Tissue Processing of Fresh Patient Normal and Tumor Specimens using the Epredia Revos Tissue Processor Results in Excellent Morphology and Isolation of High Purity Nucleic Acids

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Background

Introduction

Turnaround time for any pathology laboratory is critical and depends on the preparation and diagnosis of the pathological lesions. The rapidity advantage of the clinician in treating acutely ill patients influences the work practice of the pathologist. With the advent of modernization, tissue processing is modified from the point of tissue removal to embedding for instant histopathological diagnosis by various techniques or methods. In this regard, the Epredia Revos is an automated tissue processor designed for all types of tissue processing; the Epredia Revos tissue processor has been shown to process a variety of surgical samples ranging from 2 mm to 8 mm rapidly. Given this, we tested the ability of the Epredia Revos rotational tissue processor to process fifteen (15) fresh normal and eight (8) tumor surgical specimens adequately and provide access to high-quality nucleic acids (DNA and RNA) and excellent morphology for pathological review.

Hypothesis

Epredia Revos rotational tissue processor can provide excellent tissue morphology, sufficient amounts of nucleic acids for required downstream testing and better-quality nucleic acids for next-generation sequencing, compared to Sakura Tissue-Tek VIP 5 tissue processor and Leica HistoCore PELORIS 3 tissue processors.

Methods

A total of 15 normal tissues and 8 tumor tissues were acquired as fresh surgical specimens and placed into 10% NBF within a few minutes after surgical resection. These tissues were grossed and cut into three equal parts and processed on the Epredia Revos tissue processor using the routine surgical protocol setting. After processing, tissues were embedded into low melting point paraffin and cut for histology slides or used for nucleic acid isolation. Up to 40 µm of tissue were processed using the Qiagen FFPE kit, following the manufacturer's instructions to isolate both DNA and RNA. Additional slides were stained for H&E and digitized using the E1000 Dx Digital Pathology Solution[1].

Acquiring patient material - tumor and normal

Fresh surgical tissues were placed in 15 ml of 10% NBF (fixed overnight @ 4°C). All tissues were weighed and using a scalpel cut into three equal parts all weighing the same (approx. 20-100mg of tissue).

- Samples were divided into three (3) equal pieces of equal weight, divided samples then processed on each of the three tissue processors: Epredia Revos, Sakura Tissue-Tek VIP 5, and Leica HistoCore PELORIS 3>
- After processing all samples were embedded, and microtomy was performed using Epredia HM 355S microtome.
- Samples were then processed to Isolate DNA/RNA from 40 µm of sample and repeated as needed.
- Its important to note, 40 µm of sample were processed in the standard clinical isolation kit-Qiagen according to recommended manufacturers specifications.
- Hematoxylin and Eosin (H&E) staining was performed using a Leica Auto Stainer XL (WFIRM staff prepares all the reagents needed for the autostainer fresh weekly as recommended in operators manual).

Protocol for FFPE Nucleic Acid Isolation from Normal/Tumor tissue samples

Excise tissue samples from patient.

Immediately place tissue sample into 10% NBF (3x's volume of the tissue) and allow to fix for a maximum of 24 hours for proper fixation.

Remove sample, place into tissue cassettes and then move into 70% EtOH until ready to process.

Process samples in the Epredia Revos tissue processor on the recommended routine surgical program.

Embed tissue samples using a low melting point paraffin.

Store the FFPE block at 4°C until ready to use.

Cut four (4) sections, each at a thickness of 10 µm and place into a 1.5 mL microcentrifuge tube containing 640 µl of deparaffinization solution (Qiagen).

Proceed with the AllPrep DNA/RNA FFPE Kit (Qiagen Cat# 80234) for isolation of nucleic acids as per manufacturer directions.

Elute RNA in 20 µL, and the DNA in 50 µL.

Nanodrop all samples to obtain RNA/DNA concentrations and 260/280 ratios.

Routine Surgical Program	Standard Clinical Program	Standard Clinical Program
Epredia Revos	**Sakura VIP 5**	**Leica Peloris 3**
75% EtOH for 19 min	70% EtOH for 45 min	70% EtOH for 45 min
90% EtOH for 19 min	80% EtOH for 45 min	80% EtOH for 45 min
95% EtOH for 26 min	95% EtOH for 45 min	95% EtOH for 45 min
100% EtOH for 19 min	100% EtOH for 19 min	95% EtOH for 60 min
100% EtOH for 19 min	100% EtOH for 45 min	100% EtOH for 45 min
100% EtOH for 28 min	100% EtOH for 45 min	100% EtOH for 60 min
Xylene for 21 min	Xylene for 45 min	Xylene for 35 min
Xylene for 21 min	Xylene for 45 min	Xylene for 45 min
Xylene for 21 min	Paraffin for 45 min	Paraffin for 45 min
Paraffin for 21 min	Paraffin for 1 hour	Paraffin for 45 min
Paraffin for 21 min	Paraffin for 1 hour	Paraffin for 45 min
Paraffin for 21 min		

[1]E1000 Dx Digital Pathology Solution is 510k pending.

Figure 1: Tissue morphology from the collected specimen used in this study

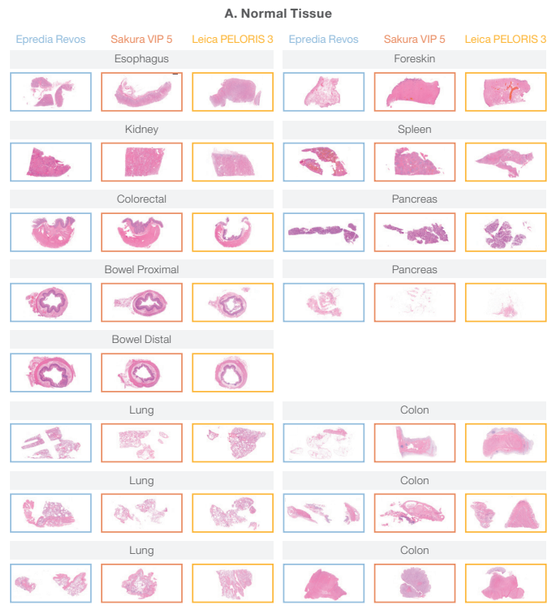


Fig 1A. Normal tissue morphology of the collected specimen used in this study. Fresh surgical specimens were collected and processed using the Epredia Revos tissue processor(Blue Rectangle), Sakura Tissue-Tek VIP 5 (orange rectangles), and Leica HistoCore PELORIS 3 tissue processor (yellow rectangles) using the routine surgical protocol. After processing, samples were embedded cut, and slides were stained with H&E. As shown above, we collected approximately 15 normal tissue specimens, including 2 normal colon and 3 normal lung specimens. We performed automated H&E staining, and the resultant slides were imaged using the E1000 Dx Digital Pathology Solution. Epredia Revos tissue processor exhibited excellent tissue processing and good morphology compared to tissue processed on the Sakura VIP 5 and Leica Peloris 3 tissue processors.

Fig 1B. Tumor tissue morphology of the collected specimen used in this study. Fresh surgical specimens were collected and processed using the Epredia Revos (Blue), Sakura Tissue-Tek VIP 5 Tissue processer (orange), Peloris 3, Leica Biosystems tissue processor (yellow) with the Routine Surgical protocol. After processing, samples were blocked and cut using an Epredia Microtome, aand specimen slides were created for histology. As shown above, we collected approximately 8 tumor specimens that included various tissues. We performed automated H&E staining, and the resultant slides were imaged using the E1000 Dx Digital Pathology Solution. The tissue processed on the Epredia Revos tissue processor exhibited excellent tissue processing and overall good morphology compared to Sakura Tissue-Tek VIP 5 and Leica HistoCore PELORIS 3 processed tissues.

Figure 2A: Revos Normal Tissue DNA concentration (total ng) and 260/280 ratios

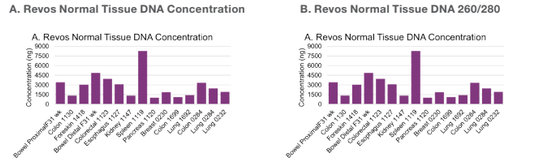


Fig 2A. Normal tissue DNA concentration and 260/280 ratio on 15 fresh tissues. Fresh surgical samples were placed in 10% NBF fixed overnight and processed in the Epredia Revos tissue processor using the Routine Surgical protocol. After processing, tissues were embedded and cut, and 40 µm of tissue was processed to isolate nucleic acids following the manufacturer's protocols. As shown on the left panel (panel A) we isolated and collected more than 1 microgram of total DNA from all tissues, and importantly, when performing 260/280 measurements, an indicator of DNA purity, most tissues exceeded the minimum threshold of a 2 ratio (red line), demonstrating excellent purity of the collected DNA samples.

Figure 2B: Revos Normal Tissue RNA concentration (total ng) and 260/280 ratios

Fig 2B. Normal tissue RNA concentration and 260/280 ratio on 15 fresh normal tissues. Fresh surgical samples were placed in NBF and fixed overnight. Then, they were processed in the Epredia Revos tissue processor using the Routine Surgical protocol. After processing, tissues were embedded and cut, and 40 µm of tissue was processed to isolate nucleic acids following the manufacturer's protocols. As shown on the left panel (panel A), we isolated and collected more than 0.5 micrograms of total RNA from all tissues; we then performed 260/280 measurements (panel B), an indicator of RNA purity; most tissues had adequate purity of RNA samples.

Figure 3A: Revos Tumor Tissue DNA concentration (total ng) and 260/280 ratios

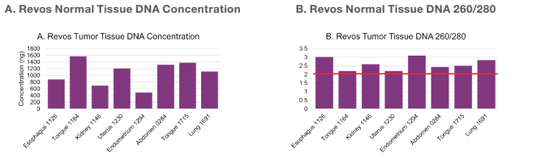


Fig 3A. Tumor tissue DNA concentration of normal or tumor tissue samples processed using the REVOS tissue processor. This chart presents total DNA (panels A), or 260/280 ratio(panel B). We collected 8 fresh surgical specimens from the OR and immediately fixed them in 10% NBF overnight (@4°C). These samples were processed using the Routine surgical protocol on the Epredia Revos tissue processor, and samples were blocked and cut for nucleic acid purification or histology. As shown in Panel A, we collected greater than 0.4 micrograms of total DNA. From each sample, and notably, the 260/280 ratio, an indicator of sample purity, was greater than a ratio of 2 (red line), suggesting excellent purity of the DNA

Figure 3B: Revos Tumor Tissue RNA concentration (total ng) and 260/280 ratios

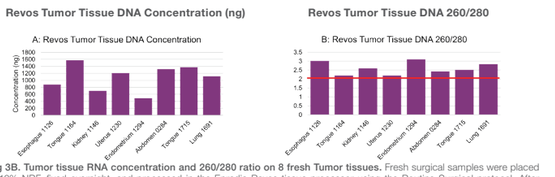
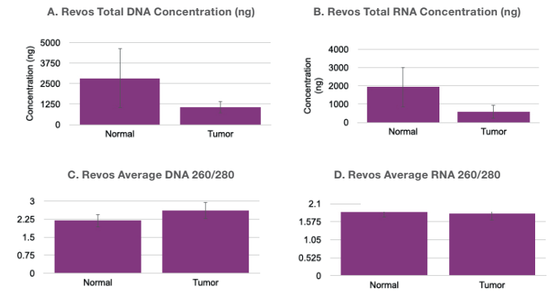


Fig 3B. Tumor tissue RNA concentration and 260/280 ratio on 8 fresh Tumor tissues. Fresh surgical samples were placed in 10% NBF, fixed overnight, and processed in the Epredia Revos tissue processor using the Routine Surgical protocol. After processing, tissues were embedded and cut, and 40 µm of tissue was processed to isolate nucleic acids following the manufacturer's protocols. As shown on the left panel (panel A), we isolated and collected more than 0.2 micrograms of total RNA from all tissues; we then performed 260/280 measurements (panel B), an indicator of RNA purity; most tissues had adequate purity of RNA samples.

Results

The routine surgical protocol on the Epredia Revos tissue processor took under 7 hours for each sample run. This run is approximately 2-4 hours shorter than the processing times of the current clinical competitors. The Epredia Revos tissue processor resulted in excellent tissue processing with no unprocessed tissues or tissue damage from all tissues (normal and tumor) observed macroscopically and microscopically. We were able to obtain excellent morphology on all tissues (normal and tumor), as observed microscopically by H&E stained slides processed on the Epredia Revos tissue processor, using the routine surgical protocol setting. Importantly, we could isolate DNA and RNA from the FFPE samples, with enough material (> 0.5 micrograms of total DNA) for Next-Gen sequencing or molecular testing. The 260/280 ratio of the purified DNA was greater than 2 units, suggesting excellent purity. Also interesting was that the total amount of tumor DNA was less than half of the DNA concentration from normal tissue. On the research side, we could isolate sufficient amounts of RNA of purity necessary to use for expression library creation.



Complied nucleic acid data from all tissue samples processed using the REVOS tissue processor. Total median DNA (panels A&C) or total median RNA (panels B&D) +/- STD from all tissues (both normal and tumor)are presented on graphical format. As seen in panel A, median DNA was greater than 2.5 micrograms in normal tissues versus 1 microgram of total DNA in tumor tissue. Median purity measurements (260/280 ratio) (panel C) of DNA demonstrated a greater than 2 ratio(red line), suggesting excellent purity of DNA. We also determined the total median amount of RNA in normal and tumor tissue (panel B), as well as the median 260/280 ratio for RNA in both normal and tumor tissue (panel D).

Conclusions

Both normal and tumor **tissue processed using the Epredia Revos rotational tissue processor** on the routine surgical program setting **demonstrated excellent tissue processing and excellent tissue morphology**, as observed by microscopic examination of the H&E-stained slides.

The **Epredia Revos routine surgical program setting utilized was more than two hours shorter per run** than the Sakura Tissue-Tek VIP 5 and Leica HistoCore PELORIS 3 tissue processors.

We observed better morphology and better-quality H&E stained slide images from tissue processed using the Epredia Revos Rotational Tissue Processor over the Sakura Tissue-Tek VIP 5 and Leica HistoCore PELORIS 3 tissue processors. We were able to isolate sufficient quantities of both DNA (>0.5 micrograms) and RNA (> 0.4 micrograms) from Revos-processed tissue that had excellent 260/280 ratios (> 2), suggesting excellent purity of the samples. These DNA samples should be enough starting material to perform next-generation sequencing and downstream molecular testing. Future studies hold great promise, as we plan to conduct immunohistochemical staining for tumor-related molecular markers and phenotypic analysis. The continued collection of additional surgical specimens will further enhance our confidence in DNA/RNA isolation and next-generation sequencing.

References

1. Singla K, et. al., Comparative evaluation of different histoprocessing method. Int. J of Health Sciences., 2017, 11(2), 28-34.
2. Sultana. A., et. al. Tissue Processing Techniques: a comparative Study. Eur. Chem. Bull. 2023., 12 (special issue 10) 2831-2846.
3. Thong, QX., et. al. Epredia REVOS Clinical Study Brief.

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