Comparative study of microwave tissue processing versus conventional tissue processing

Running title: Microwave tissue processing versus conventional tissue processing

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Abstract

Background: Conventional Tissue Processing (CTP) is the gold standard method for tissue processing, but the method is relatively time-consuming. Microwave Tissue Processing (MTP) reduces the turnaround time and gives comparable histomorphology to CTP. The objective was to compare the Laboratory-grade Microwave Processing (LMP) and Domestic Microwave tissue Processing (DMP) with CTP in terms of histomorphology and Immunohistochemistry (IHC) staining.

Methods: Three tissue bits from 89 formalin-fixed resection specimens received in the histopathology laboratory were processed by CTP, LMP, and DMP processing methods. The formalin used was 10% neutral buffered formalin. The sections were stained with Hematoxylin and Eosin stain. In subgroup analysis, IHC was done on 17 relevant cases using two antibodies, Ki67 (nuclear) and Pancytokeratin (membranous and cytoplasmic). Parameters such as the clarity of section, cytoplasmic details, nuclear details, color intensity, and interface of epithelium and connective tissue were studied. Parameters like color intensity, localization of antigen, background staining, and crispness of staining were studied on IHC. The results were analyzed by using Kappa statistics.

Results: There was a fair to moderate agreement between conventional tissue processing and laboratory microwave tissue processing. There was a slight to fair agreement between conventional tissue processing to domestic microwave tissue processing, and laboratory microwave processing to domestic microwave processing.

Conclusion: Microwave tissue processing reduced the turnaround time. The overall quality of LMP tissue was better than DMP and was equally good as that of CTP**.**

Keywords: Tissue Processing, Microwave, Conventional, Turnaround Time

Introduction

Conventional Tissue Processing (CTP), which includes fixation, dehydration, clearing, and impregnation, has been the gold standard method used for centuries. It takes a lot of time to complete these steps in conventional practice. Early diagnosis and treatment, rapid tissue processing, and staining of histologic specimens are of predominant importance (1, 2).

Rapid tissue processing for histopathological diagnosis has become more desirable in recent years. The use of microwaves in histopathology has become more widespread over the last decade (3). In contrast to conventional heating, microwaves create heat from within, evenly warming the tissue specimen and allowing chemicals to diffuse more rapidly. The benefits of microwave processing include reduced turnaround time and histomorphologies that are comparable to those produced by conventional tissue processing (4).

Many studies have highlighted the importance of microwave tissue processing and showed that tissue processed in a microwave tissue processor gives comparable morphology on histology and immunohistochemistry as conventionally processed tissue. In this study, we investigated whether Microwave Tissue Processing (MTP) (laboratory and domestic) can provide the same quality as conventional tissue processing in terms of histomorphology and immunohistochemistry staining in a shorter time.

Methods

This cross-sectional study was conducted in a histopathology laboratory at tertiary health care center in South India over one-year period. The sample size was calculated as 89 for each tissue processing method using nMaster 2.0 software. A waiver of consent was obtained from the Institute Ethics Committee (JIP/IEC/2021/095).

The resection specimens were fixed in 10% neutral buffered formalin for 24 hours then three tissue bits were taken from each specimen measuring 0.5x2x2cm. One bit was processed by the conventional tissue processing method, labeled as CTP. The second tissue bit was labelled as LMP, which is processed by a laboratory microwave tissue processor, and the third bit labeled as DMP was processed by using a domestic microwave oven. After tissue processing and embedding in paraffin, all three sections were cut and stained with Hematoxylin and Eosin (H&E) stain. In subgroup analysis, Immunohistochemistry was performed on 17 cases. Using two antibodies, Ki67 (nuclear) and Pancytokeratin (membranous and cytoplasmic), were used for IHC on relevant tissues. The Leica TP1020 open-type tissue processor was used for conventional tissue processing, and its turnaround time was around 18 hours.

Laboratory microwave processing (LMP)

For laboratory microwave processing, it was standardized first according to the Thermo Scientific tissue wave microwave processing protocol. Thermo Scientific (Model No: 3486 M) Shandon Tissue Wave 2 was used for processing. Up to 74 cassettes can be processed at a time in this microwave processor. During the study, we used to process only 20 -30 cassettes at a time. The processing time was 48 minutes, and the schedule can be found in Table 1.

Domestic microwave processing (DTP)

The Samsung MS23F301T, microwave oven with a capacity of 23L, was used. The microwave oven was operated at a minimum power level of 100W. The tissue was placed in plastic cassettes and placed in a 500 ml glass beaker containing reagents. The opening of the jar was covered with aluminum foil. The beaker is then placed in the center of the rotating table in the microwave oven. The tissues were processed in a microwave in four steps: two 30-minute intervals in isopropyl alcohol followed by two 30-minute intervals in paraffin wax.

The comparison between CTP versus LMP, CTP versus DMP, and LMP versus DMP in terms of histomorphology and IHC staining was done by two pathologists independently based on the following parameters as poor (score 0), good (score 1), and excellent (score 2). They were blinded to the tissue processing method to avoid bias in interpretation.

Parameters analyzed on histomorphology:

1. Clarity of section
2. Cytoplasmic details
3. Nuclear details
4. Color intensity of staining
5. Interface of epithelium and connective tissue (IECT)

Parameters analyzed on the morphology of IHC:

1. Crispness of staining
2. Localization of antigen
3. Intensity of staining
4. Background staining

Statistical analysis

The data obtained were analyzed using SPSS 19.0 software, and the scores that were obtained for all three techniques were presented as a frequency distribution. The agreement between the three methods was analyzed by Kappa statistics.

Results

The quality of three different tissue processing methods was evaluated based on histomorphology using H&E staining on 89 samples.

CTP versus LMP:

For parameters like Cytoplasmic details, nuclear details, and color intensity, LMP is superior to CTP (Figure 1). For clarity of section CTP and LMP got an equally good result. In terms of the interface of epithelium and connective tissue, CTP is better. Overall, there was a fair to moderate agreement between the CTP and LMP methods for various histo-morphological features examined (Table 2).

CTP versus DMP:

For parameters like cytoplasmic details and interface of epithelium, the results of DMP were slightly poor, and for other parameters like the clarity of section, color intensity, and nuclear details, the results of DMP were poor when compared to CTP (Figure 2). Overall, there was a slight to fair agreement between the CTP and DMP methods for various histo-morphological parameters assessed, with CTP having more frequency of excellent scores (Table 3).

LMP versus DMP

In our study, the clarity of section, cytoplasmic details, nuclear details, color intensity, and interface of epithelium and connective tissue were found to be better in LMP when compared to DMP (Figure 3). Overall, there was a slight to fair agreement between the LMP and DMP methods for various histo-morphological parameters (Table 4).

IHC staining: CTP versus LMP & LMP versus DMP

IHC was done on 17 cases in each group (17x3=51 specimens) using two antibodies. The comparison of IHC staining of CTP and LMP methods, LMP and DMP methods (Table 5) in terms of four different parameters in IHC-stained slides was done (Figure 4). A detailed comparison of DMP and LMP with existing literature is provided in Tables 6 and 7.

1. Localisation of antigen and background staining had consensus in all 17 cases, so kappa statistics were not performed.
2. The parameters, localization of antigen, and background staining showed a 100% agreement with a p-value of >0.001, which means there is no background staining and localization of antigen is very good for IHC-stained slides by CTP and LMP methods, LMP and DMP methods.

Discussion

Most studies on MTP compare CTP with DMP. Only a few studies in the literature compare

conventional processing with laboratory-grade microwave processing (LMP). In this study, the protocol for DMP followed established methods from similar studies (1, 5). A Samsung microwave oven (Model No. MS23F301T) was used, operating at a minimum power level of 100 W despite its maximum output of 800 W. Processing tissues at a low power level helps prevent damage, which aligns with previous findings (5–7). Notably, microwave processing was significantly faster (48 minutes for LMP and 2 hours for DMP) than conventional processing (16 hours), enabling same-day diagnosis—a result consistent with other research (1,4-7).

Our study demonstrated that the histomorphological outcomes of LMP were highly comparable to those of CTP, showing fair to moderate agreement. These findings support earlier studies (8-10). For instance, one study comparing laboratory-grade microwave and conventional processing in 158 paired tissues found no substantial difference in overall section quality (10). The superior results of LMP in our study can be attributed to uniform heat distribution and vacuum-assisted processing, which enhance tissue preservation. In contrast, CTP versus DMP showed only slight to fair agreement, indicating that DMP yielded poorer histomorphological outcomes than CTP. This contrasts with some studies that reported superior or similar results for DMP (1,5,6).

Additionally, our study revealed that DMP histomorphological results were comparable to those of LMP. The enhanced performance of LMP can be explained by the agitation and vacuum features absent in DMP. To our knowledge, no prior studies have directly compared DMP and LMP, making this the first such comparison. For immunohistochemistry (IHC) staining, CTP, LMP, and DMP all exhibited excellent staining for parameters such as background clarity and antigen localization. All three methods performed equally well in terms of staining crispness. However, staining intensity was better in CTP and LMP than in DMP. Another study reported similar IHC staining quality between LMP and CTP (10), supporting our observations.

Overall, our findings indicate that laboratory microwave processing produces histomorphological and immunohistochemical results on par with conventional processing, while outperforming domestic microwave processing. Microwave processing offers additional advantages, including reduced processing time, lower reagent costs, and the elimination of harmful substances such as formalin and xylene. Thus, microwave tissue processing achieves three key goals: faster processing, cost efficiency, and reduced toxicity.

Conclusion

LMP yields histomorphological and immunohistochemical results comparable to CTP. DMP, however, produced slightly inferior histomorphological outcomes compared to both CTP and LMP. Nevertheless, all three techniques performed equally well in IHC staining.

Acknowledgement

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Ethical statement

The Institutes Ethics Committee has granted waiver of consent for this study (JIP/IEC/2021/095).

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

Mrs, Karthika VJ : Execution of the study, Review of literature, Analysis and Manuscript writing
Arthy Raman : Execution of the study and Interpretation of slides
Subhashini Rammoorthi : Execution of study and Interpretation of slides
Debasis Gochhait : Interpretation of slides and Revision of manuscript
SreeRekha Jinkala : Conceptualization and Design of study, Result analysis and Manuscript writing

Data availability sources

Available on request

Strengths of the study:

- Both laboratory microwave and domestic microwave methods of tissue processing drastically reduced the turnaround time
- The conventional method and laboratory microwave method maintained comparable quality in the following aspects: clarity of section, cytoplasmic details, nuclear details, staining intensity, and the interface of epithelium and connective tissue
- Isopropyl alcohol was used for dehydration and clearing steps in Microwave tissue processing, thereby reducing environmental pollution/ exposure to laboratory personnel.

Limitations of the study:

- There was no temperature control panel in the domestic microwave to maintain temperature during tissue processing
- During microwave tissue processing, there was evaporation of reagents and hence they cannot be reused. This results in an excess reagent requirement as compared to conventional tissue processing.

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Table 1. Processing schedule for laboratory microwave oven

Steps	**Power level**	**Temperature**	**Time**	**Vaccum/Agitation**
Dehydration	825W	65°C	10 mins	OFF/ON
Clearing	825W	70°C	8 mins	OFF/ON
Impregnation I	825W	60°C	15 mins	ON/ON
Impregnation II	825W	65°C	15 mins	ON/ON
Total time	48minutes			

Table 2. Agreement between CTP versus LMP (n=89)

CTP LMP				
2a		Clarity of section		
	Poor	**Good**	**Excellent**	**Total**
Poor	0	0	0	0
Good	0	20 (54.1%)	17 (45.9%)	37
Excellent	0	17 (32.7%)	35 (67.3%)	52
Total	0	37 (41.6%)	52 (58.4%)	89
K= 0.214, Fair agreement, p value=0.04				
2b		Cytoplasmic details		
	Poor	**Good**	**Excellent**	**Total**
Poor	0	0	0	0
Good	0	21 (61.8%)	13 (38.2%)	34
Excellent	0	13 (23.6%)	42 (76.4%)	55
Total	0	33 (37.1%)	56 (62.9%)	89
K=0.402, Moderate agreement, p value=0.001				
2c		Nuclear details		
	Poor	**Good**	**Excellent**	**Total**
Poor	0	0	0	0
Good	0	20 (54.1%)	17 (45.9%)	37
Excellent	0	17 (32.7%)	35 (67.3%)	52
Total	0	37 (41.6%)	52 (58.4%)	89
K=0.455, Moderate agreement, p value=0.001				
2d		Color intensity		
	Poor	**Good**	**Excellent**	**Total**
Poor	0	0	0	0
Good	0	23 (52.3%)	21 (47.7%)	44
Excellent	0	15 (33.3%)	30 (66.7%)	45
Total	0	38 (42.7%)	51 (57.3%)	89
K=0.19, Poor agreement, p value=0.071				
2e		Interface of Epithelium and Connective Tissue (IECT)		
	Poor	**Good**	**Excellent**	**Total**
Poor	0	0	0	0
Good	0	22 (68.8%)	10 (31.3%)	32
Excellent	0	17 (29.8%)	40 (70.2%)	57
Total	0	39	50	89
K=0.371, Fair agreement, p value=0.001				
CTP- Conventional Tissue Processing, LMP — Laboratory Microwave Processing				

Table 3. Agreement between CTP and DMP (n=89)

CTP	DMP			
3a	Clarity of section			
	Poor	**Good**	**Excellent**	**Total**
Poor	0	0	0	0
Good	0	30 (81.2%)	7 (18.9%)	37
Excellent	0	31 (59.6%)	21 (40.4%)	52
Total	0	61 (68.5%)	28 (31.5%)	89
K=0.196, Slight agreement, p=0.032				
3b	Cytoplasmic details			
Poor	0	0	0	0
Good	0	27 (79.5%)	7 (20.6%)	34
Excellent	0	26 (47.4%)	29 (52.7%)	55
Total	0	53 (59.6%)	36 (40.4%)	89
K=0.29, Fair agreement, p=0.003				
3c	Nuclear details			
Poor	0	0	0	0
Good	0	31 (75.6%)	10 (24.4%)	41
Excellent	0	32 (66.7%)	16 (33.3%)	48
Total	0	63 (70.8%)	26 (29.2%)	89
K=0.086, Slight agreement, p=0.35				
3d	Color intensity			
Poor	0	0	0	0
Good	0	36 (81.8%)	8 (18.3%)	44
Excellent	0	29 (64.5%)	16 (35.6%)	45
Total	0	65 (73.0%)	24 (27.0%)	89
K=0.173, Slight agreement, p=0.06				
3e Interface of Epithelium and Connective Tissue				
Poor	0	0	0	0
Good	0	29 (90.6%)	3 (9.5%)	32
Excellent	0	33 (58.0%)	24 (42.0%)	57
Total	0	62 (69.7%)	27 (30.3%)	89
K=0.271, Fair agreement, p=0.001				
CTP- Conventional Tissue Processing, DMP- Domestic Microwave Processing				

Table 4. Agreement between LMP versus DMP (n=89)

LMP			DMP	
4a			Clarity of section	
	Poor	**Good**	**Excellent**	**Total**
Poor	0	0	0	0
Good	0	30 (81.2%)	7 (18.9%)	37
Excellent	0	31 (59.6%)	21 (40.4%)	52
Total	0	61 (68.6%)	28 (31.5%)	89
K=0.19, Slight agreement, p=0.032				
4b			Cytoplasmic details	
Poor	0	0	0	0
Good	0	29 (87.9%)	4 (12.1%)	33
Excellent	0	24 (43.0%)	32 (57.1%)	56
Total	0	53 (59.6%)	36 (40.4%)	89
K=0.40, Moderate agreement, p=0.001				
4c			Nuclear details	
Poor	0	0	0	0
Good	0	30 (81.1%)	7 (19.0%)	37
Excellent	0	31 (59.6%)	21 (40.4%)	52
Total	0	61 (68.5%)	28 (31.5%)	89
K=0.196, Slight agreement, p=0.032				
4d			Color intensity	
Poor	0	0	0	0
Good	0	32 (84.3%)	6 (15.8%)	38
Excellent	0	33 (64.7%)	18 (35.3%)	51
Total	0	65 (73.0%)	24 (27.0%)	89
K=0.179, Slight agreement, p=0.04				
4e			Interface of Epithelium and Connective Tissue	
Poor	0	0	0	0
Good	0	32 (82.2%)	7 (17.9%)	39
Excellent	0	30 (60.0%)	20 (40.0%)	50
Total	0	62 (69.7%)	27 (30.3%)	89
K=0.207, Fair agreement, p=0.025				
LMP- Laboratory Microwave Processing, DMP- Domestic Microwave Processing				

Table 5. Agreement between CTP versus LMP and LMP versus DMP for IHC

CTP		LMP		
5a		Crispness of staining		
	Poor	**Good**	**Excellent**	**Total**
Poor	0	0	0	0
Good	0	1 (50.0%)	1 (50.0%)	2
Excellent	0	1 (6.7%)	14 (93.3%)	15
Total	0	2 (11.8%)	15 (88.2%)	17
K=0.433, Moderate agreement, p=0.04				
5b		Intensity		
Poor	0	1 (100%)	0	1
Good	0	2 (100%)	0	2
Excellent	0	0	14 (100%)	14
Total	0	3 (17.6%)	14 (82.4%)	17
K=0.805, Substantial agreement, p=0.001				
LMP		DMP		
5c		Crispness of staining		
	Poor	**Good**	**Excellent**	**Total**
Poor	0	0	0	0
Good	0	1 (50.0%)	1 (50.0%)	2
Excellent	0	1 (6.7%)	14 (93.3%)	15
Total	0	2	15	17
5d		Intensity		
Poor	0	0	0	0
Good	1 (33.3%)	2 (66.7%)	0	3
Excellent	0	4 (28.6%)	10 (71.4%)	14
Total	1 (5.9%)	6 (35.3%)	10 (58.8%)	17
K=0.351, Fair agreement, p=0.06				
CTP- Conventional Tissue Processing LMP- Laboratory Microwave Processing, DMP- Domestic Microwave Processing				

Table 6. Comparison of domestic microwave tissue processing schedule of similar studies with our study

Steps	**Reagent**	**Rao et al. [4]**	**Devi et al. [5]**	**Patil et al. [3]**	**Amrutha et al. [1]**	**Our study**
Dehydration	Isopropyl alcohol	20	65 (100% IPA+acetone)	30	30	30
Clearing	Isopropyl alcohol	20	35 (Xylene)	30	30	30
Infiltration	Paraffin wax 1	20	30	30	30	30
Infiltration	Paraffin wax-2	20	Nil	30	30	30
Total time		1 hour 20 minutes	2 hours 10 minutes	2 hours	2 hours	2 hours
IPA- Isopropyl Alcohol						

Table 7. Comparison of laboratory microwave tissue processing schedule results of similar studies with our study

Steps	Emerson et al. [8]	Temperature (℃)	Shrestha et al. [7]	Temperature (℃)	Our study	Temperature (℃)
Dehydration	100% ethanol-10 minutes	67	100% Ethanol-10 minutes	30	100% IPA-10 minutes	65
Clearing	100% IPA-10 minutes	74	100% IPA-10 minutes	25	100% IPA-8 minutes	70
Infiltration	Paraffin wax-10 minutes	75	Paraffin wax- 40 minutes	75	Paraffin wax-15 minutes	60
Infiltration	Paraffin wax-10 minutes	80	_	_	Paraffin wax-15 minutes	65
Total time	2 hrs 56 min			2 hrs	4 hrs 20 min	
IPA- Isopropyl Alcohol						

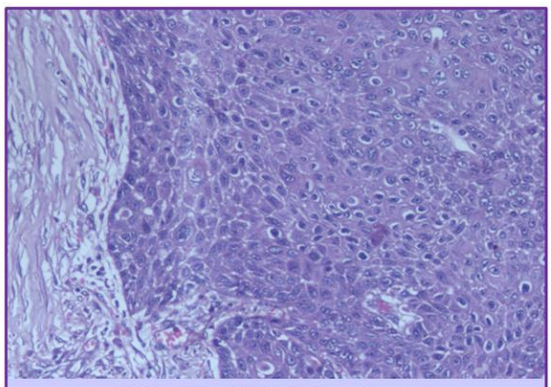

Fig 1a – Conventional tissue processing

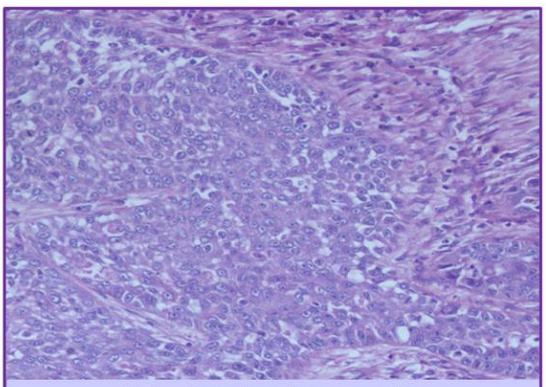

Fig 1b – Laboratory tissue processing

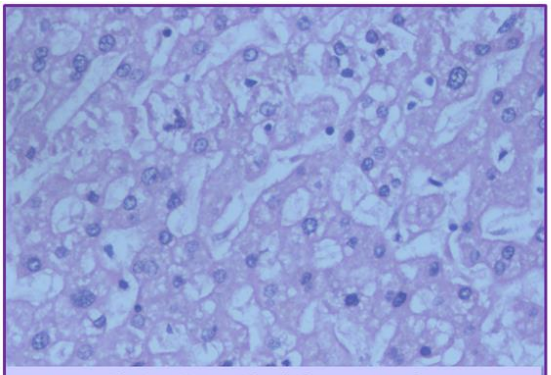

Fig 2a – Conventional tissue processing

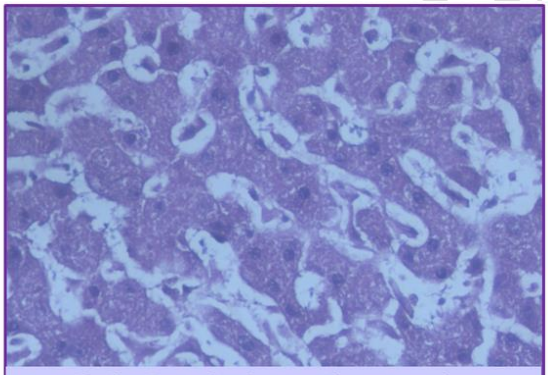

Fig 2b – Domestic microwave tissue processing

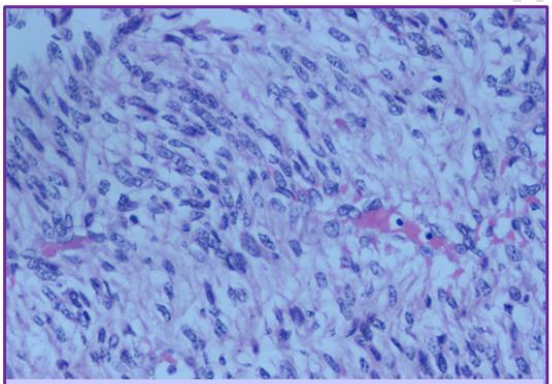

Fig 3a – Laboratory tissue processing

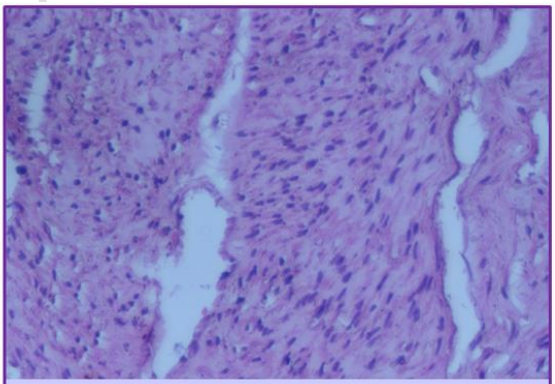

Fig 3b – Domestic tissue processing

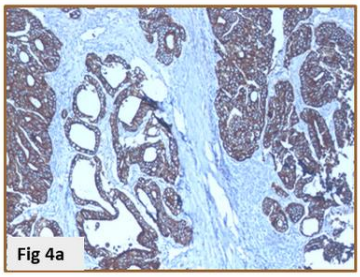

Fig 4a

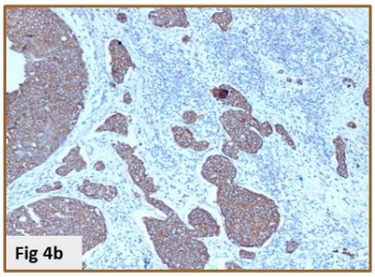

Fig 4b

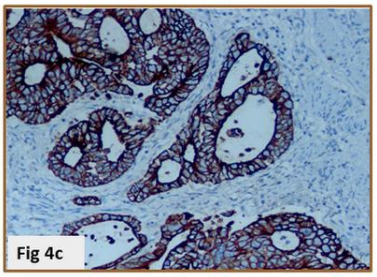

Fig 4c

Figure legends:

Figure 1. Comparison of histomorphology of a slide of squamous cell carcinoma - Conventional tissue processing (a) and laboratory microwave processing (b) show equally good morphology with a fair to moderate agreement (H and E 100x)

Figure 2. Comparison of histomorphology of a slide of normal liver parenchyma - Conventional tissue processing (a) and domestic microwave processing (b) show good morphology in conventional tissue processing. The agreement was slight to fair (H and E 100x)

Figure 3. Comparison of histomorphology of a slide of leiomyoma uterus - laboratory microwave processing (a) and domestic microwave processing (b) shows good morphology on laboratory microwave processing. The agreement was slight to fair (H and E 100x)

Figure 4. Comparison of IHC of a slide of adenocarcinoma with Pancytokeratin - Conventional tissue processing (a), laboratory microwave processing (b), and Domestic microwave processing (c) shows equally good intensity and localization of antigen (DAB 100x)