solute. For some solutes, such as NaCl, the molecular/formula weight and equivalent weight are the same. In cases where the valence is greater than 1, as in H_3PO_4 with a valence of 3, the equivalent weight will be equal to the molecular weight divided by the valence.

General formula for determining the normality of a solution

Normality (equivalents/liter) x Equivalent Weight (grams/equivalent) x Volume (liters) = Grams of solute

This formula will calculate how many grams of a pure solute will be needed to make a specific volume of a solution at a particular normality.

This formula can be rewritten as:
Weight (grams) = N (equivalents/liter) x V (liters) x FW (grams/equivalent) ÷ positive valence (which is the number of replaceable or dissociable hydrogen atoms)

Determining the normality of a given solution

Normality = Grams ÷ (Equivalent Weight x Volume)

What is the normality of a solution made by dissolving 8.5 grams of H_2SO_4 in enough water to make 500 mL of solution?

First determine the number of equivalents of H_2SO_4 per liter using the equivalent weight of H_2SO_4:

Hydrogen = 2 x 1 = 2

Sulfur = 1 x 32 = 32

Oxygen = 4 x 16 = 64

FW.....98

EW..... = 98/2 = 49

8.5 grams x 2 equivalents/98 grams = 0.17 eq H_2SO_4

To determine the normality, we then divide the equivalents of H_2SO_4 by the liters of solution:

0.17 eq ÷ 0.500 L = 0.34 eq/L = $\underline{0.34 \text{ N}}$

Other Solution Formulas

Percent solution to molarity: M = (% x 10) + Molecular Weight

Percent solution to normality: N = (% x 10) + Equivalent Weight

Molarity to normality: M = Normality + valence

Normality to molarity: N = Molarity x valence

Summary

Working with solution calculations at the histology bench doesn't require an advanced understanding of mathematics. This article reviews selected basic mathematical concepts and formulas that can be utilized for manual solution preparations in a simple format. Understanding solution calculations is primarily about seeing patterns and relationships between various measurements. Taking the time to review these fundamentals and working some practice problems should make you feel more confident when you are called upon to manually prepare reagents and solutions, and may also be helpful practice for anyone preparing to sit for the ASCP registry exam.

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IN MEMORIAM
NANCY L. KLEMME
April 4, 1946 – January 3, 2012

Histology Loses a Dear Friend

Histology loses a treasure with the passing of Nancy Klemme on January 3, 2012. Nancy enjoyed a 40-year career in histology, was a consummate teacher and mentor, and also served as managing editor for *HistoLogic*® for many years.

Thank you, Nancy, for touching so many of us with your kind, generous heart, your radiant smile, and your indomitable spirit!

✔ Mark Your Calendar!
Educational Opportunities in 2012

JANUARY

20 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Quality Management in the Histology Laboratory**
Speaker: Joelle Weaver, MAOM, HTL(ASCP)
Vanderbilt Medical Center
Nashville, TN

25 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Assessment of HER2 (c-erbB2) Status: A Comparison of Commonly Used IHC and ISH Methods Used in Histopathology Laboratories**
Speaker: Rhonda Henshall-Powell, PhD
Biocare Medical
Concord, CA
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

FEBRUARY

17 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Dermatopathology: A Guide for the Histologist**
Speaker: Clifford M. Chapman, MS, HTL(ASCP)QIHC
Strata Pathology Services
Lexington, MA

22 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Success or Failure of Implementing New Equipment**
Speaker: Katja Lehmann, PhD
Leica Microsystems
Bannockburn, IL
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

MARCH

2-3 **Indiana Society for Histotechnology**
Site: Hilton Indianapolis North
Indianapolis, IN
Contact: Debbie Wood
Phone: (317) 491-6311
Email: demwood@iupui.edu

16 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **The Role of Immunohistochemistry in Carcinoma of Unknown Primary**
Speaker: Prashant A. Jani, MD, FCAP, FRCPC
Thunder Bay Regional Health Sciences Centre
Thunder Bay, Ontario, Canada

24 **Rhode Island Society for Histotechnology**
RISH's Carnival Mardi Gras Conference
Site: Providence, RI
Contact: Nancy Heath
Phone: (401) 444-3246
Email: president@rihisto.org

28 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Beginning Chemistry for the Histotech**
Speaker: Peggy A. Wenk, HTL(ASCP)SLS
William Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

APRIL

13-15 **Texas Society for Histotechnology**
Site: Omni San Antonio Hotel
San Antonio, TX
Contact: Donna Willis
Email: donna@milestonemed.com

20 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Carbohydrate Histochemistry**
Speaker: J. A. Kiernan, PhD, DSc
University of Western Ontario
London, Ontario, Canada

20-21 **Colorado Society of Histotechnology**
Site: Ameristar Hotel and Casino
Black Hawk, CO
Contact: Janet Maass
Email: jmaass@frii.com

25 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Decalcification?**
Speaker: Diane Sterchi, MS, HTL(ASCP)
Covance Inc
Greenfield, IN
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

26-28 **Region II Symposium**
Site: Williamsburg Marriott
Williamsburg, VA
Contact: Janice Alvarez or Olga Kochar
Email: jalvare@jhmi.edu
omsp@hscmail.mcc.virginia.edu

26-28 **North Carolina Society of Histopathology Technologists**
Site: Hilton Hotel at Research Triangle Park
Durham, NC
Contact: Brandon Taylor
Email: bctaylor@gmail.com

27-28 **Region I Annual Meeting**
Site: Islandia Marriott Long Island
Islandia, NY
Contact: Mary Georger
Email: mary.georger@rochester.edu

MAY

2-4 **Tri-State Meeting (Iowa, Minnesota, Wisconsin)**
Site: Madison Concourse Hotel
Madison, WI
Contact: Judy Stasko (IA), Lois Rowe (MN), or Jean Mitchell (WI)
Email: judith.stasko@ars.usda.gov
rowe.lois@mayo.edu
jmitchell@uwhealth.org

3-6 **California Society for Histotechnology**
Site: Bahia Resort Hotel
San Diego, CA
Contact: Kathy Hardy
Email: kathy@oncomdx.com

17-20 **Florida Society for Histotechnology**
Site: Grand Hyatt Tampa Bay
Tampa, FL
Contact: Susan Clark
Email: sclark@fshgroup.org

18 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Basic Detection of Microorganisms in Tissue**
Speaker: H. Skip Brown, M.Div, HT(ASCP)
Leica Biosystems
Wauconda, IL

23 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Immunofluorescence: How to Make Your Skin Glow in the Dark**
Speaker: Patty Molner, MT(ASCP), CG(ASCP)
Beaumont Health Systems
Royal Oak, MI
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

✔ Mark Your Calendar!
Educational Opportunities in 2012

JUNE

8-9 **Tennessee Society for Histotechnology**
Site: The Edgewater Hotel
Gatlinburg, TN
Contact: Michelle Foster
Email: mchilds2010@hotmail.com

15 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Process Improvement in the Histology Laboratory: LEAN**
Speaker: William DeSalvo, BS, HTL(ASCP)
Laboratory Sciences of Arizona
Tempe, AZ

27 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Controlling Your IHC Stains**
Speakers: Debbie Siena, HT(ASCP)QIHC
StatLab Medical Products
McKinney, TX and
Kathleen A. Dwyer, HT(ASCP)
AmeriPath
Midlothian, TX
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

JULY

20 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Why Do the H&Es Look Different Today?**
Speaker: Ada Feldman, MS, HTL(ASCP)
Anatech Ltd
Battle Creek, MI

25 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Sentinel Lymph Nodes: A Look at Their Significance From the Histotech's Perspective**
Speaker: Heather Renko-Montes, BS, HT(ASCP)QIHC
Leica Microsystems
Bannockburn, IL
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

AUGUST

17 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Efficiency in Anatomic Pathology**
Speaker: William DeSalvo, BS, HTL(ASCP)
Laboratory Sciences of Arizona
Tempe, AZ

22 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Hirschsprung's Disease: Histologic Techniques for Diagnosis**
Speaker: Sarah Bajer, HTL(ASCP)
William Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

SEPTEMBER

19 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Assessing Competency in the Histology Laboratory**
Speaker: Joelle Weaver, MAOM, HTL(ASCP)
Vanderbilt Medical Center
Nashville, TN
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

21 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **The Role of the Pathologist in the Management of Breast Cancer**
Speaker: Prashant A. Jani, MD, FCAP, FRCPC
Thunder Bay Regional Health Sciences Centre
Thunder Bay, Ontario, Canada

28-Oct 3 **National Society for Histotechnology Symposium/Convention**
Site: Vancouver Convention & Exhibition Centre
Vancouver, British Columbia, Canada
Contact: Aubrey Wanner
Phone: (443) 535-4060 or register online at www.nsh.org
Fax: (443) 535-4055
Email: aubrey@nsh.org

OCTOBER

19 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Applications of Molecular Pathology in Colon and Lung Cancer**
Speaker: Sally Lewis, PhD, MLS(ASCP)HTL, MB
Department of Medical Laboratory Sciences
Tarleton State University
Coppell, TX

24 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Emergency Preparedness: Would Your Histology Lab Survive a Disaster?**
Speaker: Marcia Fisher, MEd, HT(ASCP)
El Centro Regional Health Center
El Centro, CA
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

NOVEMBER

16 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Today's Artifacts—Tomorrow's Facts?**
Speaker: M. Lamar Jones, BS, HT(ASCP)
Emory University Hospital
Atlanta, GA

28 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Reagent Alcohol—Can't Drink It, So What Is It?**
Speaker: Pam Marcum
University of Arkansas for Medical Sciences
Little Rock, AR
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

DECEMBER

19 **NSH Teleconference 1:00 pm Eastern Time**
Title: **There's a Fungus Among Us**
Speaker: Josh Fink, HTL(ASCP)
Detroit Medical Center
Detroit, MI
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

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