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Technical Note

Microwave-assisted tissue processing: real impact on the histology workflow

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Abstract Tissue processing is just one of many tasks in the histology workflow and accounts for 3% to 34% of the total time; it is technology dependent, but all other tasks are independent of this step and constitute the remaining 66% to 97%of the total time. The best histology workflow is one of fewer than 50 specimen loads processed sequentially in the shortest time possible, with postprocessing tasks completed in approximately the same time as the preprocessing and processing steps combined. Larger loads determine a total workflow too lengthy for an efficient continuous operation. This objective may be obtained with microwave-assisted instruments or by optimizing conventional instruments usage. As a major capital equipment investment, changing technology and selecting an instrument should also include a cost-effectiveness analysis.

Keywords: Microwave assisted; Workflow; Nonprocessing tasks; Productivity; Turn-around time; Cost-effectiveness

1. Introduction

Evolving from an external mechanical means to support specimens for sectioning (embedding) to a multiple-step procedure providing intrinsic firmness to the specimens (infiltration), tissue processing (TP) underwent a drastic transformation from the latter years of the eighteenth to the early twentieth century [1].

In short, TP involves the use of chemicals to ensure their preservation, dehydration, and infiltration with a medium that will be solid at room temperature.

To obtain those goals, the number of preserving agents and mixtures (fixatives), dehydrants, and antemedia (clearing agents) to remove the dehydrants and facilitate the infiltration with the molten medium, usually paraffin wax of varying melting points, is in the hundreds [2].

This article presents an overview of the development of TP from a wholly manual task to that of the most advanced instruments, both conventional and microwaves assisted (MWA), and their effect on the specimens' turn-around time (TAT), which is of utmost importance to enhance patient care.

The statistical analysis of the data was done with standard methods [3]. Mentioning manufacturers and their instruments does not constitute any type of endorsement or evaluation, just some relevant examples of what is commercially available.

2. Conventional tissue processing

Once a very laborious and tedious manual sequence, TP used to account for approximately 80% of any histology laboratory work time.

Except for the infiltration steps inside conventional ovens heated to the paraffin melting point and an occasional step using vacuum in large desiccators to help eliminate the antemedium, manual processing is performed at room temperature and ambient pressure.

The single most effective improvement in the history of TP was the development of an automated functional tissue processor in the late 1940s, the Autotechnicon [4], based on an early experimental instrument from 1909 [1].

Automating TP not only liberated the histotech (HT) from a totally manual sequence but also introduced 2 fundamental changes: consistency and time reduction, from a minimum of 22 to 28 hours to an average of just 6 to 8 hours protocols completed overnight.

From those early instruments, there have been 2 lines of improvement: one is to assure a better infiltration, and the other is to shorten the processing time. The first objective has been accomplished by providing the instruments with

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Table 1
Conventional TP

Operation	Characteristics	Manufacturers
Manual	One or several containers per step depending on the load, TP usually cannot be completed in an 8-h shift, which will add 10 h to the procedure. Handling specimens will add 0.5 h per 100 cassettes. Specimens received from Monday to Thursday can be processed in 28 h, but if received on Friday, processing will require 96 hours because of the weekend delay.	Conventional laboratory glassware and ovens
Automated	Twenty vials to process up to 56 small specimens for electron microscopy; programmable.	EMS
	A rotating basket with specimens in/out of several stations in 1 or 2 decks, some with heat or vacuum; up to 120 cassettes per run; time controlled with 24-h clock discs; newer instrument with computerized programs; accept delays.	Autotechnicon Microm NPO Izoterm Shandon Spencers
	One or 2 retorts with reagents pumped in/out; up to 600 cassettes per run; temperature/vacuum/pressure and specimens agitation of various types per station. Programmable protocols from 1 h to several days, with or without delays	Fisher Hacker Leica Microm Sakura Shandon TBS Vision Bio Systems

temperature, pressure, and vacuum capabilities, several means of specimen agitation, and the use of more effective chemicals combinations.

The second objective has been approached by having static specimens inside a retort with the reagents pumped in and out around them, reducing the numbers of steps with the help of high vacuum to facilitate the infiltration, and the introduction of MWA tissue processors. Some manufacturers have also developed proprietary multivalent solutions that allow, for example, fix and start dehydration or dehydrate and clear simultaneously [5].

These changes have been generally introduced as marketing features of the instruments, but on 2 instances the results of tests were published. When Miles, Inc launched their first Tissue-Tek Vacuum Infiltration Processor (VIP), it was followed by a publication [6], and something similar has been done by Vision Bio Systems, Ltd for their Peloris instrument [7-9]. Even if those articles were not peer reviewed and published by the manufacturers, they contained valuable information.

There are numerous manufacturers of conventional instruments with a wide variation of operational options (Table 1).

3. Microwave-assisted TP

The instruments specially designed for MWA TP vary from small and manually operated with a wide range of characteristics, to large and automated or "walk-away" units (Table 2).

There are literally hundreds of peer-reviewed articles dealing with each aspect of MWA TP and the physics involved since this technology, widely used in food processing, chemical, pharmaceutical, and many other industries for many years, was introduced in the 1980s as a means of shortening TP times.

As in the case of the conventional TP, most of the information dealing with the characteristics of these instruments is presented in their marketing publicity with 2 exceptions.

Milestone has numerous publications summarized in a recent and comprehensive book [10] validating their manual single cavity processors, but their flagship instrument, the Pathos, has not been validated in the same way to date.

In contrast, Sakura FineTek has documented the development, validating tests and routine use of their Tissue-Tek Xpress instrument, in the form of peer-reviewed scientific publications [11-14].

Table 2
Microwave-assisted TP

Operation	Characteristics	Manufacturer
Manual	Single chamber; experimental/general usage or designed for TP; 1 or 2 magnetrons; up to 1200 W variable at intervals of 10-20 W; load or temperature controlled; fumes control and ventilation; temperature monitoring; vacuum/refrigeration capabilities; agitation; wax heating with Weflon stirrers; programmable; up to 180 cassettes processed from 15 min (biopsies) to 195 min (4-mm thick specimens). Fixation step is included.	EBS EMS Hacker Milestone MRA Shandon TBS Ted Pella
Automated	Single chamber; reagents pumped in/out; 600 W load controlled; programmable, fumes control; Weflon heat absorbers; up to 210 cassettes processed from 60 min (biopsies) to 180-240 min (5-mm thick specimens). Processing in a single batch. Fixation step is included.	Milestone Pathos
	Four chambers—1 and 2 with 60 W continuous MWA and bubble agitation; 3 and 4 are convection heat/vacuum retorts; fumes control. A robotic arm moves the cassettes in a basket between the chambers. Total process time of 60 min for up to 40 prefixed specimens 1.5-mm thick, with ancillary instruments to obtain slices that thin. Thicker slices can be processed by increasing the time. Specimens can be added every 15 min for a maximum workflow of 40/60 min, 80/75 min, 120/90 min, or 160/105 min.	Sakura Xpress

Table 3
Manual tasks other than TP for every 100 cassettes

Phase	Tasks	Time (h)	P (cst/h)
Pre-TP	Unlicensed: transport/receive/number specimens, accessioning, gross transcription, number cassettes[a], etch slides[a]	4.4	23
	Licensed: grossing; cassetting	3.5	29
	Total pre-TP tasks	7.9	13
TP	Manual or automated, conventional or MWA processing	0.4-28	[b]
Post-TP	Unlicensed: routine staining[a] and coverslipping[a], handle racks and slides, collate/distribute slides and gross transcriptions to pathologists, file sectioned blocks	2.12	47
	Licensed: embedding, prepare blocks for microtomy (remove excess wax, match with slides, check against the embedding log), trim and section.	6.4	16
	Total post-TP tasks	8.52	12
Non-TP	All unlicensed tasks	6.52	15
	All licensed tasks	9.9	10
	All non-TP tasks	16.42	6

Unlicensed tasks are those completed by auxiliary personnel or those using automated instruments. Licensed tasks are those completed by licensed personnel (HTs). P indicates task productivity (100 cassettes/time for the task).

[a] If not automated; this task requires licensed personnel.

[b] Dependent on the load and the technology.

Nonautomatic MWA processors have up to a point resulted in the HT returning to the days of manual processing that is only somewhat alleviated by fewer and shorter steps with the ability of some instruments to indicate when each has been completed, but there will be always some degree of potential inconsistency when TP is completed manually.

4. Other tasks in the histology workflow sequence

The histology workflow is not just TP; it involves many more tasks to be completed before and after the TP step to culminate with the preparation of the slides ready for diagnosis. There are 10 other fundamental tasks in the workflow to reach this goal; some are automated at different levels, but others are entirely manual.

Cassette numbering and slides etching are automated in less than 15% of histology laboratories, whereas routine staining and coverslipping are automated in approximately 70% [15]. Although there is a newly developed automatic embedding instrument, there are so few of them that embedding is a task that can be considered as totally manual. Trimming blocks or "face-off" is an operational feature of all motorized microtomes, and although it is not completed faster than manually, this type of microtome adds up to less than 20% of all microtomes.

The remaining tasks of grossing, cassetting, and preparing the blocks for microtomy are totally manual, along with the fundamental task in the workflow, microtomy.

The weighed workflow automation average shows that at least 80% of all the work is still manual, with a lower percentage in laboratories with more automated instruments. To fully minimize TAT, it is required not only to accomplish faster TP but also to use proven automated technologies for as many tasks as possible, optimizing productivity for the entire workflow.

At present, apart from the time required for TP to finish 100 routine slides from as many blocks, a total of 16.42 hours of non-TP work are needed, divided into 7.9 hours for pre-TP and 8.52 hours for post-TP tasks each with a particular productivity level: 40% is completed by either an auxiliary personnel or with automated instruments, and the remaining 60% is the responsibility of HTs (Table 3) sai [15].

All these non-TP hours have to be incorporated into the analysis of the impact of the processing technology on the overall TAT of each laboratory and their peculiar automated and productivity characteristics when deciding what type of instrument and technology to adopt.

Finally, the TAT for any laboratory has to include also the time dedicated for diagnosis and reporting and that of any additional special procedure required for some cases.

5. Processing time as a fraction of the total workflow

The whole workflow has an overall productivity that can be increased only if more cassettes are processed during a specific time, if less time is needed to process a given cassette load, or if more operations are optimized or automated.

One of the best ways of optimizing the workflow is to analyze the tasks and eliminate bottlenecks with adequate measures usually involving streamlining and personnel specialization and rescheduling around the tasks.

The time to complete non-TP technical tasks depends on the workload but will be a different fraction of the total time depending on the processing technology; it can vary from 27% for totally manual TP to 66% for some conventional automatic instruments or 97% for the most efficient MWA instruments (Table 4).

Under present average general automation conditions, the maximum productivity for non-TP tasks is 10.1 cassettes per hour [15] and the workflow productivity (P) is an inverse function of the duration of the processing period (TP) expressed as a percentage of the total time, as described by the following expression:

$$P = 10.1 - 0.0994\ TP$$
$$r = -0.997^{****}\ [t = 46.86\ (df = 14)\ P < .0001]$$

This correlation is technology and workload independent, meaning similar levels of productivity can be obtained with different technologies and workloads, with expected variations by laboratories depending on their particular characteristics.

For the 7 conventional TP schedules in Table 4, the average productivity is 8.2 ± 0.8 cassettes per hour (cst/h), and that of the 14 using MWA technology is 8.6 ± 0.6 cst/h;

Table 4
Tissue processing (all technologies) and time for associated technical tasks

Manufacturer/instrument	Load	TP		non-TP (h)		Total time (h)	P (cst/h)
		h	% of total time	pre	post		
Hacker MARS (bx)	180	0.5	3	6.3	11.5	18.3	9.8
Milestone Pathos[a] (bx)	210	1.0	5	7.4	13.4	21.8	9.6
Sakura Xpress[a] (1.5 mm)	200	2.0	9	7.0	12.8	21.8	9.2
Shandon Tissue Wave (bx)	75	0.8	10	2.6	4.8	8.2	9.1
Hacker MARS	180	2.0		6.3	11.5	19.8	
Sakura Xpress[a] (1.5 mm)	160	1.75		5.6	10.3	17.7	9.0
Vision Bio Systems Peloris[a,b] (bx)	600	9.0	13	21.0	38.4	68.4	8.8
	100	1.5		3.5	6.4	11.4	
Microm STP 420[a,b]	420	6.0		14.7	26.9	47.6	
Sakura Xpress[a] (1.5 mm)	120	1.50	11	4.2	7.7	13.7	
TBS SHUR/Wave (bx)	30	0.42	12	1.1	1.9	3.4	
Sakura Xpress[a] (1.5 mm)	80	1.25	14	3.0	5.0	9.25	8.6
Hacker RTP (4 mm)	180	3.25	15	6.3	11.5	21.1	8.5
Milestone Pathos[a] (5 mm)	210	4.0	16	7.4	13.4	24.8	
Sakura VIP[a,b] (bx)	100	2.0	17	3.5	6.4	11.9	8.4
	300	8.0	21	10.5	19.2	37.7	8.0
Sakura Xpress[a] (1.5 mm)	40	1.0	20	1.5	2.5	5.0	
TBS ATP1[a,b]	320	9.0	22	11.2	20.5	40.7	7.9
Milestone RHS-1	110	3.3	23	3.9	7.0	14.2	7.7
TBS SHUR/Wave	60	2.0	25	2.1	3.8	7.9	7.6
Microm STP[a,b] 120	120	6.0	34	4.2	7.7	17.9	6.7
Manual TP	200	28.0	57	8.0	12.8	48.8	4.1
	100		73	4.0	6.4	38.4	2.6

Unless otherwise defined by specific sizes (mm), processed specimens are of the average mixed regular sizes for any laboratory. Load indicates processed cassettes. TP indicates tissue processing in hours and % of total time. Non-TP is the time needed for all pre-TP + post-TP technical tasks (hours). Total time is the TP + non-TP times (hours). P indicates overall productivity (load/total time); (bx), small biopsies (1-mm specimens).

[a] Automated instrument; all others are operated manually.

[b] Conventional technology; other instruments are MWA.

these 2 averages are not statistically different for any $P > .30$ for a combined value of 8.6 ± 0.7 cst/h, which is 2.5 times larger than that of manual TP (3.4 ± 1.1 cst/h).

A workload of 210 biopsies will require 20.8 hours of non-TP work, whether they are processed in the MWA Pathos for 1 hour (P = 9.6 cst/h) or in a conventional VIP for 2 hours (P = 9.2 cst/h), showing that non-TP time is load dependent but TP technology and instrument productivity are independent.

This is the rationale behind some laboratory practices where conventional instruments are used twice a day on short cycles (1.5 to 2 hours) for same-day biopsy results, followed by a large overnight load for regular cases.

The ideal situation is for the post-TP tasks to require almost as much time as the pre-TP and TP times combined, with the whole workflow taking the least time possible. Both the TBS SHUR/Wave with 30 small biopsies for a workflow completed in 3.4 hours (8.8 cst/h) and the Sakura Xpress also with 30 prefixed 1.5-mm specimens completed in 4.0 hours (7.5 cst/h) meet these conditions.

The Sakura Xpress allows the addition of up to 40 cassettes every 15 minutes, but because such a batch requires 1.5 hours of pre-TP tasks, either it has to be prepared beforehand, fixation included, or up to 6 HTs have to be simultaneously preparing their share of the 40 cassettes in 15 minutes to be added to the processor in that interval.

The only comprehensive information on the routine use of the Sakura Xpress in a busy laboratory setting indicates that 60% of the annual workload was completed on the same day [13,14]. The data correspond to 271 cassettes per day requiring 7 runs of the Sakura Xpress with 40 cassettes each for a total of 35 hours of workflow, which determined the reported rescheduling of HTs and pathologists alike to allow for this workflow and same-day diagnosis [14].

For a productivity of 7.5 cst/h, existing MWA technology, and 2 hours of workflow, the load to be processed the fastest is 1 of 15 cassettes in 0.42 hours, with 0.53 hours of pre-TP and 1.05 hours of post-TP tasks. Under these conditions, 30 slides from as many cassettes can be ready in 4 hours, but with a much better workflow than with the Xpress in the same time.

For the available MWA technology, another 2 effective loads are 1 of 25 biopsies processed in 2.9 hours (8.6 cst/h) or 50 biopsies in 5.5 hours (9.1 cst/h), but more than 50 cassettes will determine a total workflow time too long for a good continuous operation (Table 5).

Those short workflows could be even more effective using just 1 manual MWA instrument operated successively by several HTs with overlapping schedules, so each is assigned to complete 1 load at a time.

6. Costs considerations

Conventional tissue processors cost from $25 000 to close to $100 000 and MWA instruments vary from the

Table 5
Total workflow duration and processing times for different productivity levels and workloads

P (cst/h)	Total time (h)				TP (% of total time)	TP (h)			
	cassettes per run					cassettes per run			
	10	25	50	100		10	25	50	100
7.5	**1.3**	3.3	6.7	13.4	25	**0.3**	0.8	1.7	3.5
8.1	**1.2**	3.1	6.2	12.4	20	**0.2**	1.6	1.2	2.5
8.6	**1.2**	2.9	5.8	11.6	15	**0.2**	0.4	0.9	1.7
9.1	**1.1**	**2.7**	5.5	11.0	10	**0.1**	**0.3**	0.6	1.1
9.6	**1.0**	**2.6**	**5.2**	10.4	5	**<0.1**	**0.1**	**0.3**	0.5

Bold indicates periods are shorter than technologically possible at present.

manual and multitasks capable ($2000 to $8000) to the more advanced and automated types ($24000 to $250000).

Being a capital expenditure, some return-on-investment indices should be considered. One is a productivity index per instrument (P) calculated by dividing the maximum load by the processing time (cst/h). The instrument cost in thousands of dollars divided by its *P* value offers an index that is more effective the smaller its value.

The cost (in dollars) can also be divided by the maximum permissible load to calculate how many times the instrument has to be used at full capacity for an investment cost of "$1 per cassette." Both are arbitrary indices but calculated and applied consistently to all instruments allowed their comparison (Table 6).

Because both conventional TP and MWA technologies have been proven to be qualitatively equivalent, costs considerations can help in deciding which type of instrument to buy and in selecting the instrument with both better workflow and return-on-investment.

It is a fact that MWA instruments can reduce TP time from 6 to 8 hours during overnight protocols to 0.4 to 4 hours completed the same day, but it is necessary to balance the processing time with all the manual tasks in the workflow.

Large capacity MWA instruments, such as Pathos, are especially valuable for reference laboratories with a constant flow of samples and the required grossing/cassetting personnel, allowing shorter TP protocols to schedule the HTs doing microtomy accordingly.

Conversely, laboratories with approximately 50000 cases per year and an average of 260 blocks per day [16] are economically better off if they prepare 1 or 2 biopsy batches using 1.5 to 2 hours TP cycles with conventional instruments or purchase a small manual MWA instrument of high productivity to take care of the daily biopsies divided into several small batches and leave the larger cases for an overnight conventional run.

Although it is impossible put a price tag on a patient's anxiety or the need to start a treatment on the of basis short notice, laboratories have to analyze the capital investment required to reduce significantly the TAT between reception of the specimen and issuance of the diagnosis report and all the required adjustments that have to do with more than just the small fraction in the workflow time corresponding to TP.

Balancing economic and patient care issues ultimately depends on the type and mission of the histology laboratory.

Table 6
Cost-effectiveness indices for tissue processors

Instrument	Cost	Load	TP (h)	P (cst/h)	Investment effectiveness (Cost/P)	Times used to get to $1 per cassette
Sakura VIP (biopsies)	37	100	2	50.0	0.7	370
		300	8	37.5	1.0	123
Microm SP 12	26	120	6	20.0	6.0	217
	85	420		70.0	1.2	202
TBS SHUR/Wave	25	30	0.42	71.4	0.4	833
		60	2.0	30.0	0.8	416
Miles RHS-1	38	110	3.3	33.3	1.1	345
Pathos (biopsies)	119	210	1.0	210.0	0.6	566
Pathos			4.0	52.5	2.3	
Sakura Xpress (1.5-mm specimens)	250	40	1.00	40.0	6.3	6250
		80	1.25	64.0	3.9	3125
		120	1.50	80.0	3.1	2083
		160	1.75	91.4	2.7	1563
		200	2.00	100.0	2.5	1250

Unless specified by type or size, specimens are of average characteristics. Cost is expressed in thousands of dollars. Load indicates processed cassettes per run; P, productivity (load/TP).

7. Conclusions

Reducing the TAT and increasing the histology workflow productivity is not a function of methodology alone because very different technologies can produce similarly productive results. It is more the result of the balance between the technology-dependent TP time and the time needed to complete all other non-TP tasks.

For a technology that allows rapid TP, such as the MWA processing, the whole workflow time has to be short, and the only way to obtain this goal is by processing small batches in a continuous flow. This goal can be obtained with different types of instruments, and the decision on which to choose has to be based on a return-on-investment index.

When selecting an MWA processor, it would be unwise to just pay attention to a large load processed quickly because that load also determines many hours of non-TP tasks.

Conversely, the strategic use of conventional processors several times during the day in short cycles for small specimens followed by an overnight cycle for regular cases is an improved use of existing resources.

Another advantage of MWA processors is in reagent savings due to fewer steps each requiring fewer reagents, although these potential cost savings can be offset by purchasing expensive proprietary solutions.

Making the whole workflow more productive with TP technology–independent measures, such as rearranging the instruments' location, reducing the number of cassettes per case, establishing well-defined "cutoff" hours, automating as many manual tasks as possible, and rescheduling the personnel, will also guarantee shorter TAT [15].

Microwave assisted TP alone does not ensure optimum results; the way the whole workflow is optimized, however, would.

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