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COST ANALYSIS OF TISSUE MICROARRAYS FOR CLINICAL DIAGNOSTIC

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Abstract

The need of issuing medical diagnoses with fast turn-around times (but without compromising accuracy) is generating technical challenges in all stages of histological processing. Because of the multiple variables related to tissue harvesting, processing, and sectioning quite often the resulting histological slides reach the pathologist with fragmented or incomplete tissue sections. In the present study we evaluated the feasibility of a new method of multiplexing tissue specimens with irregular shapes by placing them during grossing into sectionable matrices (BxFrame™ GRID). The working time required for a histotechnologist in obtaining multiplexed preparations as well as the costs of laboratory supplies was compared with conventional methods. Five different types of tissue (duodenum, brain, heart, tail, and skin) were placed in BxFrame™ matrices, and subjected to histological processing, sectioned, and stained. The new multiplexing method reduced the total working time with 45% to 70% when compared to conventional methods (depending on the type of tissue) while the cost of consumables was reduced with up to 70%.

Key words*: clinical diagnostic, clinical tissue microarray, cost reduction, laboratory consumables, sectionable matrix.*

INTRODUCTION

Delivering diagnoses with high accuracy and increasingly faster turn-around times have been a constant challenge in diagnostic laboratories all over the world. However, the use of lower-quality consumables, reductions in the budgets allocated to laboratories and the constraint of delivering medical findings in the shortest possible time sometimes can endanger the quality of diagnoses.

Multiple studies have shown that there are many categories of variations that can lead to diagnostic errors (Buesa, 2010):

a) Biopsy collection variations: sampling tools optimized for the type of excised biopsies (Wang et al., 2015), defining the minimum number of biopsies to be collected, type of fixative used (Varma et al., 2013)
b) Variations in the dehydration stage: different durations of the processing protocols, different reagents, different processors (specimen-transfer processors, fluid transfer processors, microwave assisted processors) (Gologan et al., 2021)
c) Variations at the embedding stage: various natural or synthetic waxes, paraffins with a melting range of 52°C to 64°C, epoxydic resins etc. (Suvarna et al., 2018)
d) Variations regarding microtome sectioning: sectioning one or multiple levels, sectioning blocks cooled in the freezer, sectioning blocks cooled on icy water, etc. (Xie et al., 2011).

Due to the rapid development of staining methods and molecular analysis new techniques for obtaining tissue microarrays (TMAs), for both research and diagnosis, are necessary and of major interest. In recent years, the main interest in the application of TMA methods has been directed towards clinical diagnostics. The idea of incorporating as many tissues as possible is aimed at reducing the use of consumables, specialized reagents for molecular testing, or to improve the quality of diagnosis by reducing/eliminating batch

variability in the many stages of histological workflow (Ștefan et al., 2020).

In 2013, a support matrix (BxChip™) was developed for parallel processing and sectionning of cylindrical biopsies of small diameters placed horizontally either directly by the surgeon in the operating room or transferred after arriving in the pathology laboratory. This sectionable matrix eliminates the fragmentation of the collected biopsies and increases the accuracy of the diagnosis (Farcaș et al., 2014; Jinga et al., 2012; Murugan et al., 2019; Mușat, 2013).

This paper presents a new sectionable matrix design that allows the multiplexing of tissue samples of irregular shapes and sizes and significantly decreases the required working time as well as the cost of consumables when compared with conventional histological methods.

MATERIALS AND METHODS

The first article describing the BxFrame™ sectionable matrix demonstrated that the formulation of this matrix withstands the decalcification solutions and dehydration protocols routinely used in pathology laboratories (Ștefan et al., 2021).

For this study, Themis Pathology SRL (Bucharest, Romania) provided two types of BxFrame™ GRID (Figure 1) sectionable matrices:

- Small matrices for regular histological cassettes: 22 mm x 16 mm x 2 mm (L x l x h)
- Matrices for large format cassettes: 30 mm x 22.6 mm x 2 mm (L x l x h)

The organs used for this experiment were:

- Heart, duodenum, brain, and tail from C57BL/6 mice provided by the Laboratory of Pathological Anatomy, "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine, Iași, Romania.
- Pig skin provided by APOLLO slaughterhouse, Afumați, Ilfov, Romania.

Two methods were employed for grossing of the tissue samples, the “BxFrame™ method” in which multiple tissue samples were inserted in a single matrix (from each type of organ), and the conventional method where only one specimen was placed in each histological cassette (Table 1). The insertion of the samples into the sectionable matrices was conducted by breaking some dividing walls with the help of tweezers to accommodate them snugly but without any distortion.

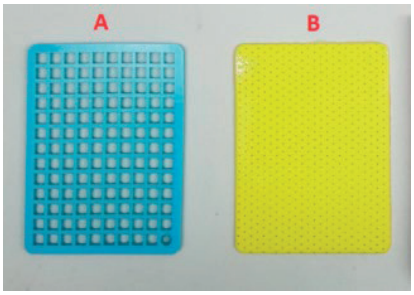

Figure 1. BxFrame™ GRID - (A) sectionable matrix; (B) sectionable perforated base

The processing protocol used had a total duration of 15 hours (Table 2) using a Sakura VIP 1000 tissue processor (Torrance, CA, USA). Before tissue processing, the mouse tails were subjected to decalcification with 5% formic acid (24 hours). All samples processed in this experiment were infiltrated and embedded in paraffin, using small and large metal moulds, and the resulting paraffin blocks were sectioned at 5 µm. During microtome sectioning, prior to facing the blocks, the yellow bases were completely removed (their role being strictly as a support for the tissues inserted in the matrix).

Table 1. Number of tissues and cassettes for each method

Method	Organs	Tissues	Cassettes
BxFrame	Duodenum	12	1
	Brain	8	1
	Tail	6	1
	Heart	4	1
	Skin	6	1
Conventional	Duodenum	12	12
	Brain	8	8
	Tail	6	6
	Heart	4	4
	Skin	6	6

Table 2. Tissue processing protocol schedule

Solvent	Time, (mins)	Temperature	P/V
NBF	60	37°C	Yes
70% Ethanol	90		
80% Ethanol	90		
95% Ethanol	60		
95% Ethanol	90		
100% Ethanol	60		
100% Ethanol	90		
Xylene	60		
Xylene	60		
Paraffin	60	60°C	Yes
Paraffin	60		
Paraffin	60		
Paraffin	60		

RESULTS AND DISCUSSIONS

Table 3 shows that the total working time required for six separate skin biopsies is almost three times longer than for biopsies processed simultaneously in a BxFrame™ GRID sectionable matrix. Although there is additional time needed during the step of loading the BxFrame™ GRID (cutting its walls and accurately loading biopsies), this extra-time is more than offset during paraffin embedding, sectioning, staining and examination of the resulting slides. In Figure 3 (Image C) it can be observed that the tissues maintain very well their position and orientation, and the use of consumables is significantly reduced.

Regarding the tail specimens it was observed that the period for processing independent biopsies is three times longer when compared to those multiplexed in the sectionable matrix. Although all tissues underwent a decalcification protocol, the sectionable matrix BxFrame™ GRID did not undergo any change in size or resilience keeping the tissues properly oriented. For heart tissue samples, the time required to process independent biopsies compared to a sectionable matrix was twice as long. The savings in terms of working time was slightly less than for the previous organs (due to the extra steps of cutting out the walls of the matrix and loading it with biopsies - since the hearts were small, they required more attention during their placement and orientation).

The working time benefit between independently processed brain samples and those placed in the BxFrame™ GRID was only 17 minutes. Similarly with heart biopsies, there was a longer time required for trimming appropriately the matrix but also for loading it with brain tissues because of its intrinsic fragile consistency. However, the time needed for sectioning a large paraffin block was half the time required for sectioning independent brain tissue. For duodenum the total working time was six times longer when processing independent biopsies versus the BxFrame™ GRID. Although it took nine minutes on average to load an array with twelve biopsies, there was no need to cut the matrix walls since the size of the BxFrame™ GRID cavities were perfectly matched to the size of the duodenum samples. Figure 2 is centralizing all the data regarding the working time needed for both methods (conventional and BxFrame™).

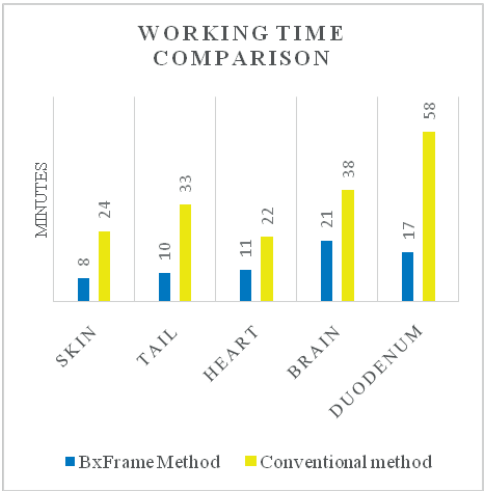

Figure 2. Comparison of total working time for the BxFrame™ technique vs the conventional method

For heart and brain samples, the total working time when sectionable matrices were used was reduced by half when compared with conventional methods.

Table 3. Detailed comparison between working time for BxFrame™ technique (A) and conventional method (B)

STEP	SKIN		TAIL		HEART		BRAIN		DUODENUM	
	A	B	A	B	A	B	A	B	A	B
Labeling tissue cassettes	10	60	10	60	10	40	10	80	10	120
Loading tissue cassette(s) with yellow base and BxFrame™GRID	15	-	17	-	23	-	25	-	20	-
Cutting walls of BxFrame™GRID	40	-	45	-	41	-	82	-	0	-
Loading tissues in BxFrame™GRID	100	-	105	-	160	-	435	-	557	-
Loading tissue cassettes with tissues	-	90	-	110	-	60	-	135	-	270
Embedding	60	209	84	260	51	146	95	270	120	539
Paraffin block cleaning	16	96	16	96	16	64	16	128	16	192
Microtome sectioning	199	695	315	1116	343	787	577	1232	280	1680
Labeling slides	6	36	6	36	6	24	6	48	6	72
Floating paraffin sections on the flotation bath	15	90	15	90	15	60	15	120	15	180
Loading the slides in the staining rack	4	25	4	25	4	16	4	32	4	48
Coverslipping	30	180	30	180	30	120	30	240	30	360
Total Time (seconds)	495	1481	647	1973	699	1317	1295	2285	1058	3461
Total Time (minutes)	**8**	**24**	**10**	**33**	**11**	**22**	**21**	**38**	**17**	**58**
Decrease (%)	**66.7**		**69.7**		**50.0**		**44.7**		**70.7**	

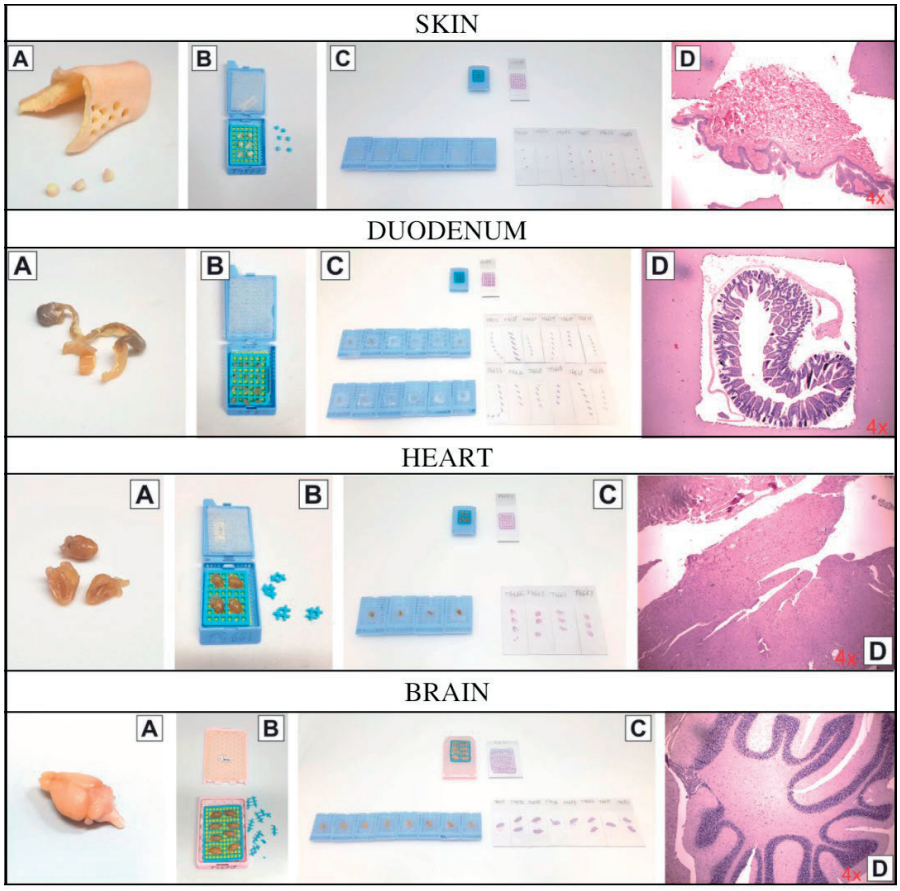

Figure 3. BxFrame™ GRID vs. conventional method: (A) Organ, (B) Loaded matrix, (C) Paraffin blocks and slides, (D) Microscopic image, 4x objective, haematoxylin-eosin stain

In the case of the tail and skin, total working time duration when employing the BxFrame™ was 3 times less than the conventional method while for small cylindrical tissue samples such as the duodenum, the savings in labour were the most spectacular since these samples can be placed directly in the receptacles of the sectionable matrix.

The analysis of the cost savings (consumables) offered by the sectionable matrix BxFrame™ GRID is presented in Table 4. The comparison was made only for tail, heart, duodenum, and skin. Mouse brains were excluded from this statistic because these tissue samples were larger in size and required sectionable matrices with different manufacturing costs, larger amounts of dehydration solvents, paraffin, etc. Prices for consumables were updated according to the 2021 Romanian market and are detailed for each histological step separately.

Tissue cassettes, sectionable matrices, histological sponges and containers with neutral formalin are consumables considered for the actual harvesting of biopsies (in the operating room) or during grossing (in the pathology laboratory). For tissue processing the working volume of reagents (dehydrants, clarifier and paraffin) was 30 ml, the minimum required for an adequate processing of the tissues, regardless of the protocol or specific tissue processor used. Paraffin block casting was estimated to require an average of 3 g of paraffin per block. Regarding glass slides all calculations were made assuming 3 slides per paraffin block will be used (2 levels for the actual diagnostic and a spare for eventual additional stains).

The skin and tail tissues required lower material costs for the BxFrame™ method versus conventional method (€13.95 versus €23.85, respectively). For heart samples, the differences are less spectacular. The material costs for the multiplexing method (€13.95) are quite similar to the costs incurred when using conventional methods (€15.90), due to the additional cost of the matrix. The advantage of using the new method in this case resides mainly in the superior preservation of the orientation of tissue samples within the matrix and the shorter processing time.

The largest cost saving is evident in the case of duodenum samples (€13.95 versus €47.70, for the BxFrame™ method versus conventional method, respectively). Figure 4 summarises the comparative costs of both methods, for all tissues examined.

A limitation of our study is the use of a single type of tissue processor and of a single dehydration protocol with predetermined conditions. However, preliminary results are suggesting that the previous observations are valid even when employing very different working situations, such as microwave-assisted tissue processors (where the protocols are much harsher: shorter dehydration protocols, solvents and paraffin heated to almost 70 degrees Celsius, and the pressure and vacuum can vary between 150 to 900 mBar).

One important aspect to be considered in the case of the BxFrame™ GRID technique is regarding the stage of loading the tissue samples within the sectionable matrix. The qualified person who performs this procedure must be careful that the matrix material does not dry out during loading, otherwise, the matrix becomes fragile, and it can become difficult to section after paraffin infiltration. In our experiments, a team of two skilled histotechnologists performed the loading of specimens in the matrices, but the learning curve for a novice who must carry out this step is quite short.

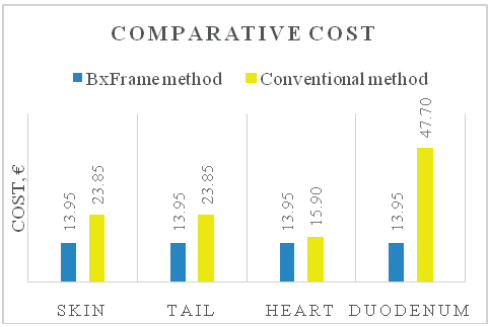

Figure 4. Comparative cost: BxFrame™ technique versus conventional method

Table 4. Cost comparison of consumables for BxFrame™ versus conventional method

Consumable	Price (€)	per	Skin		Tail		Heart		Duodenum	
			BxFrame	6 Biopsies	BxFrame	6 Biopsies	BxFrame	4 Biopsies	BxFrame	12 Biopsies
Tissue cassette	0.1	Cassette	0.10	0.60	0.10	0.60	0.10	0.40	0.10	1.20
BxFrame™ GRID	10	Unit	10.00	0.00	10.00	0.00	10.00	0.00	10.00	0.00
60 ml NBF container	2	Unit	2.00	12.00	2.00	12.00	2.00	8.00	2.00	24.00
Histological sponges	0.025	Unit	0.03	0.30	0.03	0.30	0.03	0.20	0.03	0.60
Processing cost for 1 cassette	0.25	30 ml	0.25	1.50	0.25	1.50	0.25	1.00	0.25	3.00
Paraffin (3g for one block)	0.045	Gram	0.14	0.81	0.14	0.81	0.14	0.54	0.14	1.62
Slides (2 levels and 1 spare)	0.3	Slide	0.90	5.40	0.90	5.40	0.90	3.60	0.90	10.80
Staining for 2 slides	0.25	10 ml	0.50	3.00	0.50	3.00	0.50	2.00	0.50	6.00
Coverslipping for 2 slides	0.02	Coverslip	0.04	0.24	0.04	0.24	0.04	0.16	0.04	0.48
TOTAL COST, €			**13.95**	**23.85**	**13.95**	**23.85**	**13.95**	**15.90**	**13.95**	**47.70**
Cost Decrease, %			**41.5**		**41.5**		**12.3**		**70.8**	

CONCLUSIONS

Multiplexing tissue specimens using the BxFrame™ GRID sectionable matrix reduces the working time required for histological preparations by more than half compared with conventional methods. The costs for consumables are considerably reduced up to 5 fold when compared with the costs needed for conventional methods. Tissue specimens inserted in the sectionable matrices maintain their orientation in a single plane throughout processing and embedding, inking is no longer necessary for traceability and microtome sectioning is performed without difficulty so that the histological section includes all the tissues surrounded by the BxFrame™ GRID walls.

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Conflicts of interest: Alina Elena Ștefan, Daniela Gologan and Sorin Mușat are Themis Pathology SRL employees. Matthew Okerlund Leavitt is a shareholder of LUMEA Inc (the sole proprietor of Themis Pathology SRL). The first and the second authors had equal contribution in this study.

REFERENCES

Buesa, R. J. (2010). Productivity standards for histology laboratories. *Ann Diagn Pathol*, *14*(2), 107–124.

Farcaș, C.P., Spînu, A.D., Petrescu, A., Dumitrescu, M., Vasilescu, F., Dinu, M., Mischianu, D., Mușat, S. (2014). High Throughput processing and analysis of prostate core biopsies for research applications. *Revista Română de Urologie*, *13*, 9–16.

Gologan, D., Ștefan, A. E., Stan, R., Militaru, M., Mușat, M. (2021). Factors influencing the quality of formaldehyde fixed paraffin embedded tissue samples – Review. *Revista Română de Medicină Veterinară, 31*, 87–92.

Jinga, V., Petrescu, A., Berdan, G., Tănase, F., Braticevici, B., Amuzescu, B., Mușat, S., Moișanu, D., Rădăvoi, D. (2012). C174 A comparison between classical and simultaneous processing of prostate core biopsies using Histobest Biopsy Chip™. *European Urology Supplements, 11*(4), 124.

Murugan, P., Shukla, D., Morocho, J., Smith, D., Sciacca, D., Pickard, Wahlsten, M., Gunderson, A., Konety, A., Konety, B., Khalifa, M. A., Warlick, C. (2019). Prostate Biopsy Processing: An Innovative Model for Reducing Cost, Decreasing Test Time, and Improving Diagnostic Material. *American Journal of Clinical Pathology, 152*(6), 757–765.

Mușat, S. (2013). Matrix for receiving a tissue sample and the use thereof. US Patent 9,851,349

Ștefan, A. E., Gologan, D., Leavitt, M. O., Mușat, S., Pleșea, I. E., Stan, R., Pleșea, R. M., Militaru, M. (2020). Tissue MicroArrays, brief history, techniques and clinical future. *Romanian Journal of Morphology & Embryology, 61*(4), 1077–1083.

Ștefan, A. E., Gologan, D., Mușat, S., Stan, R., Sanda, C. A., Militaru, M. (2021). Biomimetic matrices for histological processing. *Revista Română de Medicină Veterinară, 31*(3), 12–16.

Suvarna, K. S., Layton, C., Bancroft, J. D. (2018). *Bancroft's Theory and Practice of Histological Techniques* (8 ed.): Elsevier Health Sciences.

Varma, M., Berney, D. M., Algaba, F., Camparo, P., Comperat, E., Griffiths, D. F., Kristiansen, G.,

Beltran, A. L., Montironi, R., Egevad, L. (2013). Prostate needle biopsy processing: a survey of laboratory practice across Europe. *J Clin Pathol, 66*(2), 120–123.

Wang, J., Wan, B., Li, C., Wang, J., Fu, Q., Zhao, W., Zhang, K. (2015). Diagnostic yield and complications using a 20 gauge prostate biopsy needle versus a standard 18 gauge needle: A Randomized Controlled Study. *Urol J, 12*(5), 2329–2333.

Xie, R., Chung, J.-Y., Ylaya, K., Williams, R. L., Guerrero, N., Nakatsuka, N., Badie, C., Hewitt, S. M. (2011). Factors influencing the degradation of archival formalin-fixed paraffin-embedded tissue sections. *Journal of Histochemistry & Cytochemistry, 59*(4), 356–365.