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

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 dehydrating, clearing and infiltration of the tissue, the tissue morphology may be negatively affected, sectioning may be difficult at micrometry, 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 These outdated protocols often continue to be used despite flaws because of a lack of information or industry guidelines to follow to properly update them to more efficient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 rest of 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 protocol regimen of the Tissues prior processing was also noted when possible. Information about the quality of the results of the protocols 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.

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.

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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 categories. 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.

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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 quality comments to develop a set of general ratios of total protocol time (C). 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 categories. 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.