Unlocking the mystery of an effective tissue processing protocol: Using the Greenlee Ratio to Estimate Average Time (GREAT) method to determine estimated protocol length and reagent time ratio

Joshua Greenlee MBA HT/HTL (ASCP)^{cm}, Scott Webster PhD, Erico von Bueren PhD MOR

Introduction

Having an efficient tissue processing protocol is crucial for multiple reasons. With its upstream positioning in the histology process, inefficient tissue processing can have negative ramifications through the rest of the downstream process from embedding to staining. Processing inefficiencies may impact the diagnosis as well as laboratories' turnaround times (TAT).

Proper tissue processing quality is of the utmost importance for an accurate diagnosis. Without proper dehydration, clearing and infiltration of the tissue, the tissue morphology may be negatively affected, sectioning may be difficult at microtomy, and the section may not achieve proper staining for H&E, special stains, or advanced methods like IHC and molecular. At a minimum, improper processing may require additional time, cost, or rework, but at a maximum, it may result in an incomplete or inaccurate diagnosis.

Laboratories are increasingly demanded to reduce TAT. Most laboratories continue to use traditional conventional tissue processing methods, which have multiple reagent steps and may take significant time to process effectively. Inefficiencies in processing may result in longer protocol times or rework that delays TAT. Because of this, tissue processing protocols must fit in to the timeline necessary to meet laboratories' TAT expectations.

Despite the need for quality and efficiency, many laboratories settle for less than ideal quality and time of tissue processing because the protocols used have not been updated for long periods of time, in some cases, over 20 years. These outdated protocols often continue to be used despite flaws because of a lack of information or industry guidelines to follow to properly update them to more efficient versions. Conventional processing protocols consist of a host of variables, and often the laboratory does not know where to begin to make adjustments or may fear making changes that could lead to worse processing or even potentially non-diagnostic tissue.

Materials & Methods

Conventional tissue processing protocols from many different types and sizes of laboratories were collected. These laboratories included hospital, reference, university, research, and specialty laboratories, and their volumes ranged from less than 100 to more than 2,000 cassettes per day. Furthermore, published protocols from various sources, including some posted online, were collected, building a large, detail-rich library of 276 processing protocols.

Relevant information was gathered to determine how each protocol was used. The type and thickness of the tissues processed using the protocols was identified when possible if not described in the name of the protocol. The fixation regimen of the tissues prior to processing was also noted when possible. Information about the quality of the results of the protocol experienced by the laboratory was requested.

The collected protocols were grouped into categories based on the characteristics of the protocols. The protocols were then evaluated for commonalities and capabilities in terms of quality and speed.

Working with laboratories volunteering for protocol reviews and open to external assessment, the information learned was put to use to establish a set of simple, general guidelines to empower laboratories to enhance their tissue processing efficiency.

Conclusions

The GREAT method proved to be a useful tool to help guide laboratories in making protocol adjustments. Using this method, with its simple and low-risk set of guidelines, empowers laboratories to update their protocols and enhance processing efficiency, increasing quality and reducing turnaround time.

Results

The protocols received were classified as Fast (STAT), Biopsy, Routine, or Fat. Within these categories, the shortest, longest, and average protocol times were determined with the shortest time being 0 hr: 50 min and the longest as 12 hr: 30 min (Figure 1A). Six (6) categories were established based on tissue thickness and type. These categories were then associated with the general protocol time ranges (Figure 1B). The dehydration, clearing, and infiltration times of the protocols were compared to the quality comments to develop a set of general ratios of total protocol time (Figure 1C). The evaluation of the library of protocols resulted in the Greenlee Ratio to Estimate Average Time (GREAT) method to determine an initial overall protocol length based on tissue type and thickness as well as a breakdown of the ratios of time in dehydration, clearing, and infiltration for those protocols. The GREAT method was tested in several laboratories, providing more efficient protocols with better quality and faster processing like in the example displayed in Figure 1D.

A

Protocol category	Shortest sample protocol time (hr:mm)	Longest sample protocol time (hr:mm)	Average sample protocol time (hr:mm)
Fast (STAT)	0:50	3:30	1:57
Biopsy	2:15	5:35	3:56
Routine	5:30	10:46	7:31
Fat	8:25	12:30	10:09

B

Tissue thickness (mm)	Tissue type (examples)	Protocol time (hours)
< 1.5	core biopsies (prostate, liver, kidney) small GI biopsies	< 1-2
1-2	GI biopsies skin (shaves, punches, small excisions) cervical biopsies general small tissues	2-3
2-3	skin (excisions) standard grossing thickness tissues	3-4
3-4	general surgical tissues	4-6
4-5	large surgical tissues	6-8
> 3	fatty tissue (breast, colon, etc.)	> 8

C

Processing step	Ranges of ratios
Dehydration	0.40 - 0.45
Clearing	0.25 - 0.30
Infiltration	0.30 - 0.35

D

Original protocol		
Protocol type	Biopsy	
Tissue thickness (mm)	1 - 2	
Solution name	**Time (hr:mm)**	**Station**
NBF	0:30	1
NBF	0:30	2
Alcohol 70%	0:15	3
Alcohol 95%	0:10	4
Alcohol 95%	0:10	5
Alcohol 100%	0:10	6
Alcohol 100%	0:10	7
Alcohol 100%	0:10	8
Xylene	0:20	9
Xylene	0:20	10
Paraffin	0:00	11
Paraffin	0:20	12
Paraffin	0:20	13
Paraffin	0:20	14
Fixation time	1:00	
Processing time	2:45	
Total time	3:45	

GREAT time & ratio	Time (hr:mm)	Ratio
Alcohol	1:05	0.39
Xylene	0:40	0.24
Paraffin	1:00	0.36

GREAT-adjusted protocol		
Protocol type	Biopsy	
Tissue thickness (mm)	1 - 2	
Solution name	**Time (hr:mm)**	**Station**
NBF	0:30	1
NBF	0:30	2
Alcohol 70%	0:05	3
Alcohol 95%	0:05	4
Alcohol 95%	0:10	5
Alcohol 100%	0:05	6
Alcohol 100%	0:10	7
Alcohol 100%	0:10	8
Xylene	0:15	9
Xylene	0:15	10
Paraffin	0:05	11
Paraffin	0:10	12
Paraffin	0:10	13
Paraffin	0:10	14
Fixation time	1:00	
Processing time	1:50	
Total time	2:50	

GREAT time & ratio	Time (hr:mm)	Ratio
Alcohol	0:45	0.41
Xylene	0:30	0.27
Paraffin	0:35	0.32

Figure 1: Categories of the sample protocols received with the shortest, longest, and average times (A), Redesigned categories by tissue thickness with tissue type examples and general overall protocol time (B), established time ratio range of processing steps (C), a laboratory's real example of a GREAT-adjusted protocol, saving almost an hour and providing better quality (D).

Contact information

Sakura Finetek USA, Inc. | 1750 West 214th Street, Torrance, CA 90501 | T. +1 310 972 7800 | F. +1 310 972 7888 | E. info@sakuraus.com

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