High-Speed Processing of Large Specimens on the Peloris™ Dual Retort Tissue Processor

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Abstract

This paper presents the results of comparative evaluations carried out during field trials in two large public hospitals. These trials set out to examine whether the design features of the Peloris dual retort tissue processor led to reduced processing times for large specimens without compromising quality. In the context of a busy histopathology laboratory, we demonstrate that the introduction of Peloris processing would allow the processing of large, dense specimens in 6 hours leading to the completion of more runs in a working day and the reduction of turnaround times.

Ten sequential six hour and nine hour processing runs were undertaken on a Peloris processor at either 45 °C or 55 °C and the resultant blocks and sections were evaluated for quality using a comprehensive scoring system. Peloris results were compared to those achieved on a matched panel of specimens processed using a processor that represents the industry standard (the Tissue-Tek® VIP™). The results show that for large, dense specimens Peloris can produce results of an equivalent standard to an "overnight" schedule (13 hours) run on a VIP using much shorter schedules. Results also indicate that there is some advantage in processing at higher temperature (55 °C) for both the six and nine hour schedules.

Introduction

A fundamental requirement in the histopathology laboratory is to safely and effectively process specimens. Sectioning of paraffin blocks should be straightforward with the resultant sections being of a high standard demonstrating excellent morphological detail. This applies to the complete range of specimen types and sizes, ranging from tiny, delicate fragments to sizeable wedges of dense tissue. In busy laboratories these requirements must be balanced against increasing demands to reduce turnaround times, to process greater numbers of specimens and to complete more runs in the working day.

The traditional view embraces a conservative approach to processing of all specimen types. A survey of standard histology texts published in the last twenty years reveals an average processing time of 15.7 hours for "routine overnight processing" using xylene as a clearing agent. A survey of local and international laboratories shows that the average duration of a "routine overnight" schedule on an enclosed processor using xylene and excluding any additional fixation steps is 10.3 hours (see Table 1 for details). Of course these laboratories do use shorter schedules for special purposes, where particular types of specimens are to be processed (eg. endoscopic biopsies), or where specimens falling within strict size limits are included.

These field trials were conducted by Vision BioSystems (VBS). Vision Biosystems has since formed part of the Biosystems Division of Leica Microsystems. What is needed then, is a processor which can effectively process a full load of cassettes, say 200 – 300 per retort, containing the complete range of tissues normally included in a "routine overnight run", but doing so in a much shorter time, preferably within the six hour threshold that would allow them to be completed within one working day. The Peloris dual retort tissue processor has been designed with these requirements in mind. Compared to processors representing the current industry standard it has a much faster and more even heating response in the retorts, fast fill and drain actions, a basket design allowing better fluid exchange with reduced reagent carryover, and more effective agitation. These features are designed to reduce processing times. The purpose of this paper is to present the results of comparative evaluations carried out during field trials that set out to show that the design features of Peloris lead to reduced processing times without compromising quality.

Number of Labs	Types of Processor (number)	Schedule Description	Average Number of Steps	Average Number of Steps Excluding Fixation	Average Total Time Excluding Fixation (minutes)	Average Total Time (minutes)
25	Leica TP1050 (20) VIP (4) Shandon (1)	Routine overnight	13	12	620	694

Table 1. Routine overnight processing schedules

Independent field trials were carried out in two large public teaching hospitals in Melbourne, Australia, during 2003 and 2004. They were conducted in the Anatomical Pathology laboratories of the Royal Melbourne Hospital (RMH) (1200 beds) and at Austin Health (AH) (840 beds). The evaluations relating to processing speed were conducted in a similar fashion during both field trials. They involved senior histology scientists from both external laboratories together with scientists from Vision BioSystems, in the assessment of processed blocks and stained sections from a number of processing runs. Using duplicate sets of matched specimens of a variety of dimensions and types, multiple processing runs were carried out using Peloris and an "industry standard" processor in each laboratory (in both cases a Tissue-Tek VIP). Results from Peloris rapid schedules run at 45 °C and 55 °C were compared with VIP standard "routine overnight schedules" currently used in each laboratory which served as a normal control.

Processing at elevated temperatures is widely accepted as a means of accelerating processing by increasing diffusion rates in specimens (1, 2) although some authors believe that this can cause additional shrinkage and staining problems (2, 3). We have certainly not found this to be the case during extensive testing of Peloris. These trials provided an opportunity to compare results of rapid processing at 45 °C and 55 °C and to confirm that there were no adverse effects from using the higher temperatures.

The assessments were carried out independently with staff at the external sites using slightly different scoring methods, with the assessors being unaware of the schedules used to process the specimens. The scoring system used by VBS staff has been extensively used throughout the development and testing of Peloris to evaluate the quality of tissue processing and as a mechanism for optimizing standard processing protocols. A score is calculated by assessing 23 parameters and is expressed as a percentage. The complete details are provided elsewhere (4).

Method

Testing throughout the RMH and AH trials was conducted such that all processing results were compared directly to those of their existing tissue processors (RMH - Tissue-Tek VIP 4, AH Tissue-Tek VIP 5). As far as possible specimens for assessment were kept identical in terms of size, fixation and source on both instruments for each processing run. All specimens were thoroughly fixed. Not every specimen in each run was evaluated. Any specimens that were not for evaluation but loaded into retorts on Peloris to provide a representative case load comparable to the VIP, consisted of pig tissue supplied by VBS. Typical specimens used in assessment of processing and their approximate dimensions are shown in Table 2.

At each laboratory for each of 10 sequential working days, three processing runs were carried out. Two rapid schedules were run on Peloris using the two retorts. Retort A was used for the 6 hour schedule and Retort B for the 9 hour. Runs were carried out daily at either 45 °C or 55 °C. For each 6 and 9 hour run on Peloris a routine overnight run was completed on a VIP containing a normal diagnostic specimen load together with a set of the duplicate test specimens (200 – 250 cassettes). These served as our normal control group. Fresh reagents were provided for run 1 and not changed for the 10 runs on both Peloris and the respective VIP. For each Peloris run, in addition to the test specimens, cassettes containing various pig tissues were included to take the specimen number to 228, which provided an equivalent specimen load to that in the respective VIP processor (75% capacity of each retort). The processing schedules used are shown in Tables 3 and 4. Figure 1 illustrates the difference in step times between the various schedules used. Note that the total pump and drip times in the Peloris schedules are considerably shorter than those of the VIP.

Royal Melbourne Hospital (RMH)		Austin Hospital (AH)	
Tissue	Dimensions (mm)	Tissue	Dimensions (mm)
Intestine	15 x 10 x 5	Intestine	30 x 8 x 5
Liver (pig)	25 x 15 x 5	Intestine	20 x 15 x 5
Spleen	15 x 10 x 5	Liver (pig)	20 x 20 x 5
Lung	25 x 15 x 5	Liver	30 x 25 x 5
Kidney (pig)	15 x 10 x 5	Lung	20 x 20 x 5
Heart (pig)	20 x 15 x 5	Lung	20 x 15 x 5
Thyroid	20 x 10 x 5	Kidney (pig)	20 x 15 x 5
Skin	30 x 25 x 5	Kidney	15 x 15 x 5
Breast	30 x 25 x 5	Heart (pig)	20 x 20 x 5
Prostate	Chips	Heart	20 x 15 x 5

Table 2. Typical specimens used in assessment of processing

	Schedule	P1	P2				
		9 Hour Xylene	6 Hour Xylene				
Step No.	Reagent	Time (minutes)	Time (minutes)	Drip Time (seconds)	Temp ° C	P/V	Stir
1	Formalin	0	45	10	45 or 55	Off	Med
2	70% ethanol	5	10	10	45 or 55	Off	Med
3	90% ethanol	10	10	10	45 or 55	Off	Med
4	100% ethanol	15	15	10	45 or 55	Off	Med
5	100% ethanol	40	20	10	45 or 55	Off	Med
6	100% ethanol	50	20	10	45 or 55	Off	Med
7	100% ethanol	50	35	10	45 or 55	Off	Med
8	xylene	35	10	10	45 or 55	Off	Med
9	xylene	40	25	10	45 or 55	Off	Med
10	xylene	50	35	10	45 or 55	Off	Med
11	Paraffin wax	35	25	10	60	Vac	Med
12	Paraffin wax	50	35	10	60	Vac	Med
13	Paraffin wax	65	45	10	60	Vac	Med
	Total step time	505	330				
	Total processing time	531 (8.9 hours)	356 (5.9 hours)				

Table 3. Peloris processing schedules

	Schedule	V1 (RMH)			Schedule	V2 (AH)		
		13 Hour Xylene				13 Hour Xylene		
Step No.	Reagent	Time (minutes)	Temp ºC	P/V	Reagent	Time (minutes)	Temp ºC	P/V
1	Formalin	30	40	Yes	Formalin	120	45	Yes
2	Formalin	30	40	Yes	70% Ethanol	30	40	Yes
3	70% Ethanol	30	40	Yes	90% Ethanol	30	40	Yes
4	95% Ethanol	45	40	Yes	100% Ethanol	60	40	Yes
5	100% Ethanol	45	40	Yes	100% Ethanol	60	40	Yes
6	100% Ethanol	75	40	Yes	50/50 Eth/Xyl	60	40	Yes
7	50/50 Eth/Xyl	90	40	Yes	100% Ethanol	60	40	Yes
8	100% Ethanol	75	40	Yes	Xylene	30	40	Yes
9	Xylene	60	40	Yes	Xylene	60	40	Yes
10	Xylene	90	40	Yes	Xylene	60	40	Yes
11	Wax	60	58	Yes	Wax	30	58	Yes
12	Wax	60	58	Yes	Wax	30	58	Yes
13	Wax	60	58	Yes	Wax	60	58	Yes
14	Wax	0	58	Yes	Wax	60	58	Yes
	Total step time	750				750		
	Total processing time	810 (13.5 hours)				810 (13.5 hours)		

Table 4. Tissue-Tek VIP processing schedules

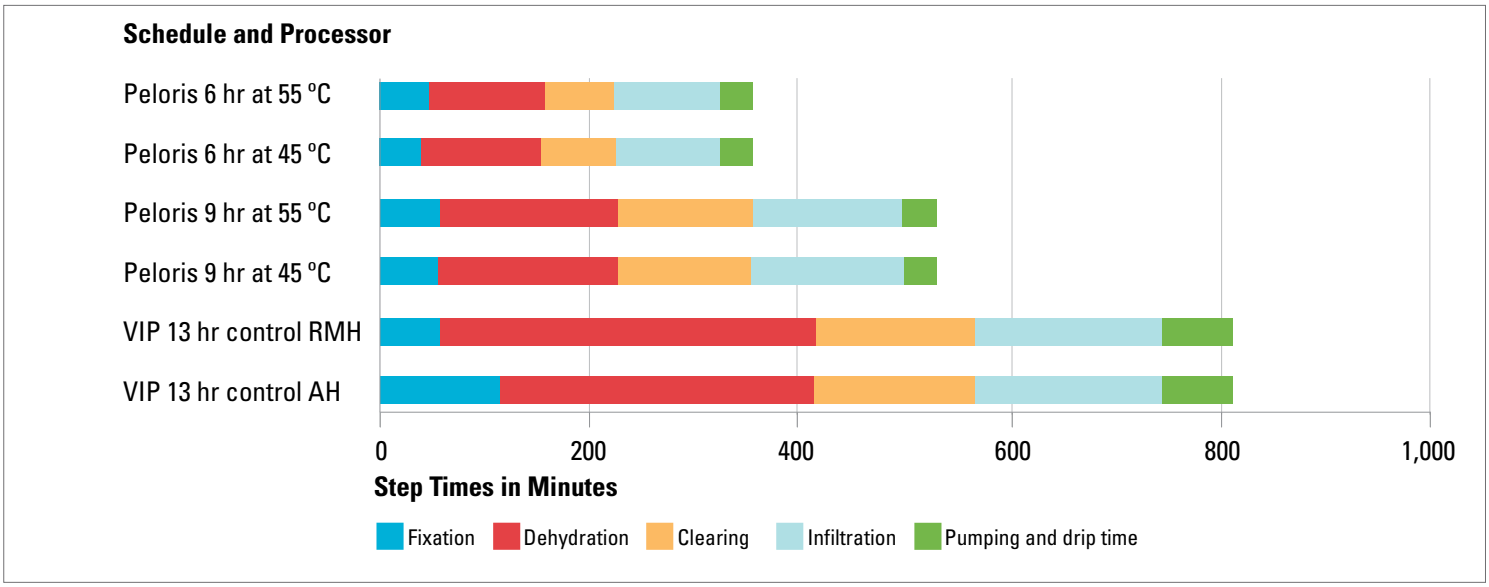

Figure 1. Routine overnight processing schedules

After each processing run the specimens were embedded, sections cut and stained with H&E using the standard methods of each laboratory. During microtomy all blocks were assessed for ease of sectioning and other parameters (4). All test sections were screened on-site by hospital scientists to check that they were of an overall satisfactory standard. Sections were deemed to be satisfactory if they scored 1 or 2 on a simple three point scale (0, 1, or 2).

A representative sample of blocks and slides from the test group of at least 4 per run, were scored by VBS staff according to the full VBS protocol (4). In using this protocol any specimen which scores < 50% in any single parameter is considered a "fail". Specimens scoring between 65% and 75% are considered to be of a good standard for diagnosis. Any specimen scoring above 80% is considered to be of exceptional quality.

Results

Table 5 shows the combined results from 20 processing runs (10 runs at RMH and 10 runs at AH). Figure 2 is included to demonstrate the consistent quality of processed blocks over 10 days for five consecutive runs, at either 45 °C or 55 °C, in comparison with the VIP control. Figures 3 and 4 are micrographs showing typical fields in the processed tissues.

Schedule	Number of Runs	Number Satisfactory/Number of Test Slides Screened (RMH & AH)	Number Passed/Number Test Slides Scored (VBS)	Average Score
RMH Evaluation				
Peloris 6hr at 45 °C	5	60/60	26/26	73%
Peloris 9hr at 45 °C	5	60/60	26/26	72%
Peloris 6hr at 55 °C	5	60/60	28/28	75%
Peloris 9hr at 55 °C	5	60/60	28/28	75%
VIP 13 hr control	10	88/90	49/51	73%
AH Evaluation				
Peloris 6hr at 45 °C	5	52/54	21/22	68%
Peloris 9hr at 45 °C	5	54/54	22/22	69%
Peloris 6hr at 55 °C	5	54/54	22/22	78%
Peloris 9hr at 55 °C	5	54/54	22/22	79%
VIP 13 hr control	10	78/78	24/24	75%
Combined results RMH and AH				
Peloris 6hr at 45 °C	10	112/114	47/48	70%
Peloris 9hr at 45 °C	10	114/114	48/48	71%
Peloris 6hr at 55 °C	10	114/114	50/50	77%
Peloris 9hr at 55 °C	10	114/114	50/50	77%
VIP 13 hr control	20	196/198	103/105	74%

Table 5. Combined results from 20 processing runs

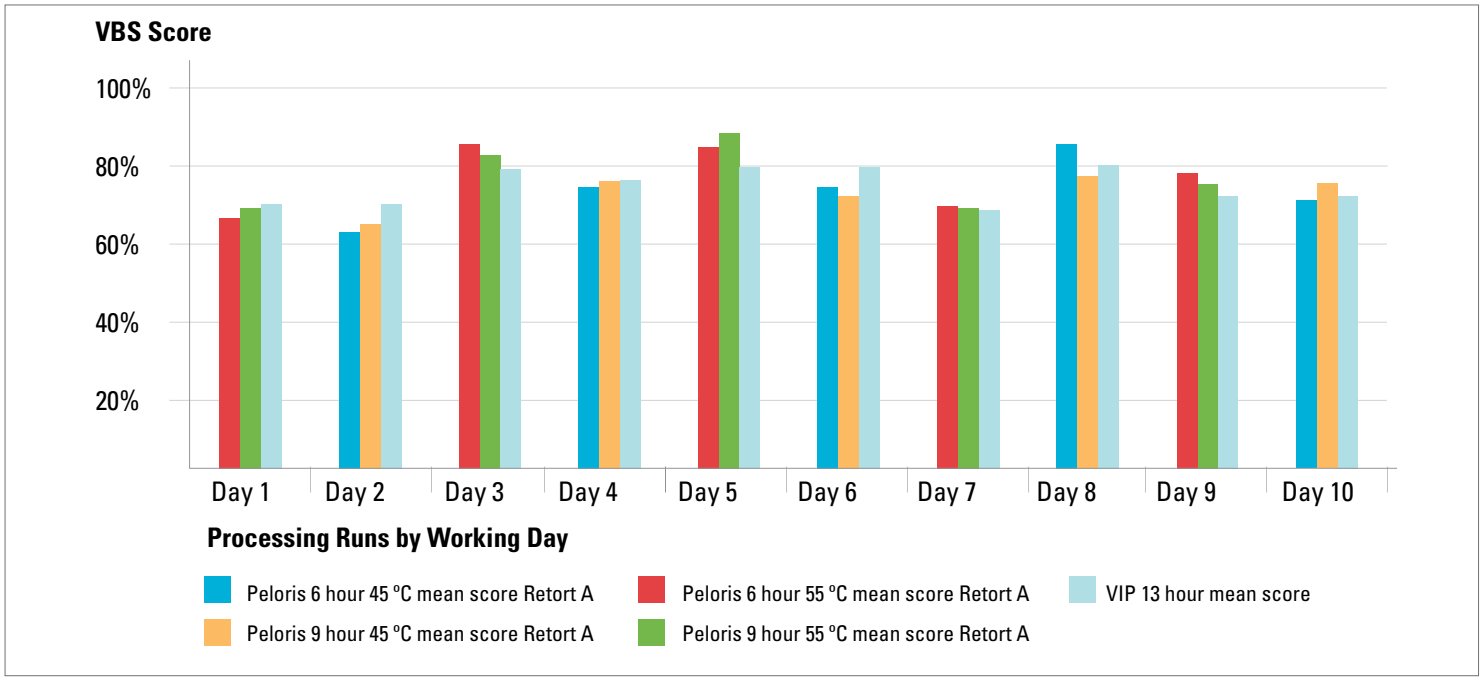

Figure 2. Graph showing the consistency of results of sequential runs for 10 days.

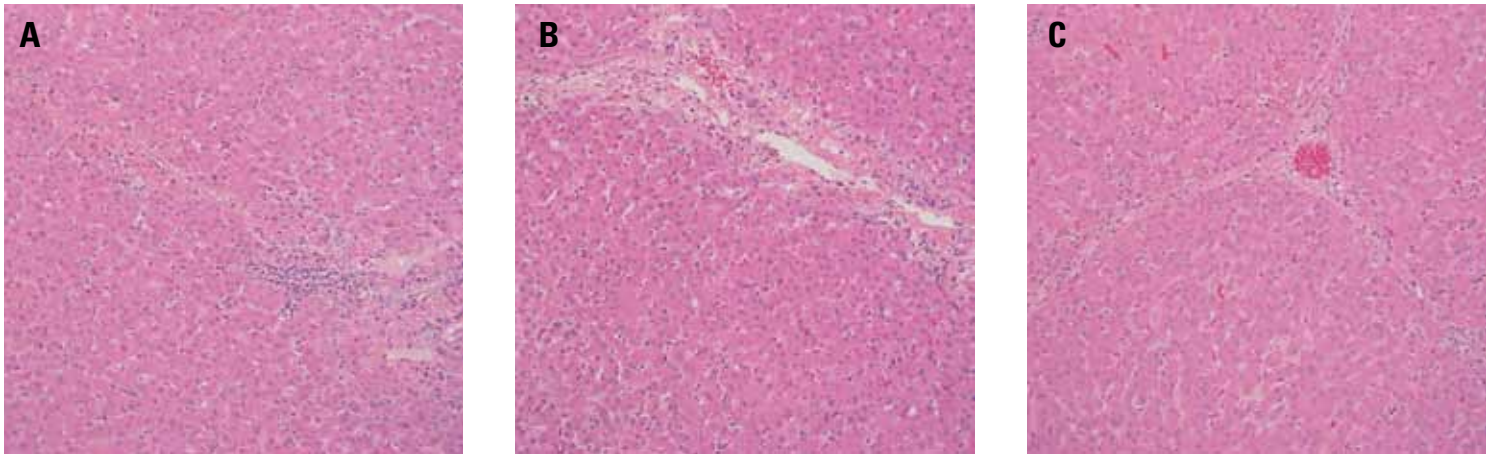

Figure 3. A comparison of typical H&E stained sections of pig liver produced using different processing schedules on three matched specimens from the same case. **A** Peloris 6 hour at 55 °C, **B** Peloris 9 hour at 55 °C and **C** VIP overnight control schedule. Note the well-preserved lobules showing minimal shrinkage with no cracking or separation from the portal connective tissue in each case. There is no discernable difference in morphological detail or staining quality between the 6 and 9 hour 55 °C runs and the 13 hour control.

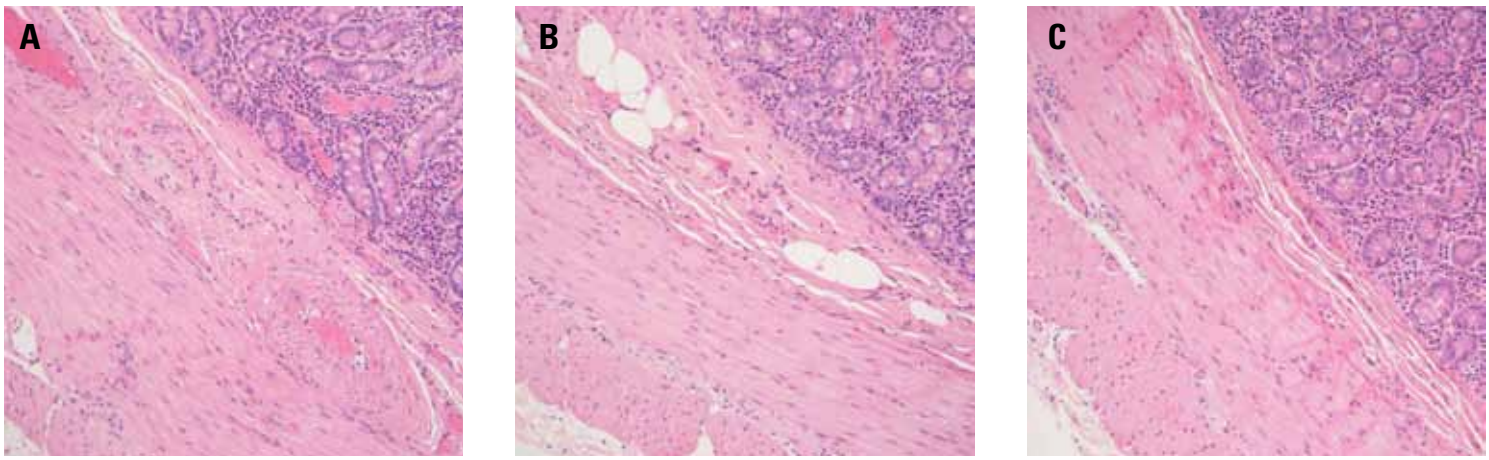

Figure 4. A comparison of typical H&E stained sections of small intestine produced using different processing schedules on three matched specimens from the same case. **A** Peloris 6 hour at 55 °C, **B** Peloris 9 hour at 55 °C and **C** VIP overnight control schedule. Note the well-preserved intestinal glands and well-defined nuclei in each. There is no discernable difference in morphological detail or staining quality between the 6 hour and 9 hour runs at 55 °C and the 13 hour control.

Discussion

The results show that Peloris can process large specimens to an equivalent standard to an "overnight" 13 hour schedule run on an industry-standard processor such as the VIP using shorter schedules – of either 6 or 9 hours. As can be seen in Table 2, the specimens used were relatively large, having characteristics that would mean in most laboratories they would be processed on on "overnight" run. The results also indicate that there is some advantage in processing at the higher temperature (55 °C) for both the 6 hour and 9 hour schedules. It is likely that for even larger and denser specimens than those used here, this improvement in processing quality would be more pronounced.

We consider the overall quality of the sections produced in these field trials to be satisfactory. When looking at the magnitude of the VBS scores, it must be remembered that, as well as reflecting the quality of processing achieved, they are limited by the initial quality of specimen fixation. As is always the case in histopathology laboratories some of the specimens showed sub-optimal fixation particularly noticeable in some larger pieces of tissue that were intentionally chosen for these trials. However, because we used matched sets of specimens for assessment we believe our results are unbiased.

Unlike other processors, in Peloris the chosen processing temperature in the retort is achieved very rapidly. This is particularly important if accelerated processing is to be achieved using short step times. It may explain why, in some processors, higher temperatures appear not to shorten processing times to the extent that is possible with Peloris.

Figure 2 indicates that rapid processing on Peloris produces very consistent results overall, at least the equivalent of the VIP. These results were achieved without changing reagents on Peloris. It should also be noted that processing on Peloris was done using the two retorts. Both the 6 and 9 hour schedules were run at the same time, the end-points being timed so that the embedding could be done sequentially. Figure 2 shows the consistency in the results achieved in each retort. This clearly demonstrates the versatility of the two-retort design that allowed the processing of at least twice as many specimens as the VIP in a shorter time.

Conclusion

The results of comparative evaluations carried out during field trials clearly show that the design features of Peloris lead to reduced processing times without compromising quality. Tissues processed at both 45 °C and 55 °C produced consistent, high-quality results with evidence that the higher temperature is an advantage for both the 6 hour and 9 hour schedules. The trials were completed efficiently, causing little disruption in the participating laboratories, due in large part to the versatility of Peloris in possessing two retorts that could be used simultaneously.

In the context of a busy histopathology laboratory, our results indicate that the introduction of Peloris processing would allow large specimens to be processed in six hours, leading to the completion of more runs in a working day and the reduction of turn-around times.

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