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# Evaluation of the Fixation Effect of Modified Larssen, Klotz, Formalin 10% and Saturated Sall Solutions on Heart Tissue

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**ABSTRACT**

By evaluating the fixation effect of Modified Larssen (MLS), Klotz (KLO), Formalin 10% (FA) and Saturated Sall (SSS) solutions on heart tissue, it was aimed to determine a suitable solution for cadaver embalming. The solutions were prepared with the knowledge of the literature. The heart organs were photographed freshly with a millimetric scale on them. Three months later, the heart organs were removed from the solutions, a millimetric scale was placed on them again and they were photographed. In addition, the solutions were examined microbiologically every week. Three months later, after photographs were taken, histological examination was performed on sections taken from four heart tissues. As a result of color measurements, it was observed that the color was preserved best in MLS solution. In histological examination, it was determined that cell and tissue integrity was best preserved in FA solution. But KLO solution also gave results close to FA solution in histological examination. In the microbial analysis, unfurtunately MLS solution is the only solution in which microbial growth occurs. On the other hand, Klotz, SSS and FA solutions are resistant to microbial growth at room temperature for 3 months. Cadaver embalming solutions are becoming more and more important day by day. The study can be included in the literature as an important study in terms of comparing many different properties (color, histological and microbial analysis) of MLS, KLO, SSS and FA solutions.

**KEYWORDS:** Cadaver Embalming, Modified Larssen, Klotz, Saturated Sall Solution, Formalin 10%

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## I. Introduction

In addition to being a mysterious and fundamental building block of anatomy education, cadaver is also an educational material whose preservation and preservation method is quite laborious (Yoo et al, 2021). The infrastructure (cadaver tank, cadaver personnel, solution chemicals, etc.) of the departments that provide cadaver anatomy training must also be well-equipped (Yoo et al, 2021; Fruhstorfer et al, 2011).

The most popular study topic that has remained on the agenda of anatomists since history is the types of cadaver embalming solutions. In fact, the most popular anatomy solution that is synonymous with the words

cadaver and embalming is formalin (Hayashi el al, 2016). Because it was easily available and acted as a shield against most infections, except prion protein (Creutzfeldt–Jakob disease) (Ogami-Takamura el al, 2022)

In recent years, new cadaver preservation solutions are being added day by day (Wolff et al, 2008). Particularly intensive surgical cadaver courses and the high interest in these courses have increased the importance and variety of cadaver solutions. Because what is desired in surgical courses is to work on a cadaver that is close to fresh tissue in terms of color and texture, almost corresponding to a living human body (Jaung et al, 2011; Homma et al, 2019).

In this study, the effects of four different solutions called Modified Larssen (MLS), Klotz (KLO), Formalin 10% (FA) and Saturated Sall (SSS) on heart tissue were studied. Comparing these four solutions with each other in terms of microbiological, histological and color may provide new cadaver fixation solution data to the literature and shed light on future studies.

## II. Material and methods

The four solutions used in the study were prepared in the light of literature knowledge. Chemicals in Klotz solution

(KLO): 1.53 mol NaCl, 0.59 mol NaHCO3, 2.41 mol $Cl_3$ $CCH(OH)_2$ , Formalin 300ml and distilled water 10 liters

(Ulmer, 1994). Content of Formalin 10% solution (FA): 10% Formaldehyde 4L, Phenol 0.4L, Glycerine 1L, Distilled water 14.6L (Hayashi et al, 2014). Content of saturated sall solution (SSS); Sodium chloride 20 kg (saturated), 20% Formaldehyde 1.0 L, Phenol 0.2 L, Glycerine 0.5 L, Isopropyl alchol 4.0 L, distilled water 19.3 L (Hayashi et al, 2014). The content of the Modified Larssen solution (MLS) is: 100 mL 10% formalin, 400 mL glycerol, 200 g chloral hydrate, 200 g sodium sulfate, 200 g sodium bicarbonate, 180 g sodium chloride, and 2 L distilled water (2500 Liters). This recipe = 1 part concentrate. This concentrated portion is completed with 3 times ml of distilled water (total: 10 liters of solution is obtained) (Da Silva et al, 2004).

**Color analysis:**

In order to observe the effect of cadaver fixation solutions on the heart tissue, 28 sheep hearts obtained from the slaughterhouse were photographed by placing the metric before being put into the solutions with a camera. Color analysis of fresh heart organs was performed with Image J software. All solutions were prepared according to the literature description and placed at room temperature (25°C) in storage containers. Then the heart organ (sheep heart) was placed in all solution containers (28 solution containers were prepared because each solution has 7

samples). For three months, all solution containers were opened once a week, the heart organs were examined, and the color and odor of the solutions were subjectively examined. At the end of three months, metrics were placed on the heart organs and their photos were taken and color measurements were made with the help of Image J software program. Color measurement was examined in two ways as the difference between the solutions at the end of three months and the difference between them with fresh tissue at the end of three months. The resulting measurement values were interpreted by comparing with the fresh tissue color. In addition to color analysis, histological and microbiological analysis were performed.

***Histological analysis:***

Heart tissues taken from FA, KLO, SSS and MLS fixatives were fixed for 3 months (90 days). Then, in order to remove the fixatives in the tissues, the tissues were washed under running water for 30 minutes, and to remove water from the tissues, they were passed through a series of 70%, 80%, 96% alcohol at 1-hour intervals. Then it was kept in 96% alcohol for 1 night. Following this, the tissues were kept in 99% alcohol for 1 hour, with two changes. Subsequently, the tissues were kept in xylene series for 15 minutes to make them

transparent, and three changes were passed through paraffin series for 1 hour in a 60 ˚C oven. Paraffin was absorbed into the tissue. The tissues were then embedded in metal cassettes and blocked. Blocked tissue samples were cut with a microtome at a thickness of 5 microns. Sections were stained with hematoxylin and eosin dye.

***Microbiological analysis:***

Solutions containing 28 heart organs used in the study were kept at room temperature (25 degrees) during the study. For bacterial isolation, 100 µl of the solutions containing the samples were taken and superficial fluid swab samples were taken, from heart tissue at the end of each week and these samples inoculated on Eosine Methylene Blue agar (EMB), Mac Conkey agar, and blood agar containing 7% sheep blood. Plates were incubated in aerobic and microaerobic environments at 37°C for 24-48 hours. Sabouraud Dextrose agar was used for fungal isolation and incubations were done at room temperature (18-22°C) and 37°C.

***Statistical Analysis***

The analyzes of the data (color measurements) obtained in our study were performed using SPSS® Statistic Version 25 (IBM®,USA). Comparisons between groups were made with the Oneway ANOVA test. Post-hoc Tukey analysis was performed for comparisons of multiple groups. As a result of the analysis, $p<0,05$ value was considered statistically significant.

## III. Results

***Color Analysis Findings:***

Four groups of solutions were compared with each other and with their fresh images after three months (Table 12).

As a result of the comparisons made at the end of three months, when the average of the red-blue-green colors was taken, it was determined that the MLS solution was closest to the fresh tissue. After MLS, KLO is the solution that comes closest to fresh tissue in terms of color. The SSS solution slightly captured the fresh color of the tissue, but FA failed considerably in terms of color.

In all groups, another evaluation was made by subtracting the differences between the color values in the photographs of fresh tissues that had not yet entered the solution and the color values after being kept in solutions for three months. In this resulting difference, the average of red-blue-green colors is parallel to the average values above. In other words, while there is a small difference in the MLS solution, the difference in the KLO solution is slightly greater than in MLS. The biggest difference (i.e., the difference value between fresh tissue and tissue remaining in solution for three months) is in the color values of heart tissues remaining in FA and SSS solutions thereafter. As can be seen from the photographs, the texture color is the faintest in FA, followed by SSS.

When the two tables obtained (Tables 1-2) were examined, a significant difference was found between the four solutions in both evaluations ($p=0.000$; $p<0.05$; Oneway ANOVA).

**Table 1.** Comparisons between groups at 3 months.

| Groups | Red Difference (Mean ± SD) | Green Difference (Mean ± SD) | Blue Difference (Mean ± SD) | (K+Y+M)/3 Difference |
|---|---|---|---|---|

| | | | | (Mean ± SD) |
|---|---|---|---|---|
| **MLS** | 171,56 ± 1,18[a] | 92,26 ± 0,80[a] | 71,50 ± 1,00[a] | 111,63 ± 1,06[a] |
| **KLO** | 143,85 ± 0,51[b] | 107,11 ± 0,39[b] | 84,85 ± 0,56[b] | 112,04 ± 0,66[a] |
| **FA** | 185,01 ± 0,96[c] | 172,06 ± 1,07[c] | 155,12 ± 0,91[c] | 171,23 ± 0,57[b] |
| **SSS** | 171,80 ± 0,86[a] | 129,48 ± 1,23[d] | 116,91 ± 0,97[d] | 140,92 ± 1,64[c] |
| **F** | 2514,027* | 9747,929* | 12362,465* | 4887,136* |
| **p** | 0,000 | 0,000 | 0,000 | 0,000 |

**p<0.05** (Oneway ANOVA). **F:** F value. **SD:** standard deviation. ***:** There are differences between groups. Different superscripts **(a,b,c,d)** in the same column indicate statistical differences between groups. Modified Larssen **(MLS)**, Klotz **(KLO)**, Formalin 10% **(FA)** and Saturated Sall **(SSS).**

**Table 2.** Difference between groups (3 Months - Fresh) comparisons.

| **Groups** | **Red Difference (Mean ± SD)** | **Green Difference (Mean ± SD)** | **Blue Difference (Mean ± SD)** | **(K+Y+M)/3 Difference (Mean ± SD)** |
|---|---|---|---|---|
| **MLS** | 2,61 ± 1,18[a] | 11,14 ± 0,80[a] | 10,42 ± 1,00[a] | 7,91 ± 1,06[a] |
| **KLO** | -25,09 ± 0,51[b] | 25,99± 0,39[b] | 23,77 ± 0,56[b] | 8,32 ± 0,66[a] |
| **FA** | 16,06 ± 0,96[c] | 90,94 ± 1,07[c] | 94,04 ± 0,91[c] | 67,51 ± 0,57[b] |
| **SSS** | 2,85 ± 0,86[a] | 48,36 ±1,23[d] | 55,83 ± 0,97[d] | 37,20 ± 1,64[c] |
| **F** | 2514,027* | 9747,929* | 12362,465* | 4887,136* |
| **p** | 0,000 | 0,000 | 0,000 | 0,000 |

**p<0.05** (Oneway ANOVA). **F:** F value. **SD:** standard deviation. ***:** There are differences between groups. Different superscripts **(a,b,c,d)** in the same column indicate statistical differences between groups. Modified Larssen **(MLS)**, Klotz **(KLO)**, Formalin 10% **(FA)** and Saturated Sall **(SSS).**

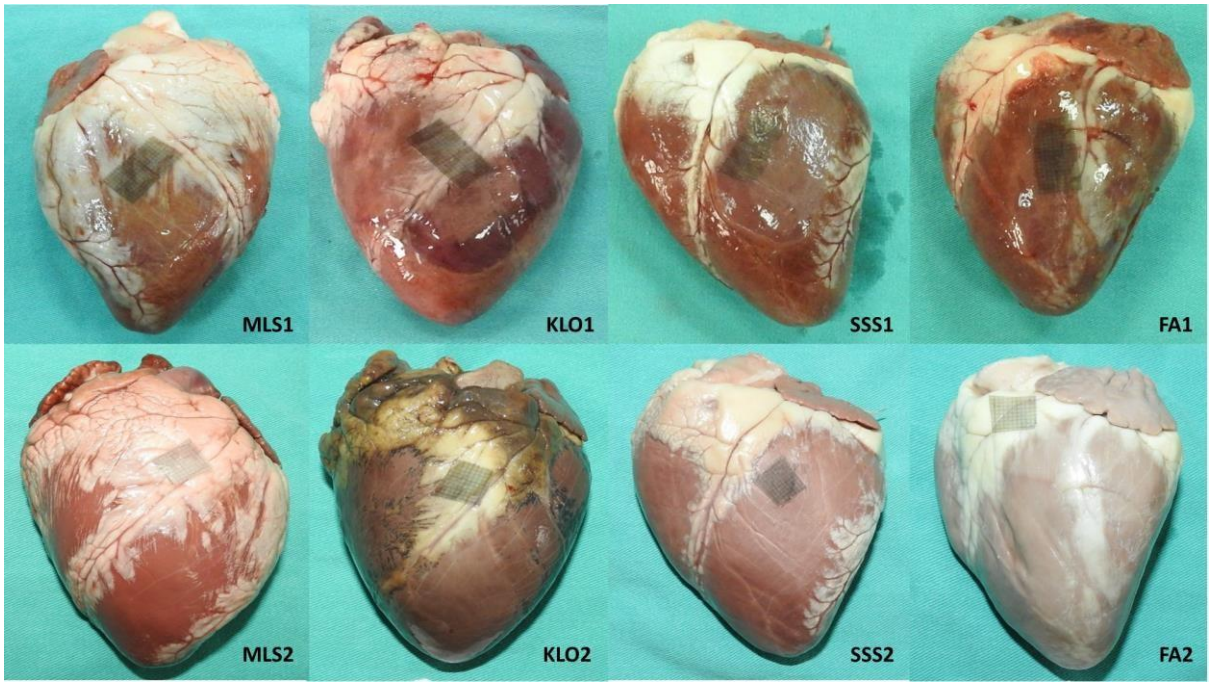


**Figure 1.**

**MLS 1;** Fresh heart that has not yet entered the MLS solution,

**MLS 2;** Heart that remained in MLS solution for three months,

**KLO 1;** Fresh heart that has not yet entered the KLO solution,

**KLO 2;** Heart that remained in KLO solution for three months,

**SSS 1;** Fresh heart that has not yet entered the SSS solution,

**SSS 2;** Heart that remained in SSS solution for three months.

**FA 1;** Fresh heart that has not yet entered the FA solution,

**FA 2;** Heart that remained in FA solution for three months.

***Histological Findings:***

When heart tissue samples fixed with four different fixatives are examined, there are separations in all tissues, muscle fibers and endomysium surrounding the muscle fibers. These separations are observed most in MLS fixative and least in formalin fixative. When KLO and SSS fixatives are compared, fewer separation areas are observed in KLO fixative.

When the cellular structure in the tissue is compared, it is observed that FA and KLO fixatives preserve the cellular structure in the tissues, but it is observed that the cellularity in KLO fixative is less than in FA fixative and the cells show eosinophilia. Although cellularity is observed to decrease in the SSS fixative, the MLS fixative has the least cellularity, severe eosinophilia and karyolysis compared to other heart tissues.
When the blood vessels in the tissues are examined, it is observed that the vascular wall structure is protected best by formalin, followed by KLO fixative, and in SSS fixative, the cellularity in the vessel wall is less than KLO and FA. However, in MLS fixative, there is severe degeneration and destruction in and around the vessel

wall. In addition, areas of vacuolar degeneration due to damage to the epicardium and myocardium in MLS fixative are also noteworthy.

When examined histopathologically, it is observed that the best fixation in the tissues is achieved with FA, KLO, SSS and MLS, respectively.

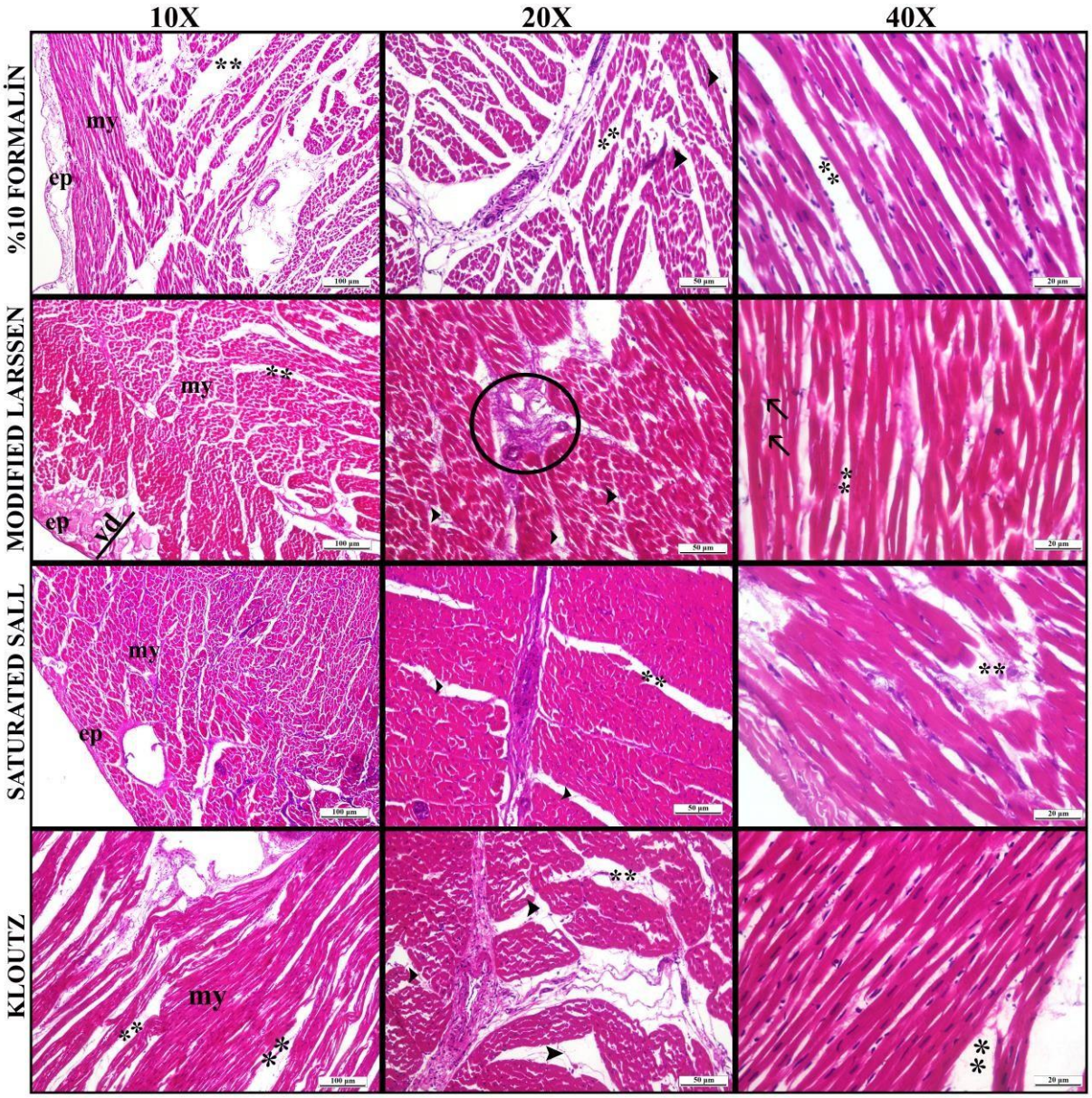


**Fig.2. ep:** epicardium, **my:** myocardium, **: muscle fiber separations, **arrowheads:** separations in the endomysium, **arrow sign:** karyolysis, **circle:** degeneration areas in the vascular wall and surrounding areas. Modified Larssen **(MLS)**, Klotz **(KLO)**, Formalin 10% **(FA)** and Saturated Sall **(SSS)**

***Microbiological Findings:***

As a result of the incubations, growth was observed in MLS fixative solutions where the heart organs were located. Colonies in the growth media were evaluated in terms of their macroscopic and microscopic characteristics. Nonhemolytic, S-type, non-pigmented colonies growing on blood agar were examined. As a

result of Gram staining, microscopic examination revealed both double and single Gram-negative coccobacilli. Classical biochemical tests were applied to colonies considered suspicious for Acinetobacter. As a result of biochemical examinations, these non-motile, sporeless colonies giving catalase positive, oxidase negative and $H_2S$ negative reactions were identified as Acinetobacter spp. Yeast growth was observed on Sabouraud agar as a result of incubation at 18-22°C.

## IV. Discussion

The common desire of anatomy departments that teach courses with cadavers is an optimal cadaver solution. If we need to elaborate on the term 'optimal cadaver solution', it can be listed as having a non-irritating odor, maximum protection against early infection and preserving the natural appearance and color of the cadaver (Kaliappan et al, 2023). Formalin 10%, the most popular solution in anatomy laboratories for years, has begun to be replaced by different solutions in recent years. Because the carcinogenic and irritant effects of formalin affect both cadaver workers, instructors and students extremely negatively (Ufelle et al, 2022). This being the case, with the development of technology day by day, new solution trials have begun to take their place in the literature. One of these is Thiel solution. In the research conducted, the positive properties of Thiel's solution have shown themselves in the literature (Beger el al, 2020; Wolff et al, 2008).

In this article, the effects of four different solutions on the heart organ were examined, based on solution studies and recipes in the literature. The heart organ is a solid organ with a very unique shape, containing both striated and unstriated muscles in its structure. Therefore, it was found suitable for analysis to show histological and anatomical changes as it is exposed to the solution.

The histological, microbial and color analysis results of these four solutions MLS, KLO, SSS and FA 10%, prepared based on the solution descriptions in the literature, revealed very important data. Especially in studies conducted with MLS, histological findings of MLS have not been included much (Da Silva et al, 2004; Kaliappan et al, 2023). In addition, color analysis has not been a parameter evaluated in almost most solution studies. Especially the color and histological findings of MLS, KLO, FA and SSS can be included in the literature with this study. In many articles, only the name of Klotz (KLO) solution was included (Zanuto et al, 2019; Brenner, 2014; Balta et al, 2015). However, in this study, the description of the KLO solution is given and its histological and microbial findings are written.

According to the results of the study, MLS can be used by freezing the cadaver, as understood from the studies in the literature. Apart from this, unfortunately, room temperature causes the growth of microorganisms. The effect of +4 degrees on MLS is not yet known. Apart from this, it provides very good protection in terms of color, but causes deterioration in the histological structure. SSS is resistant to microbial growth at room temperature, but fails like MLS in terms of color and histological structure integrity. The best solutions in terms of microbial protection and histological structure integrity are FA and KLO. FA is quite unsuccessful in terms of color. It fades the color of the tissue to a great extent. KLO, on the other hand, is a very good solution in terms of color, microbial protection and histological structural integrity. During this study, the solutions were kept at room temperature for three months. This study or studies consisting of different solutions can be tried at different temperatures and for different periods of time.

Cadaver embalming solution studies are an enormous subject that is constantly renewing itself from past to present and open to research. Since the cadaver is an indispensable element of the anatomy course, the preservation of the cadaver (both against microorganisms and in terms of color and cellularity) is an issue that requires great care and attention. Choosing a cadaver embalming solution is of great importance for a safe and comfortable anatomy course laboratory environment. Cadavers used for anatomy lessons have become important materials in surgical courses in recent years. For this reason, the subject of solution has now become a broad research field, which is studied not only in anatomy courses but also in postgraduate research and surgery courses.

## V. Conclusion

There is almost no literature in the literature that includes comparative histological examination, color analysis and microbial evaluation of the solutions used in this study. In particular, Klotz (KLO) solution is good in

terms of color preservation, tissue integrity and microbial protection (at room temperature), making it suitable for use in cadaver embalming. Studies of this solution at different temperatures can also be carried out for one or two years.

**Ethics Committee Approval:Ethics Committee Approval:** Ethics committees approval was obtained from Kafkas University Animal Experiments Local Ethics Committee KAU-HADYEK 2023/095

**Conflict of Interest:** Regarding this study, the author and/or her family members do not have a potential conflict of interest, scientific and medical committee membership or relationship with its members, consultancy, expertise, employment in any company, shareholding or similar situations.

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