Optimisation of Rapid Tissue Processing at Sunway Medical Centre Using the Epredia Revos Automated Tissue Processor

Qiu Xian Thong Department of Anatomical Pathology, Ultimed Scientific Sdn Bhd, Malaysia.

Natasha Najwa Binti Nor Arfuzir Department of Anatomical Pathology, Ultimed Scientific Sdn Bhd, Malaysia.

Muhammad Najib bin Abu Husin Department of Anatomical Pathology, Epredia, USA.
Correspondence: abuhusin.muhammadnajibbin@epredia.com

Vijayaletchumi Marimuthu Laboratory of Histopathology, Sunway Medical Center, Malaysia.

Abstract

Tissue processing is the procedure in anatomy pathology to replace water in tissue with paraffin wax, so that the tissue can be preserved for long term. However, tissue processing is a time-consuming process which often require more than 14 h. Epredia Revos automated tissue processor was designed for rapid processing. With its rotational mechanism chamber, it enhances the distribution of reagent, reduces tissue processing time and allows for high-quality processing results. To increase flexibility for the end user, the objective of this study was to optimize the protocol of rapid tissue processing at the Laboratory of Histopathology, Sunway Medical Centre. A variety of human tissue specimens with grossing thickness range from 2 mm to 8 mm were processed using the Epredia Revos automated tissue processor. The quality of tissue was then examined using H&E and immunohistochemistry (IHC) stainings. The result indicated that 2 mm non-fat-rich tissues and 3 mm all type tissues were optimally processed with 2h and 5h protocol, respectively. Lastly, thick surgical tissues up to 6 mm thickness were optimally processed with 7 h protocol. All tissues processed with Epredia Revos automated tissue processor showed good H&E and IHC stainings. As a conclusion, three rapid protocols have been successfully developed.

Introduction

Proper histological procedures are essential to provide good quality sections that can be used for morphological evaluation of tissue changes. To date, technological advances in tissue processor instrumentation has reduced the bottlenecks in laboratories by accelerating the workflow and decreasing the workforce. In current times demanding fast turnaround, advanced automated tissue processor instrumentation has unique and patented rotational agitation that breaks up fluid surface tension and promotes rapid reagent penetration (Wiederhold et al., 2009). Previous study has proven that agitation and increase temperature of the tissue in fixative can improve

fixation. However, prolonged excessive heat can damage cells and cause prominent shrinkage and hardening of the specimen (Leong, 1994).

Epredia Revos automated tissue processor was launched in year 2020. Epredia Revos automated tissue processor is built-in with canted and rotational chamber. The specimens are rotated inside the chamber, for better reagent penetration and maximize reagent utilization. The chamber is partially filled with reagent, allowing the tissue to leave the reagent, to be briefly suspended out of the reagent and re-submersed into the reagent again. With this mechanism, it creates efficient reagent flow in and out of the tissue specimens resulting in complete and thorough processing. In addition, Epredia Revos automated tissue processor chamber can accommodate up to 300 cassettes at one time processing. Epredia Revos automated tissue processor contributes to safe environment with its dual-filtration system and down-draft ventilation which helps protect laboratory staff by controlling both solvent and formalin fumes.

According to the manufacturer's manual, the rapid processing using the Epredia Revos automated tissue processor has reduced the processing time up to 50%, when compared with the 14h-conventional method. In this study, tissues from the Laboratory of Sunway Medical Center were processed in Epredia Revos automated tissue processor to optimize the protocol of rapid tissue processing and to make the same-day reporting possible.

Methodology

A variety of human tissue specimens with grossing thickness range from 2 mm to 8 mm were collected at Sunway Medical Centre, Bandar Sunway. All specimens were placed in individual cassettes and were processed in Epredia Revos automated tissue processor at different processing schedule (2h, 5h and 7h) as described in Table 1, Table 2 and Table 3. Paraffin blocks were prepared through embedding process and 3 µm thin section from the blocks were subjected to H&E staining and IHC staining to compare the quality of tissue sections at different processing schedule.

Table 1. Rapid Surgical Processing (2 h)

Step	Reagent	Temp (°C)	Time (hh:mm)	Vacuum	Drain Time (s)
1	10% Formalin	Ambient	0:07	Off	120
2	10% Formalin	Ambient	0:07	Off	30
3	Alcohol 75%	Ambient	0:07	On	30
4	Alcohol 90%	Ambient	0:07	On	30
5	Alcohol 95%	Ambient	0:07	On	30
6	Alcohol 100%	Ambient	0:07	On	30
7	Alcohol 100%	Ambient	0:07	On	30
8	Alcohol 100%	Ambient	0:07	On	30
9	Xylene	Ambient	0:07	On	30
10	Xylene	Ambient	0:07	On	30
11	Xylene	Ambient	0:15	On	30
12	Paraffin Wax	62	0:10	On	120
13	Paraffin Wax	62	0:07	On	120
14	Paraffin Wax	62	0:07	On	120

Table 2. Routine Surgical Processing (5 h)

Step	Reagent	Temp (°C)	Time (hh:mm)	Vacuum	Drain Time (s)
1	10% Formalin	Ambient	0:16	On	60
2	10% Formalin	Ambient	0:16	On	80
3	Flush	Ambient	0:00	Off	30
4	Alcohol 75%	35	0:19	On	30
5	Alcohol 90%	35	0:19	On	30
6	Alcohol 95%	35	0:26	On	80
7	Alcohol 100%	35	0:19	On	30
8	Alcohol 100%	35	0:19	On	30
9	Alcohol 100%	35	0:28	On	80
10	Xylene	35	0:21	On	30
11	Xylene	35	0:21	On	30
12	Xylene	35	0:21	On	80
13	Paraffin Wax	62	0:21	On	120
14	Paraffin Wax	62	0:21	On	120
15	Paraffin Wax	62	0:28	On	120

Table 3. Thick Surgical Processing (7 h)

Step	Reagent	Temp (°C)	Time (hh:mm)	Vacuum	Drain Time (s)
1	10% Formalin	Ambient	0:10	Off	30
2	10% Formalin	Ambient	0:10	Off	60
3	Flush	Ambient	0:05	Off	60
4	Alcohol 75%	35	0:30	On	30
5	Alcohol 90%	35	0:30	On	30
6	Alcohol 95%	35	0:40	On	40
7	Alcohol 100%	35	0:30	On	30
8	Alcohol 100%	35	0:30	On	30
9	Alcohol 100%	35	0:40	On	40
10	Xylene	35	0:30	On	30
11	Xylene	35	0:30	On	30
12	Xylene	35	0:40	On	60
13	Paraffin Wax	62	0:30	On	120
14	Paraffin Wax	62	0:30	On	120
15	Paraffin Wax	62	0:30	On	120

Results

Rapid processing of small biopsy using 2h protocol

A 2h protocol was used to rapid process small biopsy specimens. The size of the tissues was approximately 2 mm each. The result of tissue processing was listed in Table 4. All the five non-fat-rich tissues, included liver, kidney, fibroid and lungs are well processed. The fat-rich esophagus was also well processed. However, the fat-rich gall bladder and adipose tissue were not optimally processed. All tissues have good microscopic structures (Figure 2). The result indicated that the 2h protocol could well process all type of non-fat-rich tissues, whereas the fat-rich tissues are recommended to be processed using a longer protocol.

Rapid processing of routine surgical tissue using 5h protocol

A 5h protocol was used to process routine surgical specimens which have thickness of approximately 3 mm. Total seven types of tissues were processed, including omentum, gall bladder, thyroid, appendix, liver, bowel and tonsil. As shown in Table 4 and Figure 3, all tissues were well processed including the fat-rich tissues like gall bladder and omentum. The FFPE tissues were sectioned and H&E stained. The H&E results indicated that all the tissues have good microscopic structures (Figure 4).

Rapid processing of thick surgical tissue using 7h protocol

A 7h protocol was used to process routine and thick surgical specimens of various grossing thickness between 2 – 8 mm. Total ten types of tissues were processed, including colon, endometrium, thyroid, lungs, fibroid, lipoma fat, lipoma tumor, kidney, uterus, and cervix polyp. The result of tissue processing was summarized in Table 4. The FFPE blocks of some processed tissues were shown in Figure 5. The H&E staining results of the tissues were shown in Figure 6. All the tissues with thickness between 2 – 5 mm were well processed. However, colon at 6 mm and lipoma fat at 8 mm were not completely processed. Colon and lipoma fat were fat-rich tissues that require more effort for reagents to penetrate and to replace fluid in the tissues.

Worth noting, at grossing thickness of 6 – 8mm, the tissues were in close contact with the cassette lid. This had reduced the surface area of tissues exposed to processing reagents, and therefore causing reduction in the rate of reagent penetration and reagent replacement in the tissue. This could be the reason of incomplete processing of fat-rich tissue at 6 – 8mm thickness. It is recommended to trim the fat-rich tissue to the size smaller than the cassette interior volume. A longer protocol may also be applied to completely process the 6 - 8 mm fat-rich tissue. Alternatively, one could use the mega cassette for fat-rich tissue with thickness more than 6 mm, in which the processing reagents could freely move and completely surround all part of the tissues.

Figure 1. Formalin fixed paraffin embedded (FFPE) blocks of (A) liver, (B) gall bladder, (C) esophagus, (D) kidney, (E) fibroid, (F) lungs, and (G) adipose tissues, processed with the 2h protocol. All the tissues have 2 mm grossing thickness. All non-fat-rich tissues were well processed. The fat-rich gall bladder and adipose tissues are recommended to be processed with a longer protocol.

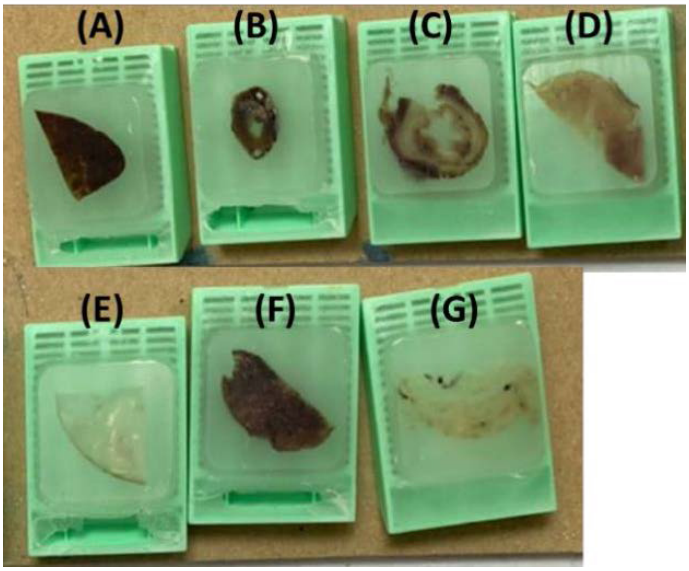


Figure 2. H&E staining of tissues that were processed with 2h protocol. The (A) Liver, (B) gall bladder, (C) esophagus, (D) kidney, (E) fibroid, (F) lungs, and (G) adipose tissues were stained well and shown good microscopic structures.

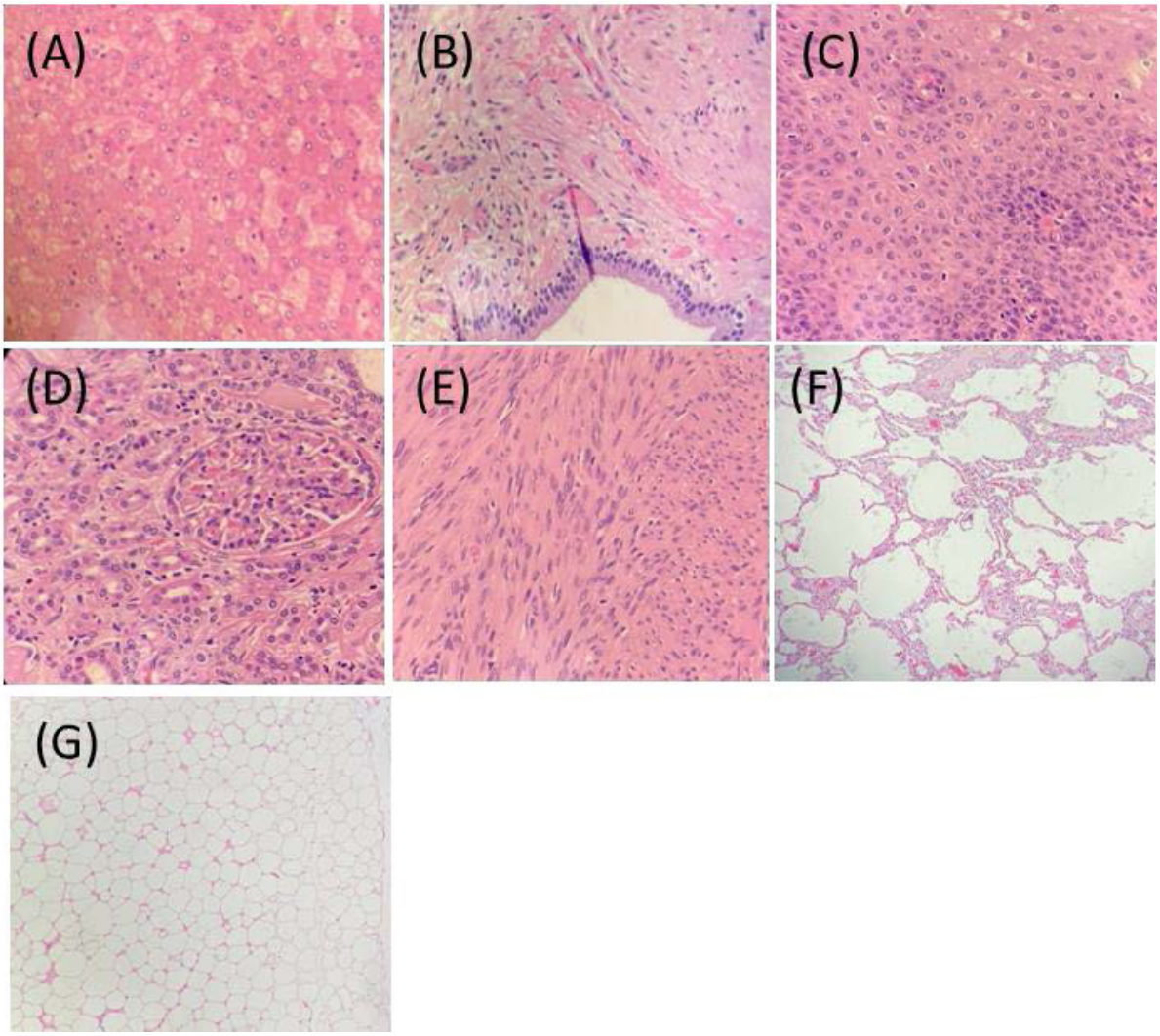


Figure 3. Formalin fixed paraffin embedded (FFPE) blocks of (A) omentum, (B) gall bladder, (C) thyroid, (D) appendix, (E) liver, (F) bowel and (G) tonsil tissues, processed with the 5h protocol. All the tissues have 3 mm grossing thickness. All tissues were well processed including the gall bladder and omentum which were fat-rich.

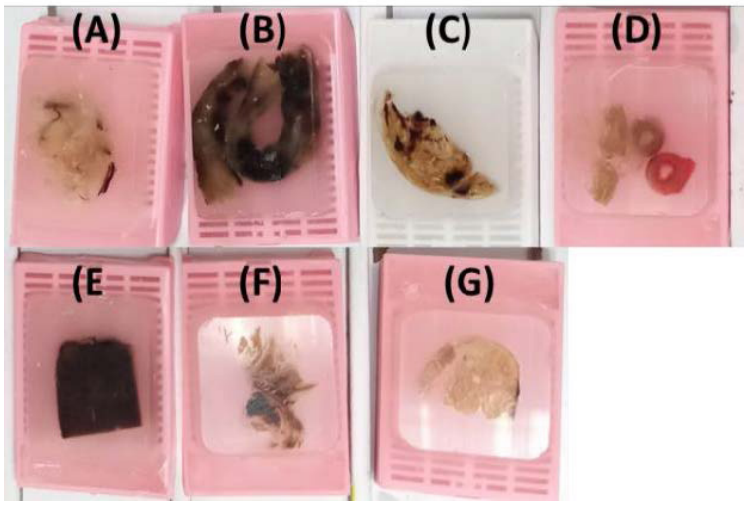


Figure 4. H&E staining of tissues that were processed with 5h protocol. The (A) omentum, (B) gall bladder, (C) thyroid, (D) appendix, (E) liver, (F) bowel and (G) tonsil tissues were stained well and shown good microscopic structures.

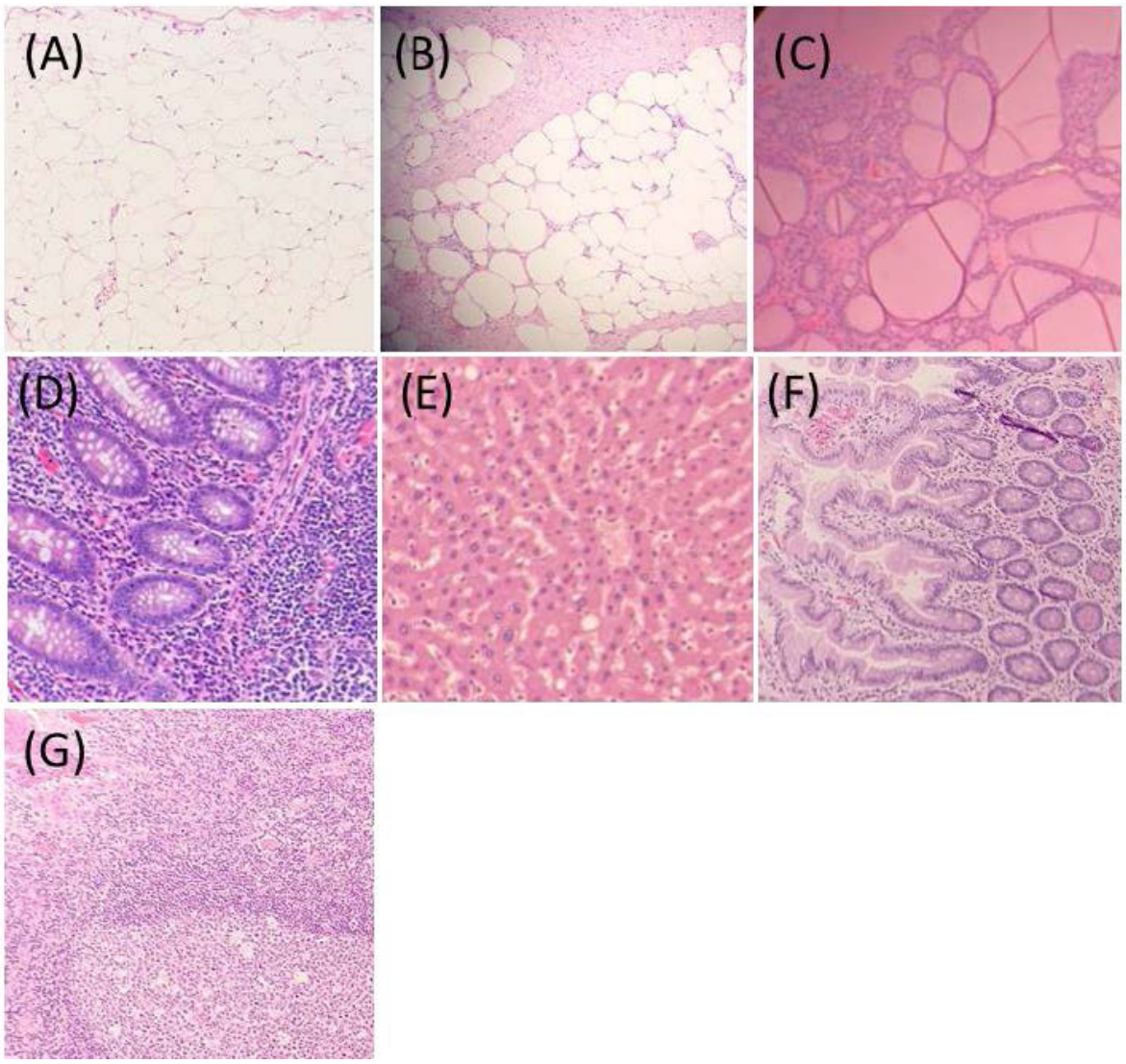


Figure 5. A 7h protocol was used to process tissues of various types with thickness 2 - 8 mm. The FFPE blocks shown (A) fibroid, (B) lungs, (C) uterus, (D) thyroid, (E) lipoma, (F) colon biopsy and (G) colon tissues with grossing thickness 2-8 mm. All tissues were well processed except the lipoma fat (not shown in figure) and the (G) colon tissues which were 8 mm and 6 mm, respectively. The blue arrow indicated that the fat component of colon tissues with grossing thickness of 6 mm was under processed. This could be due to the reduction of tissue surface area that was accessible to processing reagents when the tissue was thick and tightly covered by the cassette lid.

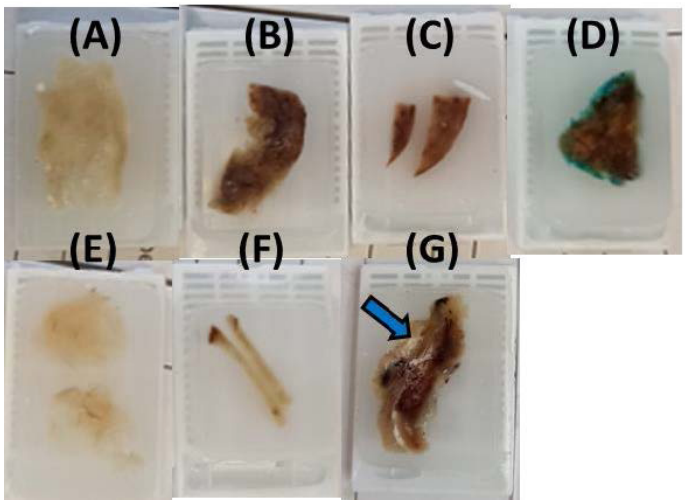


Figure 6. H&E staining of tissues that were processed with 7h protocol. The tissues were (A) lungs, (B) fibroid, (C) lipoma, (D) kidney and (E) colon. All tissues were stained well and shown good microscopic structures.

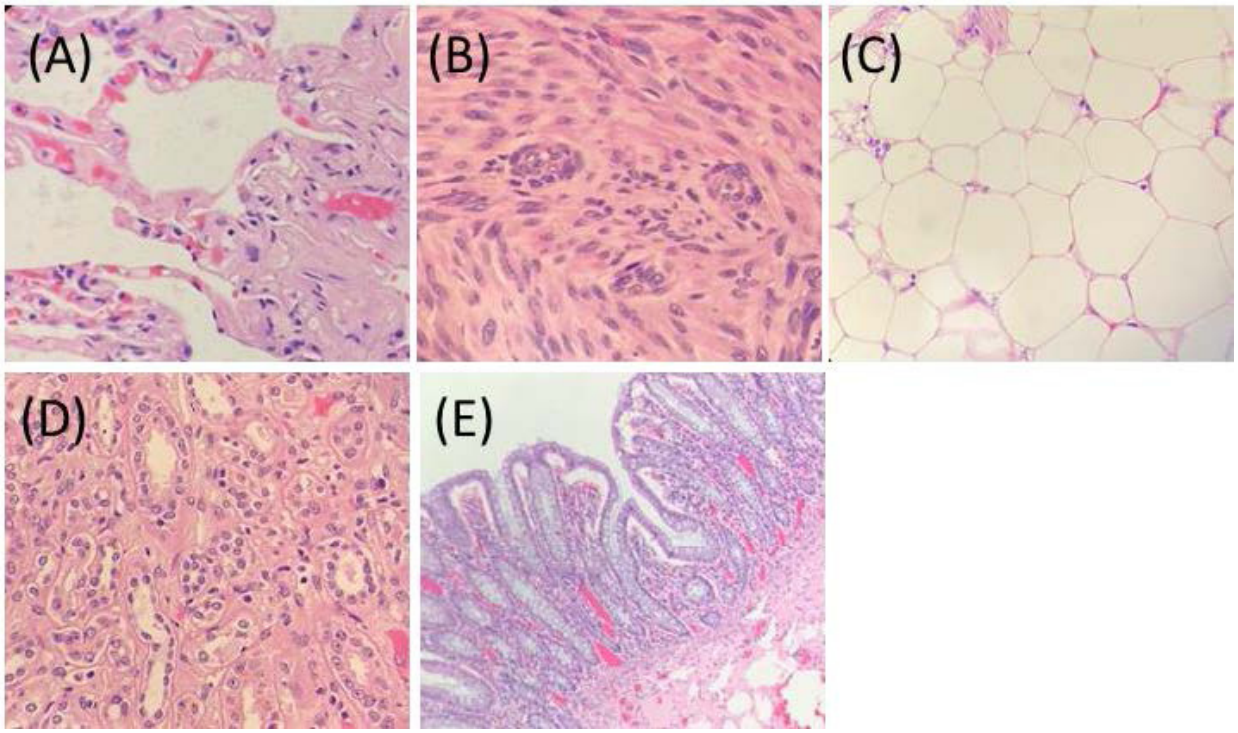


Table 4. Result of tissue processing for various tissue types and tissue thickness.
The tissues were processed with 2h, 5h and 7h protocols following specific parameters as listed in the section of Methodology.

Protocol	Tissue Type	Thickness	Result
2 h	Liver	2 mm	Processing was optimal
	Lung	2 mm	Processing was optimal
	Esophagus	2 mm	Processing was optimal
	Kidney	2 mm	Processing was optimal
	Fibroid	2 mm	Processing was optimal
	Gall Bladder	2 mm	Fatty tissue. Recommended to process with longer protocol.
	Fat	2 mm	Fatty tissue. Recommended to process with longer protocol.
5 h	Omentum	3 mm	Processing was optimal
	Gall Bladder	3 mm	Processing was optimal
	Thyroid	3 mm	Processing was optimal
	Appendix	3 mm	Processing was optimal
	Liver	3 mm	Processing was optimal
	Bowel	3 mm	Processing was optimal
	Tonsil	3 mm	Processing was optimal
	Tonsil	3 mm	Processing was optimal
	Tonsil	3 mm	Processing was optimal
7 h	Colon	2 mm	Processing was optimal
	Endometrium	3 mm	Processing was optimal
	Thyroid	3 mm	Processing was optimal
	Lungs	3 mm	Processing was optimal
	Fibroid	3 mm	Processing was optimal
	Colon	4 mm	Processing was optimal
	Fibroid	4 mm	Processing was optimal
	Lipoma	4 mm	Processing was optimal
	Endometrium	5 mm	Processing was optimal
	Kidney	5 mm	Processing was optimal
	Uterus	5 mm	Processing was optimal
	Colon	5 mm	Processing was optimal
	Colon	6 mm	Tissue too thick. Underprocessed.
	Cervix Polyp	8 mm	Processing was optimal
	Endometrium	8 mm	Processing was optimal
	Lipoma Tumor	8 mm	Processing was optimal
	Lipoma Fat	8 mm	Tissue too thick. Underprocessed.

Immunohistochemistry (IHC) staining

To further verify the quality of tissues processed by the Epredia Revos automated tissue processor, the FFPE tissue blocks were sectioned and proceeded with IHC staining (Table 2). Lungs tissues were stained with TTF-1 and Napsin A antibodies. Tonsil tissues were stained with P40 and CK7 antibodies. Breast and Thyroid tissues were stained with EMA and TTF-1 antibodies, respectively. Figure 7 shown that the location and staining intensity were insignificantly different when comparing with the IHC control tissues that were routinely used by the IHC team at the Laboratory of Sunway Medical Centre. The result indicated that the effectiveness of Epredia Revos automated tissue processor in preserving the protein structure and antigenicity of specimens.

Discussion

Tissue processing is a procedure in anatomy pathology that was done after fixation. In this procedure, water in tissues is replaced with paraffin wax to preserve the structure. The protocol involves incubation of specimens in fixative, alcohol, xylene and paraffin for an extend period of time. A typical protocol often requires 14 h to completely process a tissue. For fatty tissues, additional de-fat procedure is required if conventional tissue processor is used (Abe et al., 2021). Subsequently, patient and healthcare practitioner have to wait for a few days to receive the pathology report.

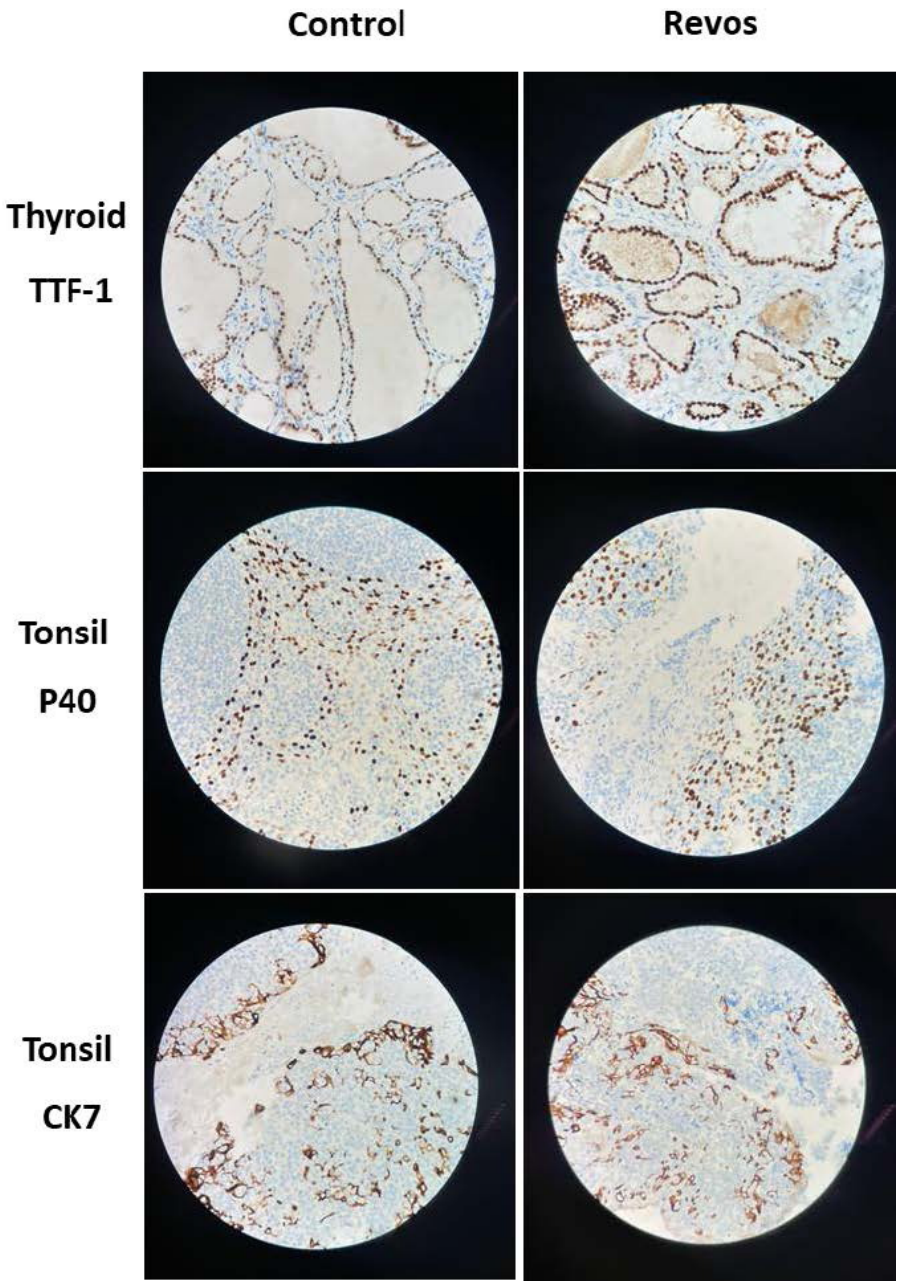
The Epredia Revos automated tissue processor has the latest technology that enables rapid tissue processing while maintaining the quality of tissue processed. The aim of this study is to optimize the protocol of rapid tissue processing and to reduce turnaround time.

The result shown that tissues of approximately 2 mm could be rapidly processed using the 2 h protocol. Besides, tissues with routine thickness which are approximately 3 mm could be processed with the 5 h protocol. For thick surgical tissues of up to 6 mm, 7 h was taken to completely process the tissue. The protocol of rapid tissue processing using the Revos automated tissue processor has reduced approximately 50% of time.

Heat was commonly used to process tissues at a faster rate. However, heating over 35 °C could adversely damage the protein (Horobin et al., 1998), DNA and RNA of tissues (Srinivasan et al., 2002). Interestingly, unlike other conventional rapid tissue processors which apply heat up to 90 °C, the Revos automated tissue processor did not solely depend on heat to achieve rapid processing.

Instead, the dehydrant and clearing reagent in Revos automated tissue processor were heated to maximum 35 °C while the paraffin was only heated to 62 °C. This innovative feature enables tissues to be processed rapidly while molecular quality to be maintained supremely. This has proven by the result of IHC staining as indicated in Table 2 and Figure 7. The finding of this study correlates well with Abe and the scientists from the Tokyo Medical and Dental University (2021), who reported time saving and excellent processing of fatty tissue using the Revos automated tissue processor.

Figure 7. The IHC staining of control tissues that were routinely used by the IHC team at Sunway Medical Centre Laboratory (namely Control) and IHC staining of tissues processed using the Epredia Revos automated tissue processor (namely Revos). The IHC staining location and intensity were insignificantly different between Control and Revos. The IHC result indicated that the effectiveness of Epredia Revos automated tissue processor in preserving the protein structure and antigenicity of specimens.



Conclusion

In conclusion, the protocol of rapid tissue processing using the Epredia Revos automated tissue processor was successfully optimised for the specimens at the Laboratory of Histopathology, Sunway Medical Centre. The outcome of this study has led to 50% reduction in the time of tissue processing, without applying heat of more than 35 °C for dehydrant and clearing reagents during processing.

References

1. Abe, S., Kitagawa, M., Inoue, M., Yoshinaga, T., and Yamamoto, K. (2021). Defatting effect of automated tissue processor with canted chamber (Revos). *Unpublished data from Tokyo Medical and Dental University.*

2. Horobin R.W. (1998). Problems and artifacts of microwave accelerated procedures in neurohistotechnology and resolutions. *Methods, 15*, 101-106.

3. Leong, A. S. Y. (1994). Fixation and fixatives. *In Woods AE and Ellis RC eds*. Laboratory Histopathology. New York: Churchill Livingstone,4.1-1-4.1-26.

4. Srinivasan, M., Sedmak, D., and Jewell, S. (2002). Effect of fixatives and tissue processing on the content and integrity of nucleic acids. *Am J Clin Pathol*, 161, 1962-1971.

5. 5. Wiederhold, G., Freeland, J. H., and Milliman, K. (2009). Fast Flex® Validation Study: Comparison of Two Tissue Processors. *Journal of Histotechnology*, 32(4), 179-185.

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