Defatting Effect of Automated Tissue Processor with Canted Chamber (Revos)

Shinya Abe Epredia Pathology Business Department, PHC Corporation

Masanobu Kitagawa Department of Comprehensive Pathology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University

Miori Inoue Department of Comprehensive Pathology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University

Tomohiro Yoshinaga Epredia Pathology Business Department, PHC Corporation

Kohei Yamamoto Department of Comprehensive Pathology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University

Abstract

In pathology laboratories, enclosed automated tissue processors are routinely used to prepare embedded specimens. However, when fatty tissue such as breast tissue is processed in a tissue processor without defatting pre-treatment, inadequate dehydration is caused by excessive fat in the tissue, making it difficult to prepare a high-quality embedded specimen. To address this problem, fatty tissue is typically pre-treated overnight using a mixture of ethanol and xylene to remove fat. It therefore takes a long time to complete the process to prepare an processed specimen of fatty tissue. In this study, the defatting effect of Revos, an automated tissue processor with canted chamber, and its usefulness for preparation of embedded specimens of fatty tissue were examined. The results of this study indicated that obtaining high-quality embedded specimens of fatty tissue with little cracking of thin sections was possible when the tissue was processed using an Epredia™ Revos™ tissue processor, even without defatting pre-treatment. Processing using Revos, which does not require defatting pre-treatment, is considered to speed up specimen preparation of fatty tissues such as breast specimens.

Objective

Currently, enclosed automated tissue processors are generally used at pathology laboratories to create formalin-fixed paraffin-embedded (FFPE) specimens. Various tissue processors from different manufacturers are commercially available for use during daily operations. However, creating FFPE specimens of fatty tissue such as breast tissue is difficult without defatting pre-treatment of the tissue. In fatty tissue,

water covered with fat remains in the tissue after the dehydration process using ethanol in a general tissue processor, resulting in an inadequately dehydrated specimen. If the specimen is inadequately dehydrated, the preparation of thin sections from a block becomes difficult and such thin sections may crack when floated on water, resulting in difficulty to prepare high-quality specimen for diagnosis. Therefore, defatting is performed in the tissue processor prior to the embedding process,

Typically, this defatting step involves the treatment of tissues overnight with a warmed mixture of ethanol and xylene or methanol and chloroform. Since fatty tissue such as breast tissue requires this pre-treatment step, it takes a long time to complete the process of preparing an embedded specimen. In this study, the tissues were processed using a Revos automated tissue processor with a canted chamber to evaluate the defatting effect.

Method

Specimens

Pork belly was used as the animal specimens. Residual breast cancer specimens collected at Tokyo Medical and Dental University Hospital were used as the human specimens. Use of human specimens was approved by the ethical review board of Tokyo Medical and Dental University Hospital (M 2018-249).

Defatting

Tissue was placed in a mixture of ethanol/xylene (1/1), which was agitated overnight for defatting pre-treatment.

Tissue Processing

An automated tissue processor with a canted chamber (Revos) and a conventional automated tissue processor were used. The processing programs are as follows:

1. 100% Ethanol, 35°C for 1 hour
2. 100% Ethanol, 35°C for 1 hour
3. 100% Ethanol, 35°C for 1 hour
4. 100% Ethanol, 35°C for 1 hour
5. 100% Ethanol, 35°C for 1 hour
6. 100% Ethanol, 35°C for 1 hour
7. Xylene, 35°C for 1 hour
8. Xylene, 35°C for 1 hour
9. Xylene, 35°C for 1 hour
10. Paraffin, 62°C for 1 hour
11. Paraffin, 62°C for 1 hour
12. Paraffin, 62°C for 1 hour

A block was prepared with an embedding process and a thin section was sliced from the block. The thin section was floated on water and observed. HE staining was performed to compare the staining results and observe the tissue orientation.

Results

Examination using pork belly specimens

Pork belly was used as the animal specimen. Pork belly specimens were fixed in 10% neutral buffered formalin and some of the fixed specimens were subjected to defatting pre-treatment. These specimens with and without defatting pre-treatment using the Revos and conventional automated tissue processors were embedded and made into blocks. When the specimens processed using the conventional automated tissue processor without defatting pre-treatment were embedded, the characteristic yellow tone of fat tissue was observed. Defatting pre-treatment could prevent the appearance of this yellow tone. On the other hand, specimens processed using Revos did not demonstrate the characteristic yellow tone of fat tissue, even without defatting pre-treatment, and fat tissue was relatively transparent as in the pre-defatted specimen (Figure 1).

These blocks were sliced to prepare thin sections and the thin sections were floated on water and compared for their condition. Thin sections derived from the block prepared using a conventional tissue processor without defatting pre-treatment cracked when they were floated on water. On the other hand, clean thin sections with hardly any cracks could be obtained from the specimen prepared using Revos, even without defatting pre-treatment (Figure 2).

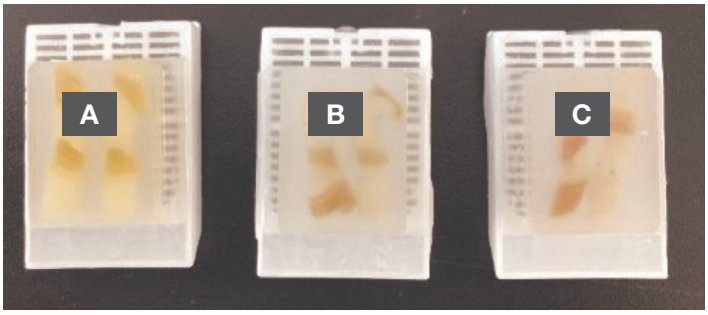

Figure 1. (A) Paraffin block of a pork belly specimen prepared with a conventional tissue processor (without defatting pre-treatment). (B) Paraffin block of a pork belly specimen prepared with a conventional tissue processor (with defatting pre-treatment). (C) Paraffin block of a pork belly specimen prepared with Revos (without defatting pre-treatment). When the specimen was embedded using Revos, the characteristic yellow tone of fat tissue faded and the tissue was transparent, even without defatting pre-treatment.

Figure 2. (A) Thin section of a pork belly specimen prepared with a conventional tissue processor (without defatting pre-treatment). (B) Thin section of a pork belly specimen prepared with a conventional tissue processor (with defatting pre-treatment). (C) Thin section of a pork belly specimen prepared with Revos (without defatting pre-treatment). Specimens prepared using Revos showed fewer cracks even without defatting pre-treatment.

HE staining was also performed on each specimen. A significant difference in staining was not observed between the specimens prepared using the conventional tissue processor and Revos. However, the specimens prepared using Revos had a clearer border between the muscle and fat tissue and showed clear orientation (Figure 3). Based on the above results, it was demonstrated that processing with Revos made it possible to prepare high-quality specimens without defatting pre-treatment.

Examination using human specimens

Next, a study using human breast tissue was conducted. Breast tissue specimens that were already fixed in 10% neutral buffered formalin were used. Some of the fixed specimens were subjected to defatting pre-treatment. The specimens were processed using Revos and a conventional automated tissue processor. When the blocks were prepared and compared, a yellow tone, which was considered to be caused by inadequate dehydration due to residual fat, was observed when the conventional automated tissue processor was used for processing without defatting pre-treatment. However, when defatting pre-treatment was performed before processing with a conventional automated tissue processor, a yellow tone was not observed and the embedded specimen was relatively transparent. On the other hand, the specimen processed using Revos without defatting pre-treatment did not show a yellow tone and was relatively transparent, like the pre-defatted specimens (Figure 4).

These blocks were sliced to prepare thin sections and the thin sections were floated on water and compared for their condition. Thin sections derived from the block prepared using conventional tissue processor without defatting pre-treatment cracked when they were floated on water. On the other hand, clean thin sections with hardly any cracks could be obtained from specimens prepared using Revos, even without defatting pre-treatment (Figure 5).

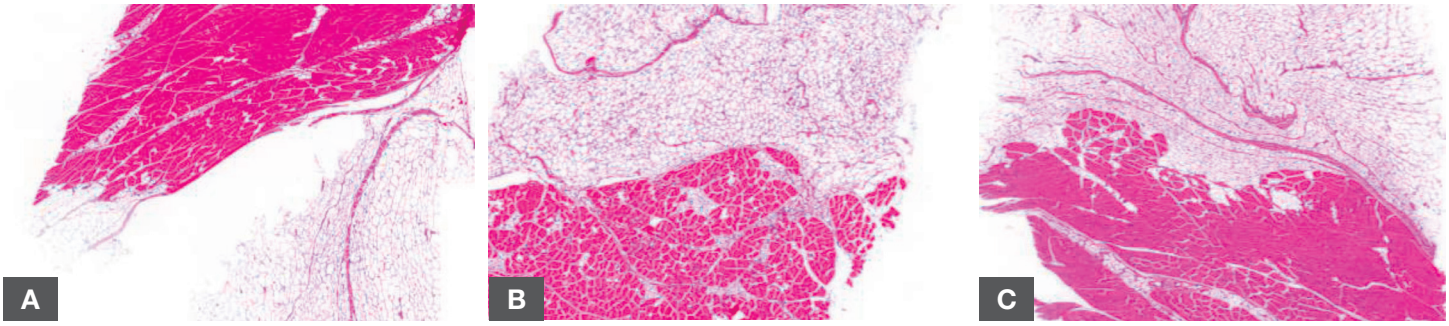

Figure 3. (A) HE stained specimen of a pork belly specimen prepared with a conventional tissue processor (without defatting pre-treatment). (B) HE stained specimen of a pork belly specimen prepared with a conventional tissue processor (with defatting pre-treatment). (C) HE stained specimen of a pork belly specimen prepared with Revos (without defatting pre-treatment). The specimens prepared with Revos had a smaller gap between fat and muscle tissues and showed clear orientation.

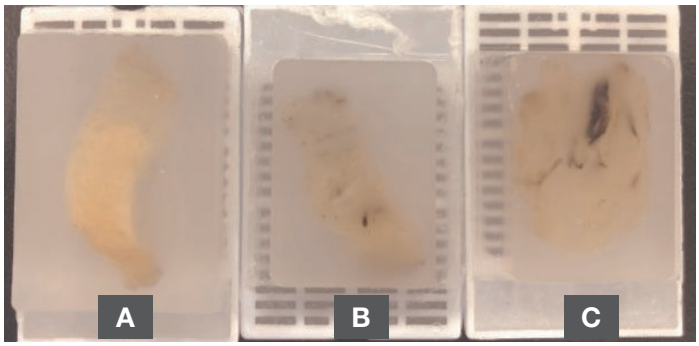

Figure 4. (A) Paraffin block of a breast tissue specimen prepared with a conventional tissue processor (without defatting pre-treatment). (B) Paraffin block of a breast tissue specimen prepared with a conventional tissue processor (with defatting pre-treatment). (C) Paraffin block of a breast tissue specimen prepared with Revos (without defatting pre-treatment).

When the specimen was processed using Revos, the characteristic yellow tone of fat tissue faded and the tissue was transparent, even without defatting pre-treatment.

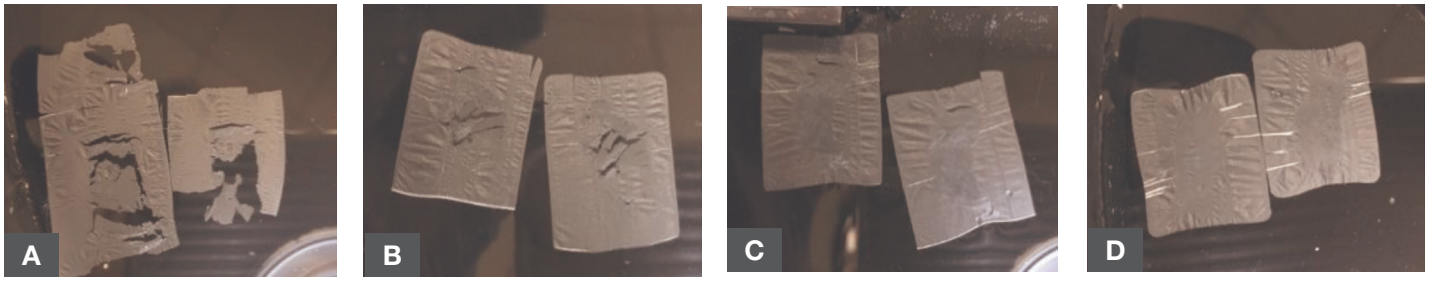

Figure 5. (A, B) Thin section of a breast tissue specimen prepared with a conventional tissue processor (without defatting pre-treatment). (C) Thin section of a breast tissue specimen prepared with a conventional tissue processor (with defatting pre-treatment). (D) Thin section of a breast tissue specimen prepared with Revos (without defatting pre-treatment). Specimens prepared using Revos showed fewer cracks even without defatting pre-treatment.

HE staining was also performed on each specimen. The specimen prepared using Revos had little cracking, resulting in better orientation of the entire specimen. A significant difference in staining was not observed between the specimens prepared using the conventional tissue processor and Revos (Figure 6).

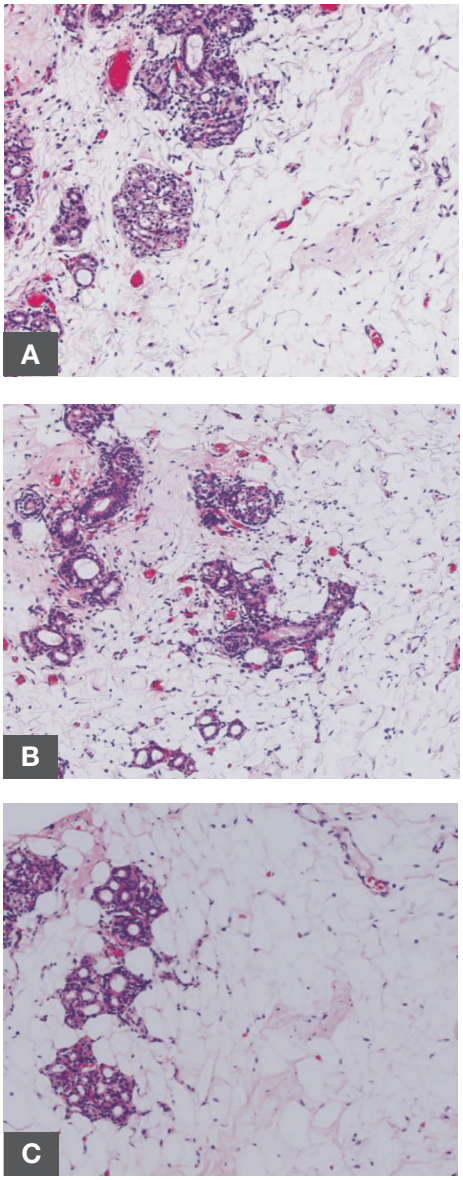

Figure 6. (A) HE staining of a breast tissue specimen prepared with a conventional tissue processor (without defatting pre-treatment). (B) HE staining of a breast tissue specimen prepared with a conventional tissue processor (with defatting pre-treatment). (C) HE staining of a breast tissue specimen prepared with Revos (without defatting pre-treatment).

Discussion

Fatty tissues such as breast tissue are usually defatted prior to paraffin embedding. However, this defatting pre-treatment requires overnight operation, which takes a longer time to complete the specimen preparation. With conventional tissue processors, such methods as agitation of specimens soaked in process reagents and circulation of the reagents are generally used to prepare processed specimens. However, due to an insufficient defatting effect of these methods, a pre-treatment step to remove fat is required before using a conventional tissue processor.

The automated tissue processor, Revos, which is used in this study has a canted chamber, which allows sufficient penetration of the process reagent into specimens that are repeatedly immersed and removed from the reagent solution. It is known that this method facilitates the penetration of reagent solution into the tissues and promotes the replacement of reagents. In this study, high-quality specimens were obtained by processing specimens using Revos, even without defatting pre-treatment. It is suggested that an automated tissue processor with a canted chamber provides appropriate agitation of tissue from which water trapped in fat is replaced by penetrating reagent and removed, resulting in adequate dehydration of the tissue.

The results of this study demonstrated that the use of Revos for processing makes it possible to prepare high-quality specimens without a defatting pre-treatment step. Therefore, it was suggested that a defatting step can be omitted for fatty tissues such as breast tissue, decreasing the amount of time required for specimen preparation, which could contribute to improved efficiency of daily pathological examinations.

References

1. Merati, P et al. Comparison of tidal agitation and rotary agitation utilizing particle image velocimetry (PIV) and planar laser induced fluorescence (PLIF) methods. Western Michigan University College of Engineering and Applied Sciences project report.*Manuscript Draft for the Journal of Histotechnology. 2011.*

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