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# EDUCATIONAL TISSUE ARRAYS FOR VETERINARY MEDICINE

## ARRAY-URI DE ȚESUT EDUCAȚIONALE PENTRU MEDICINA VETERINARĂ

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**ABSTRACT | REZUMAT**

The TMA technique allows the cost-effective and expeditious preparation and examination of histopathological specimens for both research and clinical diagnostic. Although the technique was originally used exclusively for elaborate research studies or testing of monoclonal antibodies (*e.g.*, several types of diseases or various stages of a disease grouped in one TMA, various tissues from the same species or the same type of tissue but from different species, etc.) in the last few years the TMA strategy made significant inroads in the clinical arena as well as in education (Histology, Anatomical Pathology, Forensics). This project is proposing the creation of a systematic TMA collection of educational slides generated from archived and curated paraffin blocks. The first organ with which we started this project is the kidney, to be followed later by other organs (spleen, lung, heart, skin etc.). The donor paraffin blocks were kindly provided by the Department of Pathological Anatomy, Faculty of Veterinary Medicine, Bucharest, Romania. Eight donor tissues were harvested from different paraffin blocks and assembled into a single sectionable TMA matrix. The sectionable matrices used in this project were designed with a unique identification code (USAMV), while each well belonging to a certain type of tissue is identified with an Arabic letter. The resulting histological slides were stained with hematoxylin-eosin.

**Keywords**: tissue microarray (TMA), educational slides, biomimetic materials, multiplexing tissue samples

Tehnica TMA permite pregătirea și examinarea rapidă și rentabilă a probelor histopatologice atât pentru cercetare, cât și pentru diagnosticul clinic. Deși tehnica a fost utilizată inițial exclusiv pentru studii de cercetare elaborate sau testarea anticorpilor monoclonali (de exemplu, mai multe tipuri de boli sau diferite stadii ale unei boli grupate într-un singur TMA, diferite țesuturi din aceeași specie sau același tip de țesut, dar din specii diferite, etc.) în ultimii câțiva ani tehnica TMA a făcut incursiuni semnificative atât în domeniul clinic, cât și în educație (Histologie, Anatomie Patologică, Criminalistică). Acest proiect propune crearea unei colecții sistematice TMA de lame educaționale generate din blocuri de parafină arhivate și organizate. Primul organ cu care am început acest proiect este rinichiul, urmând a fi urmat ulterior de alte organe (splină, plămân, inimă, piele etc.). Blocurile de parafină donor au fost oferite cu amabilitate de către Departamentul de Anatomie Patologică, Facultatea de Medicină Veterinară, București, România. Opt țesuturi donor au fost recoltate din diferite blocuri de parafină și integrate într-o singură matrice TMA secționabilă. Matricele secționabile utilizate în acest proiect au fost proiectate cu un cod unic de identificare (USA MV), în timp ce fiecare godeu aparținând unui anumit tip de țesut este identificat cu o literă arabă. Lamelele histologice rezultate au fost colorate cu hematoxilină-eozină.

**Cuvinte cheie**: tissue microarray (TMA), lame de educație, materiale biomimetice, probe de țesut multiplexate

Although the vast majority of TMAs are manufactured for use in research or diagnosis (6), they can also be employed with great success in medical education (1). Most techniques for obtaining tissue arrays use small-sized tissue fragments (between 0.1 mm and 5 mm) (7). There are several advantages in using TMAs in the educational environment:

1. If several organs of different pathologies are combined on a single slide, there will be more valuable information and they will be easy to compare;

2. Identifying the areas of interest on conventional microscopy slides can be time-consuming. On a TMA slide, only the areas of interest are represented;

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3. Preparations in very short supply (rare diseases, small tissue samples) can be used much more efficiently and generate vast numbers of informative microscopy slides;

4. On a single TMA slide, the student can compare expeditiously the morphology of normal (healthy) tissue from a certain organ with several pathologies, or the various stages of the same disease (2);

5. On a single TMA histological slide, several tissues from the same type of organ but from different animal species can be placed for their evolutionary comparison;

Today, almost all Pathological Anatomy departments in medical faculties use representative educational slides displaying only one tissue/organ/disease per slide.

The purpose of this project is to merge into TMAs several tissues of different pathologies that can appear in a single type of organ (kidney, lung, spleen, heart, etc.) for the facilitation and assimilation of this information by medical students.

## MATERIALS AND METHODS

For this project the following we employed:

a) Customized pre-processed BxFrame™ matrices with sectionable code (3) for identification. The "recipient" matrices were 31 mm x 16 mm x 3 mm (length x width x height) with pre-drilled round 6 mm wells (Fig. 1) provided by Themis Pathology SRL, Bucharest, Romania. Manufacturing protocols for the sectionable matrix and its benefits were described in previous articles (4, 5).

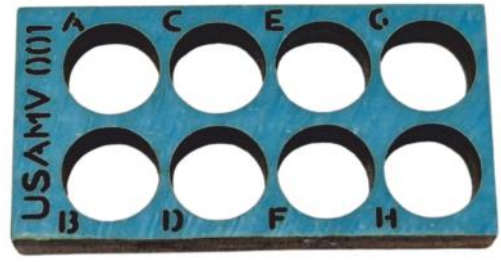


**Fig. 1.** Pre-processed BxFrame™ matrix with sectionable code

b) 8 paraffin "donor" blocks containing diseased kidney tissue and accompanying histological slides (Table 1) were provided by the department of Pathological Anatomy of the Faculty of Veterinary Medicine, Bucharest, Romania.

After receiving the "donor" paraffin blocks, they were placed on a thermostated hot plate with the temperature set at 40°C for 30 minutes for softening the paraffin blocks. This process facilitates tissue extraction using the punch, eliminating the risk of cracking the paraffin block and/or damaging the remaining tissue within the block.

After this step, each paraffin block was compared with its accompanying slide to determine the corresponding area of the tissue required to be extracted. With the help of a 5 mm dermatology punch (Kai Medical, Seki, Japan), the tissue of interest was extracted and carefully inserted in the "recipient" matrix, according to the legend. After inserting all tissues, the final, loaded TMA paraffin block was cast with fresh paraffin (Fig. 2).

**Table 1**

**Kidney paraffin blocks**

| Code assigned in TMA | Paraffin block code | Animal Data | Diagnostic |
|---|---|---|---|
| A | 20041E | Feline, Bristish Shorthair, 1 year old, Female, Unneutered | No changes |
| B | 19659 | Feline, European, 11 years old, Female, Unneutered | Enlarged glomerular filtration space |
| C | 18528 | Feline, British Shorthair, 2 years old, Male, Unneutered | Vacuolar nephrosis |
| D | 19602 | Feline, European, 5 years old, Male, Neutered | Renal cysts |
| E | 19727 | Feline, European, 3 years old, Female, Neutered | Chronic interstitial nephritis |
| F | 19640 | Canine, Half Breed, 7 years old, Female, Neutered | Renal Atrophy |
| G | 20020C | Feline, European, 8 years old, Female, Neutered | Medium cell lymphoma |
| H | 19827 | Canine, Half Breed, 8 years old, Male, Unneutered | Renal sarcoma |

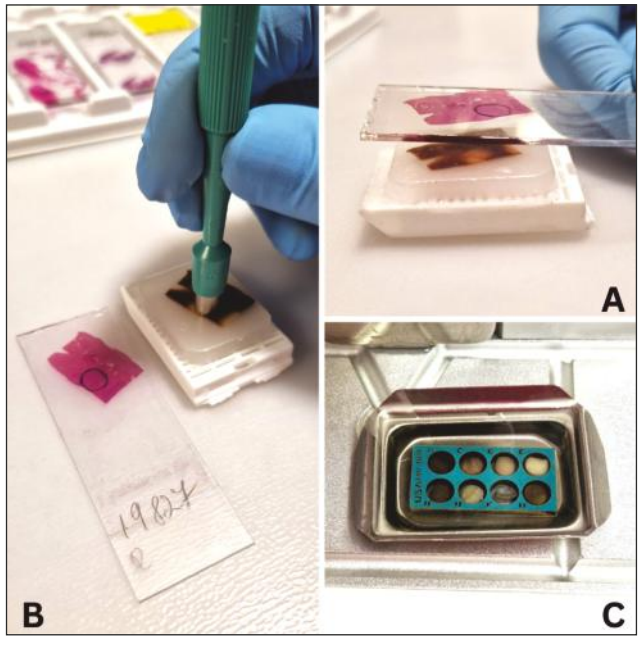


**Fig. 2.** Obtaining the educational TMA: (A) Determining the area of interest; (B) Extracting the tissue; (C) TMA block casting

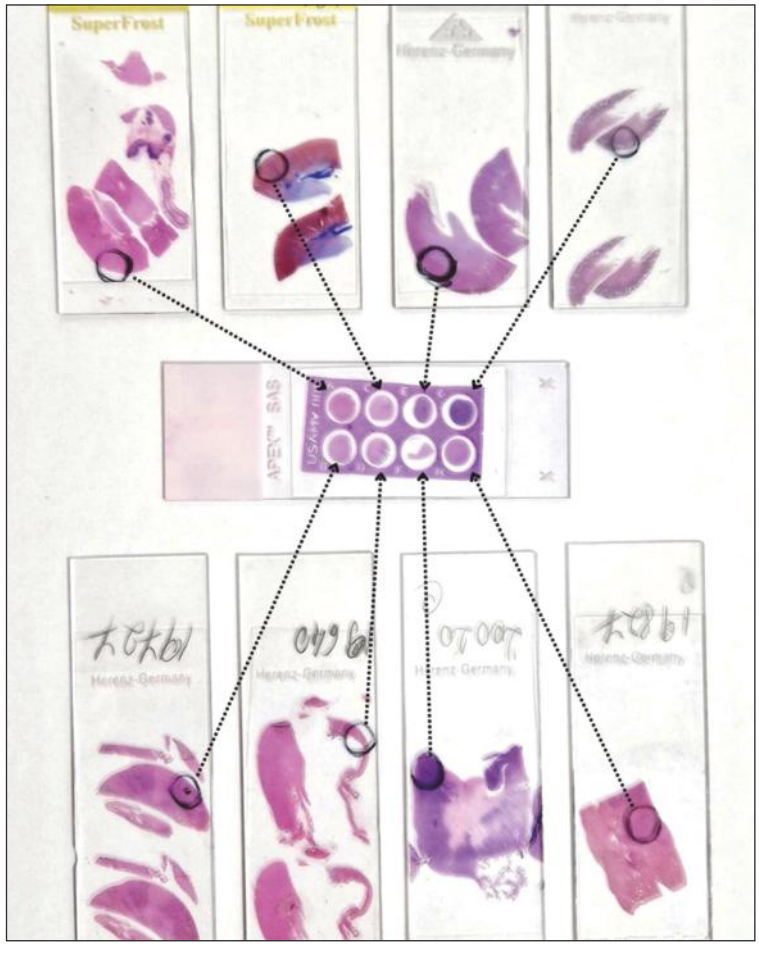


**Fig. 3.** Identification of donor tissues on the educational TMA slide

Sectioning of the TMA paraffin block was performed at 4 microns on a Leica RM2235 rotary microtome (Leica Biosystems, Wetzlar, Germany) resulting in 40 histological slides, leaving enough tissue for future re-cuts. Twenty slides were stained with haematoxylin-eosin, and the rest were left unstained for future studies.

## RESULTS AND DISCUSSIONS

As can be seen in Fig. 3, all the tissues have kept their position and orientation in the TMA, the matrix code can be read, and they are easy to identify when reading the slides.

As expected, the sectionable matrix maintained its shape, did not show folds, and did not disintegrate in the flotation bath. Although traditionally, TMAs use small donor tissue fragments, for educational TMAs, larger punches are required to be sufficiently representative of the studied pathologies.

Employing sectionable matrices instead of plain paraffin "recipient" blocks allowed the generation of unique, readable "sectionable" codes.

By functioning as a companion legend to the preparation, these codes facilitate quick and precise examination of the resulting educational slides.

Fig. 4 exemplifies well "F" of the TMA educational slide seen under a 4x microscope objective of a Picrosirius Red stain in visible light and polarized light.

## CONCLUSIONS

BxFrame™ educational arrays are a very useful adjunct for the student in veterinary Medical Histology and Pathology.

They take up less space than regular microscopy slides, and their reading under the microscope is much faster and more effective.

This project will be continued for many other types of organs and pathologies, resulting in a complete educational collection, the first of its kind in Romania.

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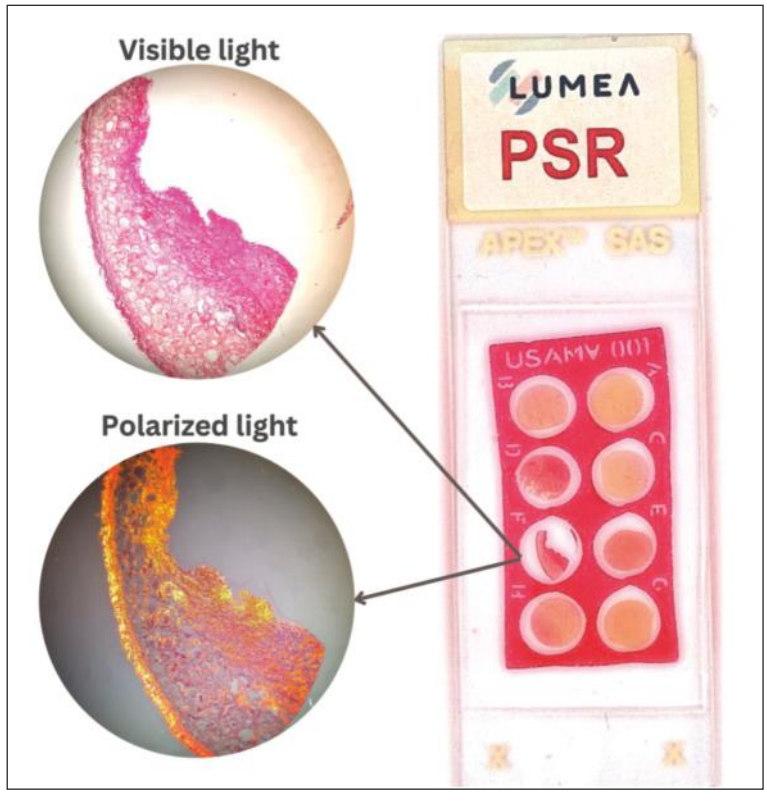


**Fig. 4.** Educational TMA slide:
Well F seen under the microscope
(Picrosirius Red stain, 4x)

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