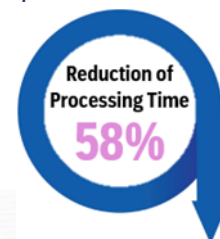


## WHITE PAPER

# 5-Hour Fatty Tissue Processing Protocol Utilizing the ActivFlo System

In combining Leica's Routine I ActivFlo cassette, Paraplast paraffin, standard reagents, and the HistoCore PELORIS 3 Tissue Processor, a 5-hour xylene tissue processing protocol has been developed for routine-sized specimens.

Histologists know all too well the challenges of processing surgical samples, especially fatty tissue specimens such as breast cases. In the current environment of staff shortages and consistently growing caseloads, the pressure of meeting turn-around-times has increased. Utilizing unique features of the HistoCore PELORIS 3 Tissue Processor and ActivFlo Routine I cassettes, the creation of this novel patent pending protocol reduces processing time of formalin-fixed tissue by up to 58% (or seven hours) when compared to standard protocols.



It is anticipated that this reduction in processing time will improve the throughput to reaching a comprehensive pathology report in under 24 hours. The study laid out below describes verification activities performed to support a 5-hour protocol being successfully used in processing several tissue types, including breast, of a recommended size of 1.0 x 1.0 x 0.4cm on the HistoCore PELORIS 3 Tissue Processor in combination with ActivFlo Routine I cassettes.



## Verification Study Protocol

A statistically relevant sample size of 240 formalin-fixed test specimens comprised of breast, GI and uterine tissue were grossed to approximately 1.0 x 1.0 x 0.4 cm and placed into ActivFlo Routine I cassettes. Four separate 5-hour processing runs were completed with 60 test cassettes in each basket. After processing was completed, tissue samples were embedded, trimmed until the tissue was accessible, and placed onto ice for no more than 30 minutes before sectioning. Each tissue block was sectioned by laying out a ribbon of at least 3-5 sections on a water bath before being mounted onto a slide and left to air dry overnight. Subsequent HistoCore SPECTRA H&E staining and microscopic examination was then conducted.



Figure 1. Breast specimen tissue blocks from Verification testing.

Each tissue block and accompanying tissue section slide was graded on 26 different parameters developed by Leica Biosystems, some of which included: cutting, mounting, microscopic quality, and block storage stability. Individual sections with a total score equal to or greater than 80% are to be considered of high quality. Overall acceptance criteria included a minimum passing rate of 95% when the number of test specimens that passed were averaged against the total sample size of 240. Sections were also analyzed by two internal Leica Biosystems Pathologists, Dr. Melissa Alexander and Dr. David Ferber, to confirm internal scoring system results and to provide further feedback.

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## Verification Study Results

Scoring results met the 95% acceptance criteria when the 227 passing test samples were averaged against the 240 total sample size, demonstrating a statistically relevant pass rate. Average scores of all test samples for each tissue can be seen below:

Breast Tissue Blocks	AVG
Cutting	75.0%
Mounting	82.5%
Block stability on storage	92.5%
Physical quality of section	79.0%
Quality of tissue preservation	89.1%
Quality of staining (chemical)	90.9%
Section prep & block storage	83.2%
Microscopic assessment	86.4%
Total score	84.9%

GI Tissue Blocks	AVG
Cutting	94.1%
Mounting	82.3%
Block stability on storage	99.7%
Physical quality of section	80.3%
Quality of tissue preservation	93.6%
Quality of staining (chemical)	90.6%
Section prep & block storage	91.9%
Microscopic assessment	88.2%
Total score	90.1%

Uterine Tissue Blocks	AVG
Cutting	93.6%
Mounting	88.1%
Block stability on storage	97.8%
Physical quality of section	76.5%
Quality of tissue preservation	94.4%
Quality of staining (chemical)	92.3%
Section prep & block storage	93.2%
Microscopic assessment	87.7%
Total score	90.5%

With each tissue type scoring above 80% overall, this demonstrates that breast, GI, and uterine tissue that underwent processing with the 5-hour processing protocol in ActivFlo Routine I cassettes were of high quality.

When viewed by pathologists, the images were found to display well preserved nuclear and cytoplasmic detail which allowed for a definitive primary read. Below are two images with comments from Dr. Melissa Alexander, and Dr. David Ferber regarding tissue-specific attributes.

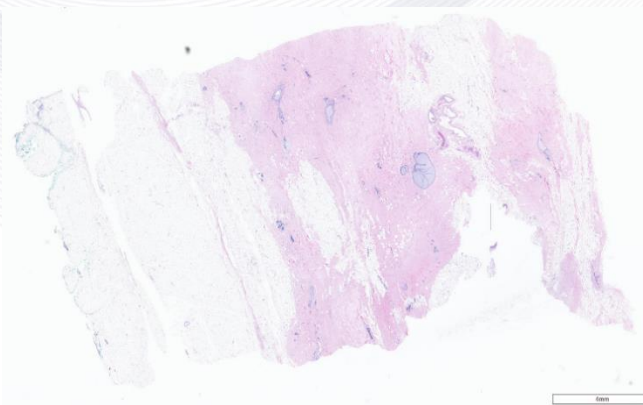


Figure 2. Dr. Melissa Alexander describes this breast tissue specimen as “displaying intact fibroadipose tissue, including an intact inked margin without tissue dropout or missing areas. This is critical for margin assessment and accurate distance measurement.”

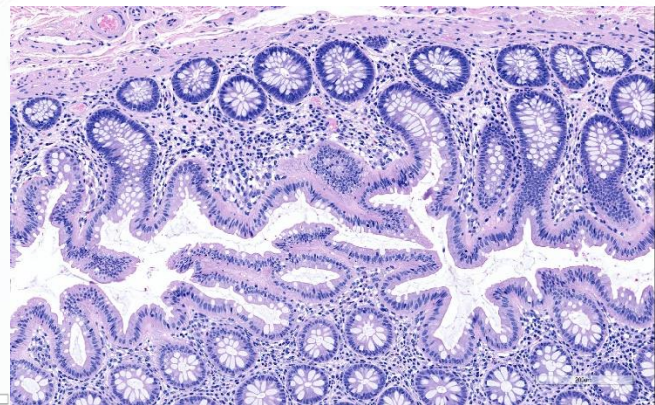


Figure 3. Dr. David Ferber describes this colon tissue specimen as “demonstrating an accurate representation of the various components of the colonic mucosa with easily identifiable nuclear and cytoplasmic detail. This preservation would not require deeper cuts or recuts to obtain a primary evaluation.”

## WHITE PAPER

When compared to current processing protocols for routine specimens in the HistoCore PELORIS 3 Premium Processing System User Manual, the 5-hour processing protocol is found to reduce processing time by up to seven hours. This decrease allows laboratories to put slides (both physical and digital) into pathologists' hands sooner, as well as workflows downstream such as advance staining. This revolutionary protocol is the first step in continued innovation with the ActivFlo System on the HistoCore PELORIS 3 tissue processor.

### Product Numbers

ActivFlo Routine I Cassettes- Product # 39LC-500-1

Paraplast Regular- Product # 39601006

HistoCore PELORIS 3- Product # 45.0001

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### References

1. HistoCore PELORIS 3 User Manual Rev A09 © Leica Biosystems Melbourne Pty Ltd 2021, p. 156.