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Modified methods to simplification histochemical, immunohistochemical, and hematoxylin-eosin staining

Ali Kalantari-Hesari[1*], Hassan Morovvati[2], Mohammad Babaei[3], Alireza Nourian[4], Kaveh Esfandiari[5], Taher Elmi[6] and Salman Soltani[7]

[1] Assistant Professor, Department of Pathobiology, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran
[2] Professor, Department of Basic Science, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[3] Assistant Professor, Department of Clinical Sciences, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran
[4] Associate Professor, Department of Pathobiology, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran
[5] Assistant Professor, Department of Basic Science, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[6] Assistant Professor, Department of Laboratory Science, Babol Branch, Islamic Azad University, Babol, Iran
[7] PhD of comparative histology, Central Laboratory, University of Ilam, Ilam, Iran

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Abstract

Most histotechnical methods and preparation of tissue sections were invented and used in the 18th and 19th centuries. Due to the colorless nature of most cells and tissues in the body, tissue staining methods were used from the beginning to differentiate cells and tissues. *Materials & Methods:* According to sources and reports in this field, the time required for each hematoxylin-eosin staining period is approximately 80 minutes. In the present study, a method of hematoxylin-eosin staining is reported that reduces the time required for staining by maintaining the quality of sections and staining from 80 minutes to less than 40 minutes. Also, considering the existing problems regarding the cost of histochemistry and immunohistochemistry kits, the Low amount of kits and the problems that exist in the drip staining method of this type of staining, the present report tries to present three creative methods in this regard. These methods increase the quality of the prepared slides, in addition to reducing consumables. Optimization of tissue staining steps can reduce expenses spent and increase the quality of tissue sections.

Keywords: Tissue sections, Histotechnique, Histochemistry, Immunohistochemistry

Introduction

After the expansion and modification of the light microscope structure by Anton van Leeuwenhoek (in 1673), the first microtome, proper for sectioning the animal tissues, was made in 1848. Also, most of the materials and methods used to prepare tissue sections, such as formalin, paraffin, tissue passage methods, tissue staining methods, etc., were invented and used in the 18th and 19th centuries (Titford, 2006). The materials that used for tissue staining in terms of the origin of production are divided into two groups of natural and synthetic dyes. Among the natural dyes, can mention

[*] **Corresponding Author**: Ali Kalantari-Hesari, Assistant Professor, Department of Pathobiology, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran
E-mail: a.kalantarihesari@basu.ac.ir

Cochineal, saffron and carmine. Methylene blue and eosin can also be mentioned as synthetic dyes. Synthetic dyes are usually compounding of benzene derivatives (Suvarna et al., 2018). Hematoxylin was first used by Wilhelm von Waldeyer in 1863 (Conn, 1928). The production of synthetic dyes for tissue staining with the production of aniline dye by William Perkin in 1856 became a growing trend and the production of many other dyes evolved in Germany. Primary dyes were mostly invented and made for the textile industry (Beer, 1956). For the first time, Schwarz in 1867 used several dyes for tissue staining. This method was used in 1868 for tissue differentiation. So, different types of hematoxylins were invented, such as Delafield's (in 1885), Ehrlich's (in 1885), and Heidenhain's (in 1892) (Bracegirdle, 1981).

Hematoxylin is obtained from Heartwood ('logwood') and *Hematoxylon campechianum* tree. The origin of this tree is in Mexico, but now it is widely cultivated in the West Indies. Hematoxylin is extracted by natural oxidation methods (exposing the hematoxylin solution to air for three to six weeks) or chemical oxidation (using oxidizing substances such as mercuric oxide and hydrogen peroxide). For tissue staining, hematoxylin alone does not have much affinity with the tissue, and in order to increase its affinity, it is necessary to have a substance called "mardant" or color stabilizer in the dye composition to give the dye an alkaline state; it well stained the acidic elements in the nucleus. Mardant consists of divalent or trivalent metal solutions that are attached to the dyes and increase their staining power. The utilization of mardant in tissue stain has several advantages, which include: when we add mardant to the dyes, the dyes will be relatively stable, and the subsequent water extraction will not cause the loss of stain. To prepare a more durable color, should be using materials that do not have an oxidizing effect or contain a weak oxidation level, such as alloaluminum, alloptasium, phosphotungsten acid, phosphomolybdic acid, and iron alum. But if the durability of the dye is not important, can be used stain that have a high degree of oxidation, such as ferric chloride and ferric acetate, but, because of that the mentioned dye loses its coloring properties within a few hours, it is necessary to prepare it just before using. The Alum salts of aluminum, potassium or iron can be mentioned among the common mardants. Hematoxylin solutions are classified as alum hematoxylins, iron hematoxylins, tungsten hematoxylins, molybdenum hematoxylins, lead hematoxylins, and hematoxylins without mardant (Morrovati and Kalantari-Hesari, 2018).

One of the types of hematoxylins is Harris hematoxylin. Mercuric oxide is used to oxidize this type of hematoxylin (Harris, 1900). But due to the high toxicity of mercury and its long shelf life and its incompatibility with the environment and the destructive effects it has on laboratory equipment, today, sodium or potassium iodate is used. This type of hematoxylin has a suitable nuclear subtraction and acid alcohol can be used for its discoloring. According to reports, 2.5 gr of hematoxylin powder, 25 ml of absolute alcohol, 50 gr of potassium alum, 500 ml of distilled water, 1.25 gr of mercuric oxide (or sodium iodate) and 20 ml of glacial acetic acid are used for its preparation. Hematoxylin powder dissolves in absolute alcohol, and potassium alum dissolves in distilled water. These two solutions are mixed together and quickly reach the boiling point. At the boiling stage, mercuric oxide (or sodium iodate) is slowly added to the solution. Then the solution is cooled and acetic acid is added to it. Adding glacial acetic acid is optional, but adding it improves the stainability of the nucleus. The prepared hematoxylin solution loses its quality after a few months due to the formation of sediments. It is better to prepare the solution monthly and filter the solution before using. The duration of

staining with this type of hematoxylin in cytological studies is 5 to 10 minutes in Harris's progressive and 30 seconds to 4 minutes in Harris's regressive (Morrovati and Kalantari-Hesari, 2018).

There are different types of eosins, but eosin Y is most commonly used as a solution in water or alcohol. Eosin is usually prepared and used as a 0.5 to 1% solution. A small piece of thymol can be used to prevent the growth of fungi in eosin stain. To increase the stain sensitivity, 0.5 ml of acetic acid is added for every 1000 ml of eosin solution. Mostly, the excess colors of eosin are removed in the washing step in water, but a part of this color subtraction is also done in the step of dehydration by alcohols (Suvarna et al., 2018). Hematoxylin-eosin staining was introduced by Wissowzky in 1875 (Mazzarini et al., 2021). In the 19th century, the process of creating and expanding tissue staining took on a growing trend. The first automatic tissue staining machine was invited in 1960. Also, in 1966, immunohistochemical staining methods were used for the first time (Titford, 2006). Hematoxylin-eosin (H&E) staining is the most common method of staining tissue sections, which is easy to use and show the well differences in tissue structures. Hematoxylin stains the nucleus of the cells in black-blue color and shows the details of the nucleus well; while eosin stains the cytoplasm and connective fibers as pink, orange, or red (Suvarna et al., 2018).

The method of staining with Harris's hematoxylin is reported in the book Bancroft's theory and practice of histological techniques as follows: slides were deparaffinized and hydrated by descending alcohols. In the next step, it is better to remove the fixative solution by washing in water. Then the slides are stained with hematoxylin solution with the appropriate time. Subsequently, washing in running water for 5 minutes or less (until the nuclei turn blue) and then subtraction in 1% alcohol acid solution (1% HCl in 70% alcohol) for 5 to 10 seconds. After that, it was washed in running water (10 to 15 minutes) or it can be placed in an alkaline solution for 4 minutes until the nuclei turn blue again. Then, the steps include staining in eosin Y solution (for 10 minutes), washing in running water (for 1 to 5 minutes), dehydration with ascending alcohols, clarification by xylene and the last step is mounting. The result of staining includes nuclei in blue to black color, cytoplasm in a wide range of pink color, muscle fibers in dark pink to red color, red blood cells in orange to red color and finally fibrin in color dark pink is visible (Suvarna et al., 2018).

Usually, the time required for hematoxylin-eosin staining in reference sources is considered to be about 80 minutes. However, the emergency hematoxylin-eosin staining method for frozen sections has also been reported. The method of this type of staining is as follows: the tissue is blocked in a frozen section embedding medium and sections with a thickness of 3 to 6 micrometers are prepared. Then, the sections are fixed in 10% buffered formalin solution for 20 seconds at room temperature and placed in water for 4-5 seconds. Afterword, it is placed inside the concentrated solution of Carazzi's hematoxylin for one minute. In the next step, washing in water for 10 to 20 seconds, staining with 1% aqueous eosin solution for 10 seconds, a few taps in water, dehydration, clarification by xylene and the last step is mounting. According to the references, the time required for each hematoxylin-eosin staining is about 80 min. In the present research, a method of hematoxylin-eosin staining is reported, which reduces the time required for staining from 80 min to less than 40 min while maintaining the quality of tissue structure and staining. Also, considering the existing problems regarding the cost of preparing histochemistry and immunohistochemistry kits and materials, the low volume of the kits and the problems that exist in the drop staining method of this type of staining, this

report tries to present three creative methods in this regard. In addition to reducing the consumption of materials, these methods also increase the quality of prepared slides.

Materials and methods

Hematoxylin-eosin staining method in a shorter time

Harris's hematoxylin stain preparing method

To prepare 100 ml of Harris hematoxylin stain: 0.6 gr of hematoxylin powder (Merck, 105175), 10 ml of absolute ethyl alcohol 99% (Merck, 107017), 10 gr of alum ammonium or potassium (Merck, 101047), 100 ml of distilled water and 0.5 gr of mercuric oxide (Merck, 104465) are needed. Then, hematoxylin powder is dissolved in alcohol and potassium alum-ammonium in distilled water in the vicinity of a mild heat and the two solutions are mixed together and quickly reached to the boiling point. Afterword, the mercuric oxide is slowly added to it and the solution is quickly cooled (by placing in cold water). The solution is dark purple in color and can be stored for several months. Due to the quality of raw materials prepared, the weight and volume of the material may be changed very slightly.

Acid alcohol preparation method: to prepare 100 ml of acid alcohol, 1 ml of 65% concentrated hydrochloric acid (Merck, 100317) is dissolved in 99 ml of 70% ethyl alcohol.

Lithium carbonate saturated solution preparation method: To prepare 100 ml of this solution, 1.25 gr of lithium carbonate is dissolved in 100 ml of distilled water. The volume of lithium carbonate should be enough to deposit some of the lithium carbonate at the bottom of the container.

Eosin satin preparation method: To prepare 100 ml of eosin color solution, 1 gr of Eosin Y powder (Merck, 115935) is dissolved in 100 ml of distilled water and 1 ml of glacial acetic acid (code 100063) is added to it.

Staining method

The sections for this method should be prepared with a thickness below 5 µm. A suitable period of time (at least 24 hours from the cutting stage) should be considered for the complete adhesion of the tissue section on the slide, so that the sections are not removed from the slide during staining. Before staining, ensure the quality of all the consumables, especially the percentage of alcohols. In this method, three containers containing xylene (Merck, 108298) is considered for 5 minutes in each dish. It is better to give a few taps on the tissue paper before transferring the slides from xylene to the alcohol container in order to prevent excess xylene solution from being transferred into the alcohol and reducing the quality of the alcohol. Then, the slides are placed in the alcohol container and the consideration time is 30-60 seconds. It is better to gently shake the slides basket every few seconds. Then, slides are placed in 80% alcohol and 70% alcohol for five dips, respectively. Increasing the duration of placing the slides in the 70% alcohol container (5 dips to 1 minute) will help to increase the quality of dehydration. After this step, the slides are transferred into a container of water (preferably running water). After 2 minutes, the slides should be checked for the quality of deparaffinization and dehydration (check point 1). At this stage, one of the slides is removed from the basket and checked. If the steps have been done well and with quality materials, at this stage only the cut tissue should be visible on the slide, without any paraffin or cloud-like halos around it. In fact, whatever, the surface of the slide is cleaner, the steps are performed more accurately.

After the washing stage, the slides are stained with Harris's hematoxylin solution for 3 to 5 min. The exact duration of placing the slides inside the hematoxylin stain depends on the preparation time of the stain and its quality; if the stain prepared freshly and carefully, it will be shortening the staining time. To check the stain quality, it

can be put a drop of hematoxylin satin into a water container and after the drop of stain spreads, its color spectrum can be observed. The blue to purple color spectrum means the correct satin quality, and the brown to black color spectrum indicates the corruption of the stain solution and should not be used. It is better to check the volume of dye inside the staining container so that it covers the entire height of the slides. Shaking the slides every 30 seconds inside the staining container will improve dye acceptability. After the end of the staining time, the slides are placed in a container of running water for two min. At this stage, after washing, the slides should be checked for the quality of staining (check point 2). If the sections on the slide have a spectrum from blue to purple (according to the volume of the nucleus in the tissue structure), the hematoxylin staining step has been done correctly (Figure 2, 1).

After washing, the decolorization process is done by two dips in an acid-alcohol container. At this stage, if the decolorization has been done correctly, the color of the tissue sections will change from dark blue to light brown (checkpoint 3) (Figure 2, 2). At this stage, the excess colors of the tissue will be washed. Slides must be transferred to water immediately after leaving the acid-alcohol, otherwise the hematoxylin color will be completely removed. Afterword, in order to stabilize the remaining color on the tissue sections, the slides are placed in saturated lithium carbonate solution for 15 seconds. If the materials and execution method are correct, the result of this work will cause the color of the slides to change from brown to light blue (check point 4) (Figure 2, 3). Then, the slides are washed in water for 2 minutes so that there is no disturbance in the cytoplasmic staining stage.

To stain the cytoplasm and some tissue structures such as fibers, the basket containing the slides is placed in the eosin container for 90 seconds. The duration of using eosin stain depends on the quality of the dye. It is better to prepare eosin stain fresh and before using it, dye inside the container are mixed completely by a spoon so that the settled material is separated from the bottom of the container. If the quality and red color of eosin is not satisfactory, one to two drops of glacial acetic acid can be added to it. It should be noted that adding more glacial acetic acid to the eosin stain causes it to become too acidic and may cause removing of the hematoxylin dye on the tissue sections (Figure 1).

Henceforth, the stained slides are washed in running water for 2 min before dehydration. This step continues until the color of the slides no longer comes out from the water. The stage after washing the stained slides with eosin and before the dehydration process is the best time to wipe the slides with a tampon. At this time, you can clean the extra textures or satin around the slide with a tampon. Also, this step is important as check point number 5 (Figure 1). If the staining method is done correctly, the color of the tissue sections can be seen in a spectrum from pink to red along with purple spots (Figure 2, 4).

After washing and drying the basket, the slides enter the dehydration stage. This stage contains ascending alcohols with percentages of 70 (two to three dips), 80 (two to three dips) and a container of absolute alcohol (for 2 min). Because 70% and 80% alcohol have eosin decolorization properties, it is recommended to pass the tissues through the mentioned two containers faster. The container of absolute alcohol plays a very important role on the quality of the slide in the dehydration stage. It is better to ensure the quality of absolute alcohol with an alcohol-meter before starting staining, otherwise it is better to add another container of absolute alcohol to the last step of dehydrating. The use of the second container of absolute alcohol is to ensure complete dehydration of tissues (Figure 1).

After dehydrating the tissues and drying the basket with tissue paper, the basket of the slides is put into the xylene container.

This operation is done for two purposes: 1- to increase the refractive index and make the tissue sections more transparent. 2- xylene has the ability to mix with entellan medium. For this purpose, it is better to use at least 2 containers of xylene for clarification. The first container is considered for 3 min and the second container for at least 5 min. If there is no time for mounting, the slides can be kept inside the container containing xylene 5 for several hours (Figure 1).

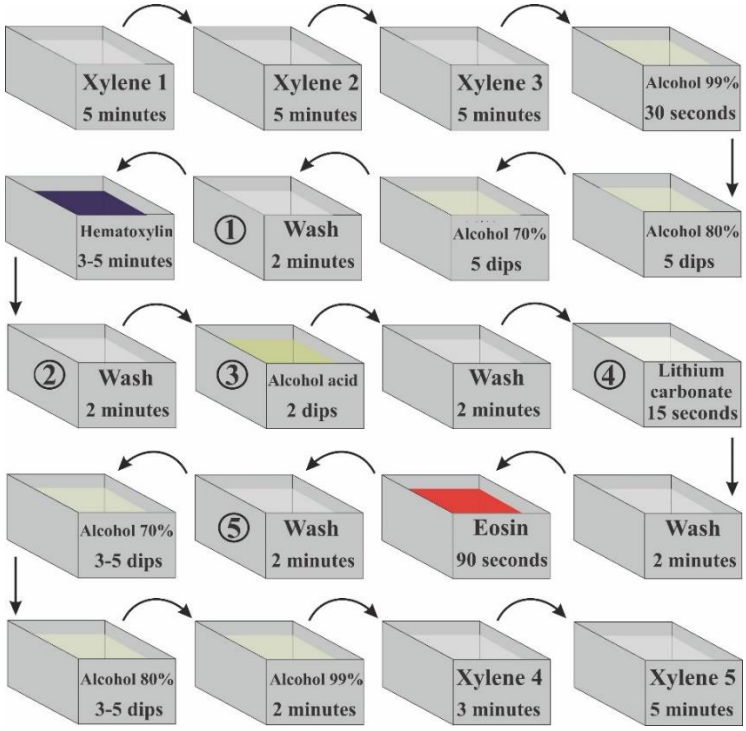

Figure 1. Modified method for declining H & E staining time

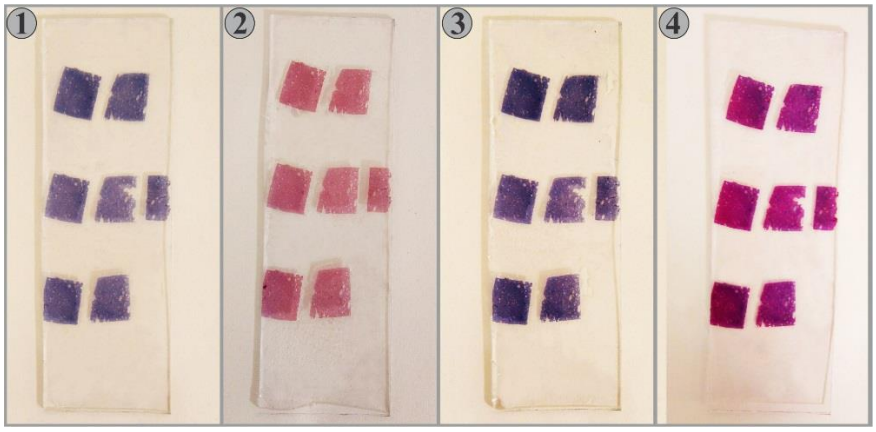

Figure 2. check points to ensure the accuracy of the staining method

1; The slides should have a blue to purple color spectrum after leaving the hematoxylin stain due to the volume of the nucleus in the tissue structure. At this stage, it is better to check the quality of the slide under a microscope; However, the duration of this check should be short to prevent the slide and tissue section from drying out. 2; The color of the slides should change from blue to brown to light red after being immersed in an alcoholic acid solution. 3; After placing the slides in a solution of saturated lithium carbonate for fixation, the color of the slides should change to a relatively dark blue again. 4; Finally, after removing the slides from the eosin stain and washing it, the color of the tissue sections should show the presence of eosin satin along with small hematoxylin spots (according to the tissue structure). At this stage, it is better to check the quality of the slide under a microscope; of course, the duration of this check should be short to prevent the slide and tissue section from drying out.

New methods to improve and increase the quality of the histochemical and immunohistochemical stainings

Certainly, the best method for histochemical and immunohistochemical staining is to immerse the slides in the stain solution. As a result, it requires a relatively high volume of stain. Since the commercial kits usually do not have a suitable volume and also the relatively high cost of the kits makes it difficult to make more than one kit. Of course, the drop method (putting a drop on the section) is routinely used, and this method has disadvantages. The disadvantages of this method are low accuracy, stain deposition and the inability to reuse the dyes. In addition, it is necessary to have a completely flat surface that does not have any slope and the drop of stain stays fix in its place. Also, due to the accuracy and time that must be spent when placing the drop on the tissue sample, the exact time indicated on the kits, which are sometimes effective in seconds, may not be complied. Some of the artifacts and disturbances created during the process of histochemical and immunohistochemical staining using the drop method are shown in Figure 3 (Figure 3). In this report, an attempt has been made to provide alternative methods of immersion. There are three methods for this.

First method: using special handmade puddles

In this method, a series of handmade puddles are used, whose height and diameter, and even the shape of the handmade puddles can be designed and built according to the needs. These puddles can be made of silicone or rubber that after being fixed, it is not possible for liquids and stain to pass under the puddles wall. Materials such as glycerin oil can be used to create a waterproof condition under the puddles well. These puddles can be fixed in its place using tape or paper clips. After placing the puddles on the slide, the desired stain can be transferred into it by a dropper. In this method, a very low volume of stain is used. There is also no possibility that a part of the tissue section is out of stain, or that a stain deposit is formed due to surface evaporation around the tissue sections. After the end of the staining, it is possible to restore the stain inside the puddles using pipettes or droppers (Figure 4).

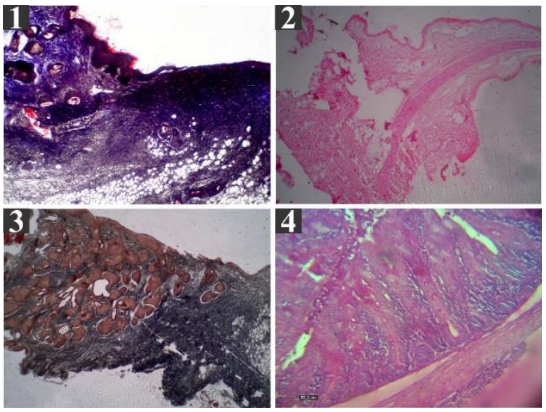

Figure 3. Some artifacts created on tissue sections in the drip staining method. 1; Severe satin sediment on the cross section of the skin stained by Masson's trichrome method. 2; Lack of proper staining in the third eyelid section of goats stained by PAS method. 3; Lack of staining and proper absorption of stain in skin tissue of the third eyelid of goats stained by Masson's trichrome method. 4; Inappropriate results in intestinal goblet cells stained by PAS method.

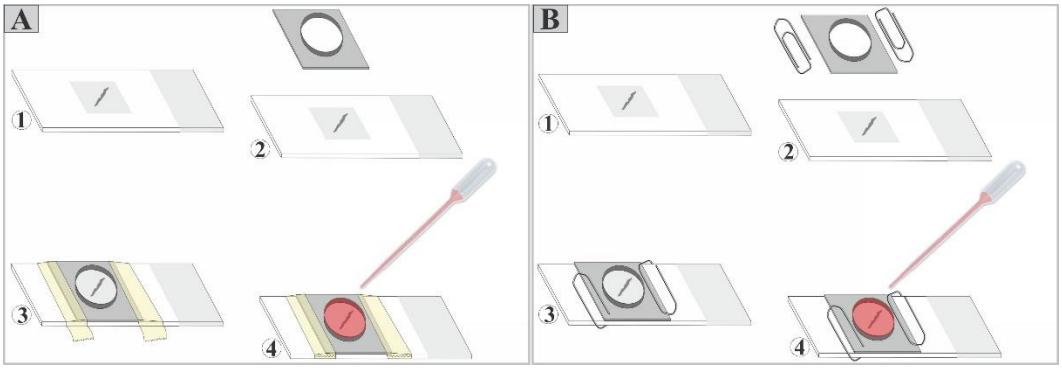

Figure 4. Schematic diagram of how to use special handmade puddles. A; use of Adhesive tape, B; use of paper clip. After preparing the tissue sections, the appropriate puddle with the tissue size is prepared. The puddles can be made of silicone or rubber that after fixing on the slide, it is not possible for liquids and stains to penetrate from inside the puddles to the outside. Oily substances such as glycerin can be used to create a waterproof state. After placing the puddles in place, the desired volume of stain is transferred into the puddles with the help of a pipette or plastic dropper. At the end, the used stain can be removed from the puddle with a pipette or dropper and utilized again. For washing the slides, the slide and the puddle connected to it can be immersed in water.

Second method: making a well around the tissue section using melted paraffin

This method is suitable for staining method that in next step, use xylene for deparaffinization. For this purpose, a paper or plastic tube whose tip is coated with glycerin is placed on the tissue section. Using a pipette, some melted paraffin is poured around the tube. This is done several times to create the desired height. After the paraffin hardens, the tube is removed and a paraffin well is created around the sample. The desired amount of color can be transferred to the well using a pipette. After finishing the work, you can carefully and slowly remove the excess paraffin from the slide using a scalpel so as not to damage the tissue section. If xylene is used in the next stages of staining, in this case xylene can eliminate the residual effects of paraffin (Figure 5).

Third method: using a container with a low height and placing the slide upside down

In this method, a container with wide surface and small volume is used (such as the lid of glass-colored jars). For this purpose, according to figure 6, stain is poured into a shallow container and the desired slides are placed upside down on that container. In this method, it should be ensured that the surface of the solution in the container is enough to be completely in contact with the tissue sections (Figure 6).

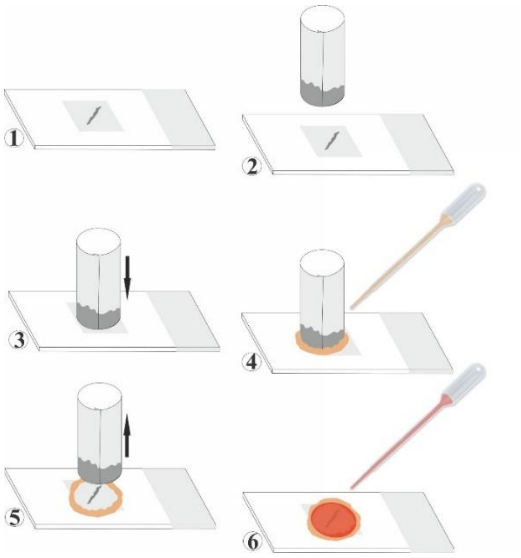

Figure 5. Schematic diagram of how to make a puddle around a tissue sample using molten paraffin. In this method, after preparing a tissue section, a paper or plastic tube whose tip is impregnated with glycerin is placed on the sample. Using a plastic pipette, pour the molten paraffin around the tube and continue until it reaches the desired height. After the paraffin hardens, the tube is removed from the sample. The desired satin is transferred into the puddle with the help of a pipette or dropper. At the end, the volume of satin inside the puddle can be recycled. For washing slides with paraffin puddles, it can be easily immersed in water.

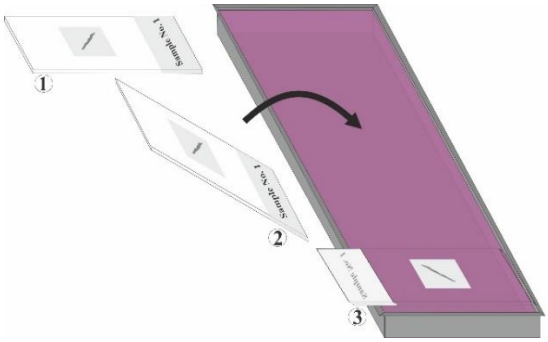

Figure 6. Schematic diagram of using a container with a low height and placing the slide upside down on it. In this method, a container with a low height is used. The volume of solution that is poured into the container should be large enough to create a convex state. In this case, by placing the slide containing the tissue section upside down on the container, the sample is in full contact with the color.

Results

Acceptable results were obtained in the hematoxylin-eosin staining method in shorter time, as shown in figure 3. So that different components and cell structure were well stained and distinguishable from each other. No color deposit was observed in this method. The structure and different components of the nucleus and nucleolus were well visible and distinguishable. This method was tested on various tissues, including tissue that have many nuclear, lymphoid tissues, and tissues with a high volume of cytoplasm, and on different animals, and results with optimal quality were obtained for all tissues (Figure 7). As a result, the suggested methods for histochemical staining had acceptable quality. No dye deposition was observed in the sections staining with these methods. Heterochromasia (pigmentation with a color other than the intended color) was not observed. The tracked components were well visible and recognizable. Also, different cellular components were well stained and distinguishable (Figure 8). Regarding the methods mentioned in immunohistochemical staining, the results were completely acceptable. The tissue sections stained with these methods did not have dye deposition, excessive stain density or lack of coloring. The tracked components were well distinguishable (Figure 9).

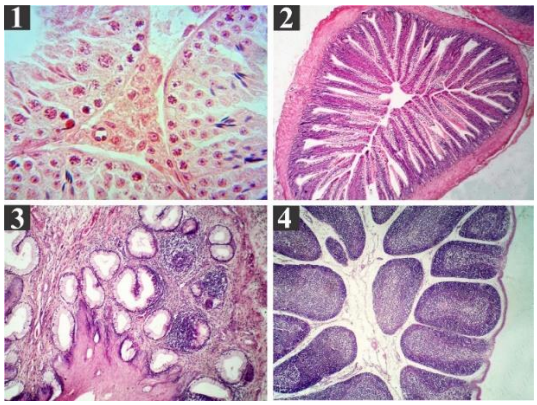

Figure 7. Photograph of sections stained by H & E staining method presented in this study. 1; Histological section of mice testis (Using the first method). 2; Cecal tonsil in turkey (Using the first method). 3; esophageal tonsil in chicken (Using the second method). 4; Bursa fabresius in Quail (Using the third method).

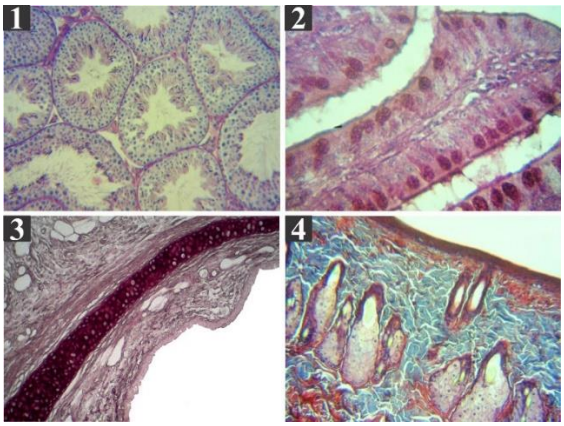

Figure 8. Tissue sections stained by histochemistry using hand-made puddle method and inversion method of slides on the container containing stain. 1; Staining of testicular tissue in mice by PAS method (Using the first method). 2; Staining of small intestine of Quail by PAS method (Using the second method). 3; Staining of third eyelid tissue of goat using Orcein method (Using the second method). 4; Staining of rat skin by Masson's trichrome method (Using the third method).

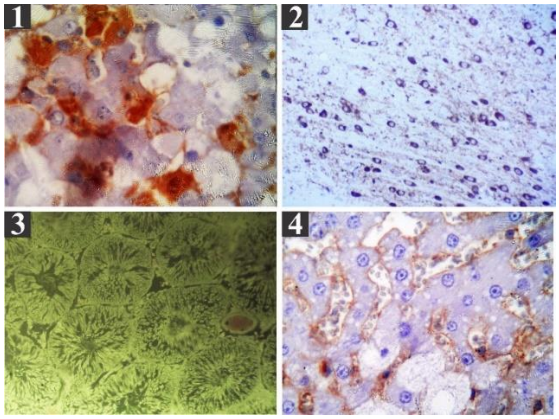

Figure 9. Tissue sections stained by immunohistochemistry using hand-made puddles and paraffin puddles. 1; Immunohistochemical staining of mice liver tissue by Ki67 antibody (Using the first method). 2; Immunohistochemical staining of neurons in rat brain nuclei (Using the second method). 3; Thioflavin S staining in rat testicular tissue (Using the third method). 4; Immunohistochemical staining of mice liver tissue by α sma antibody (Using the third method).

Discussion and conclusion

In light microscope studies, due to the colorless nature of most tissues and cells, it is necessary to stain the tissue slides for better differentiation. In the different staining methods, the hematoxylin-eosin is one of the most widely used and common staining methods in histological laboratories. This method has been used for several decades in the preparation of tissue slides and histology and histopathology studies. In recent years, the use of histochemical and immunohistochemical stainings is also due to their special application and more advantages is increasing. The advantages of these methods compared to other methods such as immunofluorescence, as follows: need less sample size and their easier shipping and handling, the possibility of better evaluation the tissue samples morphology, improve determination of the alternation sites and sediments in histopathological changes (Asgari et al., 2007).

But, one of the important factors that have always been considered in the staining of tissue sections, especially in relation to histochemistry and immunohistochemistry methods, is the high cost and sometimes the unavailability of the required satin and kits, which forces laboratory experts to consider the necessary for optimal use and saving in the consumption of these materials. Also, studies showed that different materials and compounds used during stabilization, tissue passage and staining can affect tissue structure, histochemical and histomorphometric properties (Kiernan, 1999; Slocombe et al., 1982). This issue is more prominent when using different staining methods for a one tissue. The result of an experiment in 1982 by Slocombe et al. showed that the thickness of the epithelium of the horse's esophagus was different from each other in two staining methods with hematoxylin-eosin and Masson's trichrome staining (Slocombe et al., 1982). This mentioned point shows the importance and effectiveness of the staining method in the preparation of tissue slides, and on the other hand, it is necessary to review and test the modified methods for use in these staining methods. Therefore, in the present study, which was carried out with the aim of introducing modified methods to facilitate tissue histochemistry, immunohistochemistry and hematoxylin-eosin staining, an attempt has been made to use the experiences gained by the authors in the histology laboratory, in order to reduce the cost and time of preparation of tissue sections, as well as improve the quality.

The results showed that by performing the modified method of hematoxylin-eosin staining presented in this research, the time of performing the staining steps in the introduced method is reduced to half compared to the conventional method, while the quality of the samples prepared in the new method better than usual method. This can be effective in reducing time and cost in laboratories that have a large number of samples (such as hospital and clinical pathology laboratories).

Also, in the proposed methods for performing histochemical and

immunohistochemical staining, efforts have been made to overcome the disadvantages of the usual direct drop staining method. In the common method of these stainings, in addition to the impossibility of reusing the stains, artifacts such as: intense stain deposition, lack of proper and desirable stain acceptance, inappropriate dye spreading in tissue sections and its inappropriate absorption are created. But, in the corrected methods that using stain puddles and placing the reverse of the tissue slide on the stain, despite the fact that a very low volume of stain is used and after the staining process is finished, it is possible to restore the dye inside the puddles using pipettes or drops. Also, there is no possibility that a part of the tissue sample will be out of stain, or that a stain deposit will form due to surface evaporation around the tissue. For this reason, the possibility of artifacts created in conventional methods is reduced in these methods. Therefore, the use of these corrective methods, which is the result of the experiences of the authors of this research, can be effective and useful in different histology and histopathology laboratories, in order to reduce the cost and time, as well as improve the quality of the prepared tissue slides, and it is suggested by these researchers.

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Conflict of interest

The authors declare that they have no conflict of interests.

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روش‌های اصلاح شده برای تسهیل رنگ‌آمیزی‌های هیستوشیمی، ایمونوهیستوشیمی و هماتوکسیلین- ائوزین بافتی

علی کلانتری‌حصاری[۱*]، حسن مروتی[۲]، محمد بابائی[۳]، علیرضا نوریان[۴]، کاوه اسفندیاری[۵]، طاهر علمی [۶] و سلمان سلطانی[۷]

[۱] استادیار، گروه پاتوبیولوژی، دانشکده پیرادامپزشکی، دانشگاه بوعلی سینا، همدان، ایران
[۲] استاد، گروه علوم پایه، دانشکده دامپزشکی، دانشگاه تهران، ایران
[۳] استادیار، گروه علوم درمانگاهی، دانشکده پیرادامپزشکی، دانشگاه بوعلی سینا، همدان، ایران
[۴] دانشیار، گروه پاتوبیولوژی، دانشکده پیرادامپزشکی، دانشگاه بوعلی سینا، همدان، ایران
[۵] استادیار، گروه علوم پایه، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران
[۶] استادیار، گروه علوم آزمایشگاهی، واحد بابل، دانشگاه آزاد اسلامی، بابل، ایران
[۷] دکتری تخصصی بافت شناسی مقایسه ای، آزمایشگاه مرکزی، دانشگاه ایلام، ایلام، ایران

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چکیده

اکثر روش‌های هیستوتکنیک و تهیه‌ی مقاطع بافتی در قرن‌های ۱۸ و ۱۹ میلادی ابداع و مورد استفاده قرار گرفتند. به دلیل ماهیت بی‌رنگی که اکثر سلول‌ها و بافت‌های بدن دارند جهت تفریق سلول‌ها و بافت‌ها از همان ابتدا روش‌های رنگ‌آمیزی بافتی به کار گرفته می‌شدند. با توجه به منابع و گزارشات موجود در این زمینه، مدت زمان لازم برای هر بار رنگ‌آمیزی هماتوکسیلین- ائوزین تقریباً ۸۰ دقیقه می‌باشد. در تحقیق حاضر روشی از رنگ‌آمیزی هماتوکسیلین- ائوزین گزارش می‌گردد که مدت زمان لازم برای رنگ‌آمیزی را با حفظ کیفیت مقاطع و رنگ‌پذیری از ۸۰ دقیقه به زیر ۴۰ دقیقه کاهش می‌دهد. همچنین با توجه به مشکلات موجود در مورد هزینه‌ی تهیه‌ی کیت‌های هیسوشیمی و ایمونوهیستوشیمی، حجم پایین کیت‌ها و مشکلاتی که در روش رنگ‌آمیزی قطره‌ای این نوع از رنگ‌آمیزی‌ها وجود دارد در گزارش حاضر سعی می‌شود سه روش خلاقانه در این ارتباط ارائه شود. این روش‌ها علاوه بر کاهش مواد مصرفی، کیفیت لام‌های تهیه شده را نیز افزایش می‌دهد. بهینه‌سازی مراحل رنگ‌آمیزی بافتی می‌تواند سبب کاهش هزینه‌ها و افزایش کیفیت مقاطع شود.

کلمات کلیدی: مقاطع بافتی، هیستوتکنیک، هیستوشیمی، ایمونوهیستوشمی

*نویسنده مسئول : علی کلانتری‌حصاری، استادیار، گروه پاتوبیولوژی، دانشکده پیرادامپزشکی، دانشگاه بوعلی سینا، همدان، ایران
E-mail: a.kalantarihesari@basu.ac.ir