# Anatomical evaluation and preparation procedure of a cross-sectioned kidney plastination of a thoroughbred horse with local polyester resin

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**Abstract**

This study aimed to develop a protocol for the thin section plastination of the kidney with local polyester resin and examine the anatomical details of this specimen. The sample was fixed with 10% formalin fixation solution and then sectioned into 3 mm slices. The four stages of the polyester plastination technique were used. Firstly, the samples were kept in 99.5% acetone baths at -25ºC for the dehydration. After this process, the sections were placed in polyester resin and the impregnation process was started at room temperature (20ºC) under vacuum. Following the forced impregnation, the curing chambers were constructed and the curing process was continued under ultraviolet light. The data of each applied stage were recorded carefully and the protocol for polyester plastination of kidney sections was successfully established. The specimens were observed from a different point of view with a thin cross-sectional appearance. The anatomical morphology and the structures of the sections of the kidney such as renal parenchyma and circulatory components were preserved well. The final products could be used as educational samples for cross-sectional anatomical training of kidneys.

## Introduction

The P40 polyester plastination was first introduced in the mid-1990s (6). The P40 polyester technique follows similar classical steps of silicone plastination. However, when the processes are examined in detail, there are some important differences. The first main step of the silicone plastination technique is to remove the fixative solution applied to the specimens and replace it with some chemicals like acetone. This process is called the dehydration stage and is also involved in the polyester plastination technique. The impregnation stage that follows dehydration, is the replacement of acetone with a different polymer. This stage consists of replacing the acetone with polyester resin on the basis of polyester plastination. The last step is curing which includes polymerization of the polymer in the structure and obtaining a solid, partially flexible, and long-lasting structure. This stage varies according to the type of plastination techniques (1, 5, 6).

The polyester plastination technique is used for the durable preservation of sections of various structures. Especially due to the developing technology, improvement in imaging systems has highlighted the understanding and evaluation of cross-sectional images. As a result, the number of plastination studies has increased on cross-sectional anatomy (1, 2, 4, 6, 9, 10).

The objectives of this study were to produce cross sectional polyester plastinated kidney slices, to develop a polyester plastination protocol with local polyester resin for kidney specimens and examine the anatomical details of the plastinated horse kidney.

## Materials and Methods

The present study was carried out in the Plastination Laboratory of of Ankara University Faculty of Veterinary Medicine Department of Anatomy. Also, additional support was received from TOBB University Faculty of Medicine Department of Anatomy. Ethical procedures were confirmed by Ankara University Animal Experiments Local Ethics Committee (Decision no. 2021-21-189, Ankara, Türkiye). Nomina Anatomica Veterinaria (12) was used for anatomical terms. All the processes were schematically given with photographs in Figure 1.

***Specimen Preparation**:* The exenteration of a right kidney from a thoroughbred horse was performed and used in this study. The sample was taken from one-week stayed cadaver after perfused with 10% formaldehyde. An incision was made into the renal capsule of the kidney and soaked in 10% formaldehyde for further 7 days to increase penetration of the fixative solution into the kidney.

After the fixation stage, the kidney was cut into thin slices with a deli slicer. The thickness of the sections was 2-3 mm. A total of eight sections were obtained and all serial sections were left in running water for one day to remove excess formaldehyde in the organ. All sections were then placed between square grids and stacked one after another. These grids were tied in a bundle to ensure that sections not to displace. This grid bundle was used in the plastination processes, respectively.

***Dehydration**:* The dehydration process is carried out in a cold environment (-25°C) to increase the quality of the final product (6). Sections were kept in three consecutive 99.5% acetone baths at -25°C. Throughout the dehydration process, acetone concentration was measured and monitored daily with an acetonometer. The bundle of the sections was stirred gently in acetone every day for 10 days. The duration of the first acetone bath took 5 days. At the end of the first acetone bath, the acetone value decreased to 92%. The second acetone bath took 3 days and the acetone value dropped to 97%. The final (third) acetone bath remained stable at 99.5% for 2 days. At this stage, the dehydration process was considered complete. Due to the texture of the kidney, no degreasing process was performed.

***Impregnation:*** Forced impregnation of kidney sections was performed using local full-clear moulding polyester resin (Turkuaz Polyester, Türkiye) at room temperature (20-22ºC). No activator was used while preparing the polyester resin in the impregnation process. The grid bundle was placed in the prepared polymer and kept inactive for 12 hours (vacuum pump stopped) overnight. Then, the forced impregnation process was applied active for 12 hours (vacuum pump running continuously). During this procedure, the samples were carried out in a dark environment to prevent the reaction of any ultraviolet light. This stage took 3 days; during this 3 days the internal pressure was reduced from the initial measurement of 912 mbar to 20 mbar. At this point, it was observed that the bubbles, which are the indicator of acetone output, decreased on the surface of the polyester bath in which the samples were located. When this amount of pressure was reached, the forced impregnation stage was considered completed.

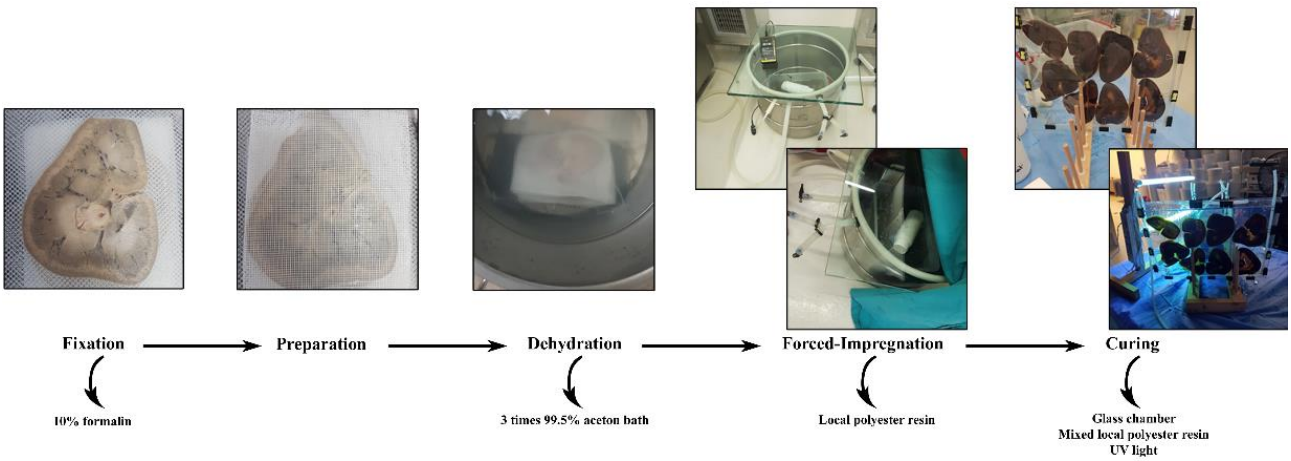


**Figure 1.** An overview of the polyester plastination procedure workflow from the fixation to the curing stage. Specimen observation in fixation and preparation phase. Three consecutive aseton bath with being started 99.5% concentration in dehydration phase. Local polyester resin being used with dark environment and vacuum in forced-impregnation phase. Vertical glass chamber with kidney sections being filled mixed local polyester resin and UV light application as a catalyst on the kidney slices in curing phase.

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***Curing:*** After the forced impregnation process was completed, the kidney sections were taken from the polyester resin to the curing room for hardening. Every section was placed in a glass chamber. The glass chamber was prepared between the 5 mm thick glass plates on both sides. All sides, except the top, were wrapped with a gasket between two glass plates and fixed with clamps. Then the sections of the kidney were placed inside and filled with a new modified local full-clear moulding polyester resin solution (Polyester / 1% Cobalt / Methyl Ethyl Ketone Peroxide: 99.6% / 0.1% / 0.3%). In order to prevent any air bubbles from forming in the polyester, the opening of the upper edge was used for the exit of air bubbles. In the curing room, glass chambers were placed under a ultraviolet light (30 minutes on and 30 minutes off) placed from all directions were placed from all direction of the sample at a distance of approximately 30 cm. In addition, it was tried to provide temperature distribution and protection to high temperatures with the help of a fan. The hardening of the polyester resin in the sections was completed in about 12 hours.

After curing was finished, the surrounding glass plates were removed from the sections. The sample was wrapped with plastic wrap for protection.

## Results

The protocol of section plastination prepared by using polyester resin was successfully applied to the kidney specimen. The local polyester plastinated sections were found to be durable and semi-transparent. These plastinated specimen is a good educational material in which anatomical features can be evaluated conveniently.

In the plastinated cross-sectional slices of the kidney, the anatomical details were preserved well and easily identified. In each layers of the kidney, renal cortex (cortex renalis), renal medulla (medulla renalis), and renal pelvis (pelvis renalis), were easily distinguished (Figures 2 and 3). On the plastinated sections the renal parenchyma consisted of two main parts, renal cortex and renal medulla. The outer zone of the renal medulla was darker than the inner zone of the renal medulla (Figure 2b and c). The radial striations belonging to the inner zone were visible and these lines extend to the renal sinus (Figure 3c and f). The endpoint of the medullary structure fused to form the renal crest (crista renalis) (Figure 3e). Papillary ducts were opened to the specific regions of the renal crest (Figure 3d, e, and f). It was seen that one of the large terminal recesses located at the end poles of the renal pelvis and many papillary ducts (ductus papillares) opened on this one to transfer the urine (Figure 3g).

Even though the kidney consists of the fused type of the cortical and medullary substances in the equine, it was determined that lobulation of the kidney can be seen due to the interlobar vessels when the parenchymal tissue was examined (Figure 2). The interlobar vessels and renal columns (columnae renales) were identified on the section image of the polyester plastination (Figure 2k).

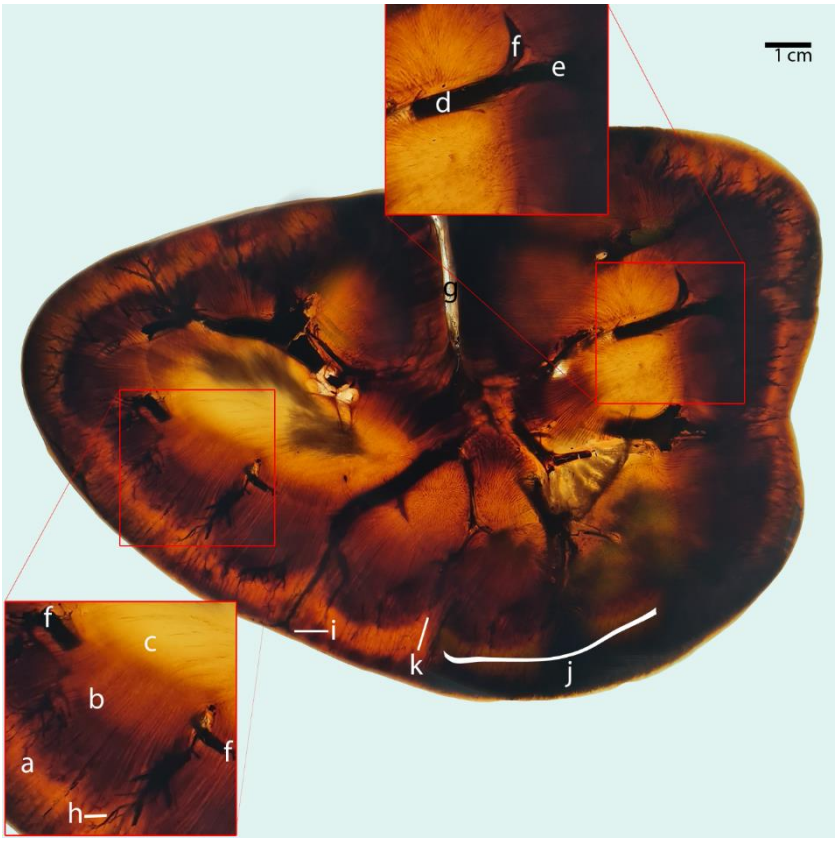


**Figure 2.** The polyester plastinated equine kidney slice.
a, renal cortex;
b, external zone of the renal medulla;
c, internal zone of the renal medulla;
d, interlobar vessels;
e, interlobular vessels at the crossing of the arcuate vessels;
f, arcuate vessels;
g, renal hilus;
h, efferent glomerular artery (arteriae glomerularis efferens);
i, stellate veins (venulae stellatae);
j, fused pyramidal shaped lobes;
k, renal column.

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**Figure 3.** The polyester plastinated equine kidney slice.
a, renal parenchyma;
b, renal cortex (cortex renis);
c, renal medulla (medulla renis);
d, renal pelvis (pelvis renalis);
e, renal crest (crista renalis);
f, papillary duct (ductus papillaris);
g, terminal recess (recessus terminalis);
* interlobular arteries (arteriae interlobulares).

Interlobar vessels were determined at the level of the renal hilus (hilus renalis) and it was observed that they proceed between the renal lobes (lobi renis) within the parenchymal tissue. Arcuate vessels were also detected in the corticomedullary region of the kidney and they were divided from the interlobar vessels. The part that kept on towards the renal cortex continues by taking the name of interlobular veins (Figure 2d, e, and f). These veins formed by the unity of the smaller efferent arterioles (arteriola glomerularis efferens) and capillary network (Figure 2h). Stellate veins (venulae stellatae), the subcapsular branches of the interlobular veins, were easily seen on the outer line of the renal cortex (Figure 2i).

## Discussion and Conclusion

Sheet plastination is a type of plastination that is considered to be a vital tool in the enhancement and clarification of concepts of cross-sectional anatomy and relationships previously often difficult to appreciate (15). The introduction of sheet plastination has provided us an opportunity to combine modern cross-sectional imaging techniques with corresponding slices of humans and animal tissues. Cross-sectional plastination samples have been previously proven to be used for educational purposes. However, it may be limited due to the long processing time required to obtain plastination samples and the use of high-cost chemicals during the collection of samples (3, 8, 16). When the sections preserved in the cured local polyester resin were evaluated in terms of their use, it was observed that they were easy to handle, biosafe, and cost-efficient.

The plastination protocol applied to the kidney sections of the polyester resin in this study can be used both for the preservation of kidney sections and for the differentiation of different layers in the sectioned kidney. As mentioned before, polyester plastination can be applied not only to the brain sections but also to the different organ sections (1, 5). The result of this study is in line with the above findings. In a previous study, students confirmed the educational advantages of P40 plastinated specimens in practical lessons. It was pointed out that 60% of students preferred plastinated specimens in the anatomy lessons (13).

In our study, plastinated samples illustrated the anatomical features of the kidney section very well. It can be used for demonstration of cross sectional part of kidney for students in practical section and for museum exhibition. Although it was not among the aims of this study to conduct research on student opinions, our results demonstrated that local polyester plastinates were good examples for museum specimens as well as for anatomy education, in addition to their affordable cost. These can be exhibited as very interesting and attractive examples for students and visitors.

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A uniform kidney structure is observed in domestic mammals due to the fused type of cortical and medullary substances. However, when the parenchymal tissue is examined, lobulation of the kidney can be seen apertly with a determination of the interlobar vessels (11). This explanation is also similar to our study. Renal columns were identified on the section image of the plastinated specimens. The parenchymal tissue is divided into two part. These are renal cortex and renal medulla with their morphological differences. The endpoint of the renal medulla is fused in the non-lobulated type of kidney. This area is called the renal crest (7) and it was visible at the renal pelvis end of the medullary structure of polyester plastinated slices in kidney of the horse.

The kidney contains vessels to ensure blood flow. In this plastinated sectioned specimens, the branches of renal blood vessels were visible. These branches are distributed within parenchyma of the kidney. The main root divided into several interlobar vessels at the hilus of the kidney. These branches further divided into arcuate vessels between the different renal lobes in the corticomedullary region of the kidney. Other branches, interlobular vessels, raised from the renal medulla to the renal cortex, and there interlobular vessels gave branches to support the renal corpuscles. These veins formed by the unity of the smaller efferent arterioles (arteriola glomerularis efferens) and capillary network. Blood vessels distribution and branching through paranchyma of kidney in our finding was incorresponding with studies conducted previously (7, 11).

One of the stages required for the preparation of a plastinated specimen is the dehydration phase. Aceton was used for dehydration of the slices since it is the best intermediate solvent for dehydration. Studies have shown that the acetone level should be at least 99% at the end of the acetone baths and it is recommended to be applied under the cold chain in order to prevent both biosecurity and tissue shrinkage (5, 14). In a previous study, it was revealed that the dehydration process carried out under the cold chain had a less shrinkage ratio (14). The same procedure is used for this study; 99.5% acetone in each bath and they were kept under the cold chain. In our study, the dehydration process was completed by passing the samples through three 99.5% acetone baths. The last value was estimated as 99.5% before proceeding to the next step. Degreasing may or may not be applied in order to remove the adipose tissues in the structure between the dehydration and impregnation stages (1, 6). In one of the previous study, the degreasing process was evaluated on the pig kidneys of the S10 plastination. It was stated that dehydrated and degreased kidney slices of the silicone plastination were shown distinct differentiation of the renal structures due to the presence of fat (16). In this study, the degreasing was not applied both by taking into account the amount of the fat tissue and to prevent any shrinkage that may occur. The distinction of the layers of the kidney were shown clearly in the polyester plastinated specimens.

The impregnation step was carried out in a dark environment to preserve the color of the kidney sections and to prevent undesired curing. In this way, any possible polymerization can be avoided as UV light acts as a catalyst of such polymers (6). In our study, the forced impregnation was applied at room temperature, and finished when the pressure reached 20 mbar (15 mmHg) at the end of the third day. In a study conducted by Guerrero et al. (5) on brain sections, 20 mmHg pressure is recorded at the end of the first day. Although the measurement result was in parallel with our study, the appropriate value was reached on the 3$^{rd}$ day. This situation is thought to be related to the applied passive pressure time. Since these types of polymers contain styrene, the applied pressure should be controlled frequently (6). In this study, the pressure was continuously monitored electronically to avoid the loss of styrene in the polymer at the forced impregnation stage.

In our study, fixation and dehydration stages were applied adequately and the values of the dehydration solution were checked every day. For this reason, local polyester resin was applied without using any catalyst. Another positive aspect of this is that polyester will thus be usable for a long time. It is thought that the high cost of plastination methods, which is the most discussed part, can be avoided with such approaches. It is known that samples with adequate fixation can be applied without using catalyst in the impregnation stage. The most important disadvantage of the use of impregnation solutions with a catalyst is the shortening of the polymer usage time (6). In addition, in a previous study, it was stated that the polyester bath not mixed with the activator could be used for a long time (1). In impregnation stage of our study, acceptable results were obtained.

In previous studies, it has been stated that no problems were encountered with the horizontal position of the samples in the curing process. However, in the same studies, it was stated that the specimens could be displaced vertically or curved curing process. For this reason, it has been suggested that attention should be paid to such problems (1, 6). The procedure in which the organ and polymer are placed in a vertical chamber is called the "vertical chamber method" (1). In this study, the curing process was applied with the vertical chamber method with a 15º inclination without any moving of the samples. No displacement of the samples in polyester was encountered.

The findings of this study showed that the polyester plastination method can be used to evaluate anatomical structures in kidney sections. It was observed that

polyester plastinated specimens can be used in thin sections obtained from various organs other than brain sections. One of the most important conclusion of this study is that using local full-clear moulding polyester for the preparation of plastinated slices of kidney can be used for demonstration of cross-sectional part of kidney for students in practical section and for museum exhibitions with an affordable cost.

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## Conflict of interest

The authors declare no conflict of interest.

## Author Contributions

CB conceived and planned the experiments. CB, HAY, BB and ST carried out the experiments. CB and HAY planned and carried out the simulations. CB, HAY, BB and ST contributed to sample preparation. CB, BB and OE contributed to the interpretation of the results. CB and HAY took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

## Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

## Ethical Statement

The present study was approved by the Ankara University Animal Experiments Local Ethics Committee (Decision No. 2021-21-189, Ankara, Türkiye).

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