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Original Article

Assessing the use of Lawsonia Inermis and Hibiscus Sabdariffa Aqueous Extracts as a Possible Substitute for Eosin Stain in Paraffin-embedded Tissues

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ABSTRACT

Eosin has been widely used as a counterstain to hematoxylin; however, it is a synthetic stain that can pose a possible threat to the ecosystem because it is toxic and non-biodegradable. Continuous use of this dye can cause various health hazards to the laboratory technicians, pathologists, and scientists working in the laboratory. The dye is also known to be costly. Since eosin is known to be a synthetic stain that can be harmful, costly, and not readily available, and development of natural histological stain is justified if the natural stain is harmless, cheaper, and readily available. Therefore, this research work may bring out natural dye products which may serve as a counterstain replacing eosin in the Hematoxylin-Eosin staining technique. With the rising attention given to the ecosystem, the need to replace synthetic dyes with naturally available dyes due to their eco-friendly nature is pertinent. Premised on the result of this study it was concluded that henna (Lawsonia inermis) and roselle (Hibiscus sabdariffa) can be used as substitutes of eosin stain. The naturally acidic nature of their aqueous solutions at the tested concentrations enabled their staining of cytoplasm. Further studies for suitable mordant and/or accentuators that would increase the staining intensity as well as reduce the staining time, thus making henna and roselle a potent stain are recommended. Aqueous extracts of henna and roselle should be considered for use as an alternative when there is a shortage or absence of eosin in histopathology laboratories because they stained tissue just like eosin does.

Keywords: Lawsonia Inermis, Hibiscus Sabdariffa, Stain, Tissue.

INTRODUCTION

The extensive evolution of histopathology can be mainly attributed to the availability of a wide array of stains. Staining has made possible the identification of various tissue structures under the microscope aiding in appropriate diagnosis. However, the present era of increasing importance to ecology has necessitated the requisite for natural dyes. Unlike synthetic dyes, natural dyes are less toxic, biodegradable, cheap and are eco-friendly (Raju et al., 2018).

The hematoxylin-eosin stain is the most important routine histological stain that has been used in most laboratories. It is a combination of the natural hematoxylin stain and the synthetic eosin stain. Hematoxylin is a basic dye that stains acidic components of the cell; eosin is an acidic dye that stains the basic cytoplamic components of the cells (Hashim, 2006). Since eosin is a synthetic dye, attempts have been made to replace it with a natural dye in the routinely used H&E stain (Raheem et al., 2015).

Lawsonia inermis Linn, commonly known as Henna, is a shrub belonging to the Lythraceae family. It is seen as a perennial shrub in sub-tropical and tropical areas of the Middle East, Africa, Southern Asia, Northern Australia, and other semi-arid areas. One of its large-scale uses includes those for cosmetic purposes as pigments to color hair and nails imparting a reddish yellow tint. Other uses are namely, in textile industries for dyeing wool and nylon, traditionally practiced medicinal plant for the treatment of various ailments (Alawa et al., 2015). Of late, attempts have been made to use henna as a biologic stain for plants and micro-organisms. The staining property of henna is mainly attributed to a naphthoquinone compound named as lawsone which is seen in abundance in the dried leaves. The

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compounds in these dried leaves impart a brown color as they have chemical properties that are analogous to tannic acid and thus its name hennotannic acid (Raju et al., 2018).

Hibiscus sabdariffa (HS) is a plant belonging to the vascular flowering plants, known as Roselle or Red Sorrel in English. The plant has several uses; the outer thick red and fleshy cup-shaped leaves, for example, are commonly used in the production of several food products and are consumed worldwide as a cold beverage and as a hot (sour tea) drink (Ibnouf et al., 2014).

Aqueous extract of Hibiscus sabdariffa is red in color and acidic in taste. Previous investigations used aqueous extract of Hibiscus sabdariffa in medical studies and food industry. Others used its aqueous extract to stain blood film, fungi and plants (Hashim, 2006).

Aqueous extract of Hibiscus sabdariffa has been recently used to stain lymph node and kidney biopsies. The trial was however without clearly defined treatment (Egbujo et al., 2008). Due to the acidic properties of henna and Roselle plants from existing literatures, this present study aims at exploring the possible use of henna and Roselle stain as a natural substitute to eosin and the objectives are to determine the potentials of aqueous leaf extract of Lawsonia inermis as a natural histological stain; to determine the potentials of aqueous flower extract of Hibiscus sabdariffa as a natural histological stain and to evaluate the staining efficacy of Lawsonia inermis and Hibiscus sabdariffa aqueous extracts in comparison with eosin stain.

METHODOLOGY

Study Area: The extraction and preparation of the staining solutions were done at the biochemistry laboratory of the Federal college of animal health and production technology Vom, while the staining investigations were carried out at the histopathology laboratory of the National veterinary research institute Vom, Plateau state.

Plants collection and identification: Fresh leaves of L. inermis (Henna) and H. sabdariffa (Roselle) flowers were collected from Just relax garden, Jos. The plants were taken to the College of Forestry Jos for identification and confirmation by a botanist.

Source of animal tissues: Albino rats were obtained from the experimental animals section of the National Veterinary Research Institute Vom, and parts such as intestine, skin, lung, and kidney were removed and used for tissue sections.

Preparation of plant materials: Per the procedure used by Hashim (2006), the plant materials were washed with clean sterile water and oven-dried at 40˚C ± 2˚C for 5 days. The plant materials were then powdered into coarse granules for extraction.

Extraction from henna powder: The extraction was done according to the method outlined by Raju et al., (2018); 100 g of the dried powdered henna leaves was weighed and soaked in 1000ml of distilled water and was allowed to stand for 24 hours, the solution was then filtered with a filter paper. The filtrate was dried in a hot air oven for three days. The dried residue was scraped and stored in a dried airtight container.

Extraction from roselle powder: The extraction was done according to the method outlined by Raju et al., (2018); 100 g of the dried powdered roselle was weighed and soaked in 1000ml of distilled water and was allowed to stand for 24 hours, the solution was then filtered with a filter paper. The filtrate was dried in a hot air oven for three days. The dried residue was scraped and stored in a dried airtight container.

Preparation of staining solution from a henna extract: The staining solutions were prepared per the technique used by Alawa et al., (2014) in which 2g and 5g of the aqueous extract was dissolved in 100ml of distilled water in four different bottles, each as follows: 2g of an extract with mordant (Aluminum sulfate), 2g of extract only, 5g of an extract with mordant (Aluminum sulfate), 5g of extract only. The pHs of the solutions were determined using a pH meter.

Preparation of staining solution from Roselle extract: The staining solutions were prepared following the technique used by Alawa et al., (2014) in which 5g and 10g of the aqueous extract was dissolved in 100ml of distilled water in four different bottles, each as follows: 5g of an extract with mordant (Aluminum sulfate), 5g of extract only, 10g of an extract with mordant (Aluminum sulfate), 10g of extract only.

Preparation of tissue sections: As carried out by Raheem et al., (2015), the specimens were fixed in 10% formalin for 24-48 hours, and then processed at the histopathology laboratory to make thinly sliced sections from paraffin-embedded tissue blocks of the skin, intestine, kidney, and lung.

Experimental trial of henna staining solutions: The routine procedure for staining with hematoxylin-eosin as described by Ochei and Kolhatkar (2007) was used but slightly modified by replacing the counterstain (eosin) with the henna extracts solutions to check its efficacy. The different solutions which were prepared at different percentages (2% and 5%) with and without mordant respectively were used to check which is more active. The counterstain was done for 30 minutes.

Experimental trial of roselle staining solutions: The routine procedure for staining with hematoxylin-eosin as described by Ochei and Kolhatkar (2007) was used but slightly modified by replacing the counterstain (eosin) with the Roselle extracts solutions to check its efficacy. The different solutions which were prepared at different percentages (5% and 10%) with and without mordant respectively were used to check which is more active. The counterstain was done for 30 minutes.

Staining of control slides: Some of the tissue sections were stained with the routine hematoxylin and eosin stain; to serve as a positive control for the work.

RESULT AND DISCUSSION

The hydrogen ion concentration of the different solutions of henna and roselle extracts is shown in Table 1. The result showed that the solutions of henna and roselle devoid of additives at normal room conditions are acidic whereas the addition of Aluminum sulfate reduces the pH.

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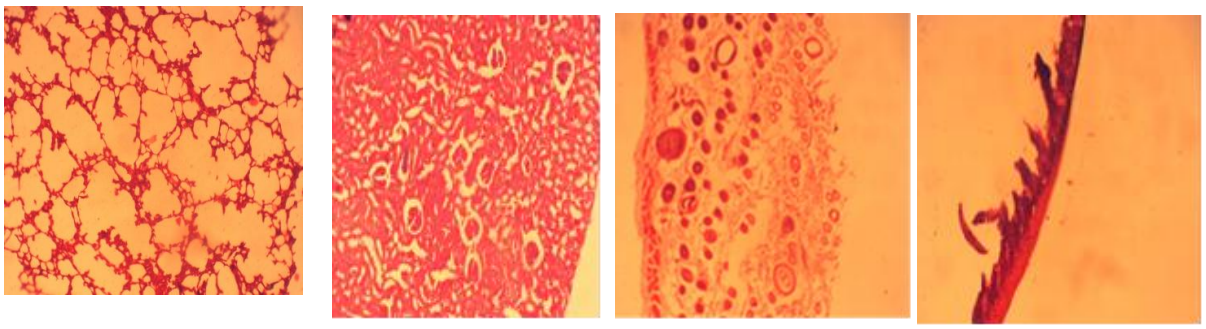
Table 1: Hydrogen ion concentration of various solutions of henna and roselle extracts.

Solution	pH value
2% henna solution	4.5
2% henna solution with aluminium sulfate	4.2
5% henna solution	4.0
5% henna solution with aluminium sulfate	3.9
5% roselle solution	3.6
5% roselle solution with aluminium sulfate	3.4
10% roselle solution	3.2
10% roselle solution with aluminium sulfate	2.8

The Photomicrographs in Figure 1, shows the appearance of the alveoli of the lung(A); the glomeruli as seen in the kidney(B), the layers of the skin(C), and the finger-like projections of the intestine(D). The control (H and E) demonstrate the cytoplasm as pink-red. Counterstaining with 2% henna solutions gave Photomicrographs in Figures 2 and 3. Figure 2 is a product of staining without mordant while figure 3 is got with mordant addition. Likewise, the counterstaining with 5% henna solutions gave the photomicrographs in Figures 4 and 5 respectively. Figure 4 are the outcome of staining without mordant while those in figure 5 are with mordant. The counterstaining with 5% roselle solutions on the other hand are shown in Photomicrographs of Figures 6 and 7 respectively. Figure 6 are products without mordant while those in figure 7 are with mordant. Again, photomicrographs in Figure 8 and 9 were counterstained with 10% roselle solutions, those in figure 8 are without mordant while those in figure 9 are with mordant. The Photomicrographs in Figure 2-9 shows that both the henna and roselle solutions give the cytoplasm a brownish color, unlike the control (eosin) in Figure 1 which gives the cytoplasm a pink-red color.



A: LUNG X200 **B: KIDNEY X200** **C: SKIN X200** **D: INTESTINE X200**

FIGURE 1: HEMATOXYLIN AND EOSIN STAINS

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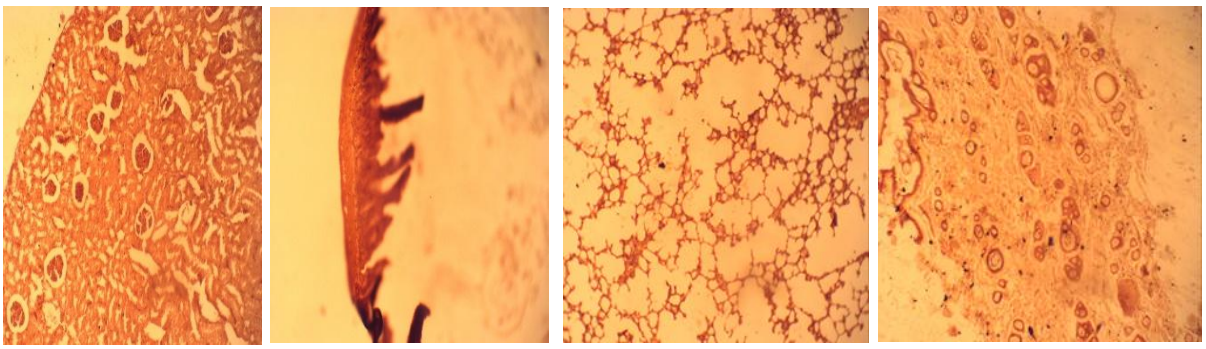
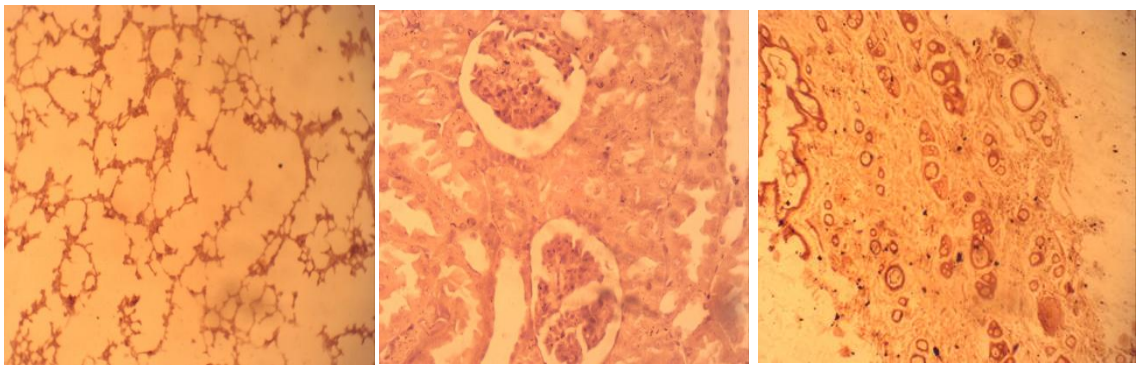


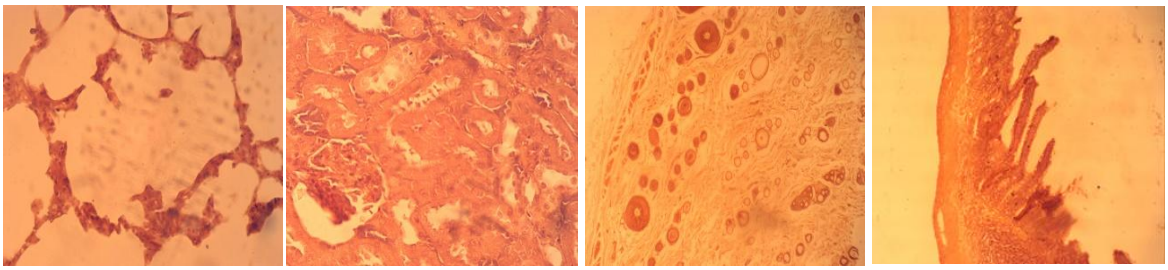
A: KIDNEY X200 B: INTESTINE X200 C: LUNG X200 D: SKIN X200

FIGURE 2: COUNTERSTAINING WITH 2% HENNA SOLUTION WITHOUT MORDANT.



A: LUNG X200 B: KIDNEY X400 C: SKIN X200

FIGURE 3: COUNTERSTAINING WITH 2% HENNA SOLUTION WITH MORDANT.



A: LUNG X400 B: KIDNEY X400 C: SKIN X200 D: INTESTINE X200

FIGURE 4: COUNTERSTAINING WITH 5% HENNA SOLUTION WITHOUT MORDANT.

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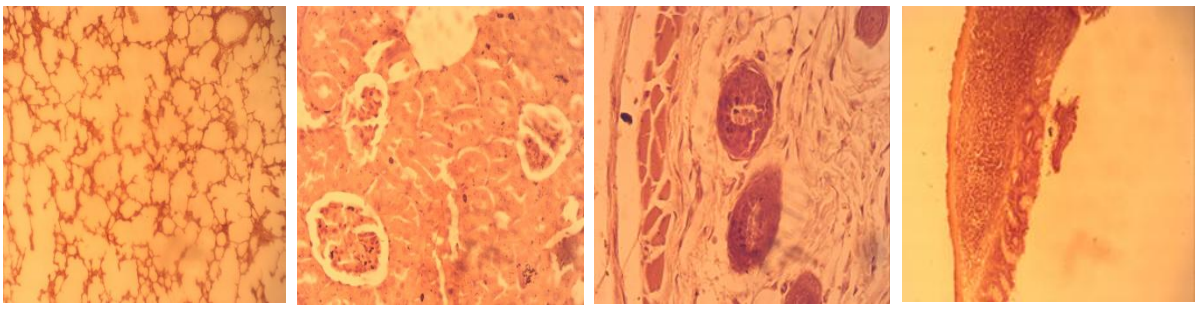
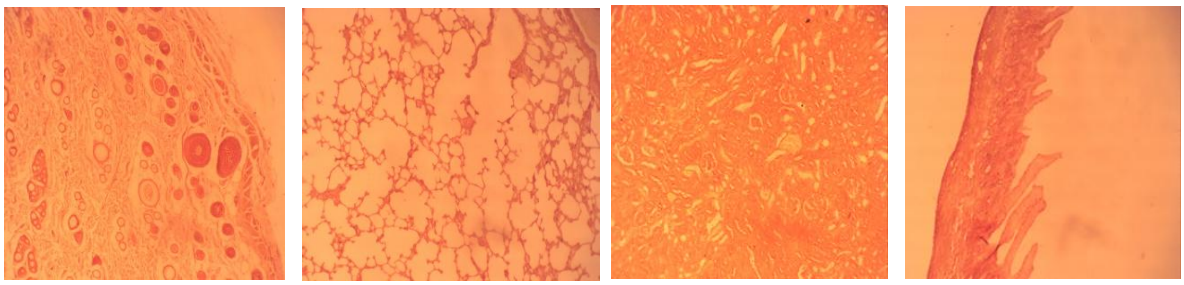


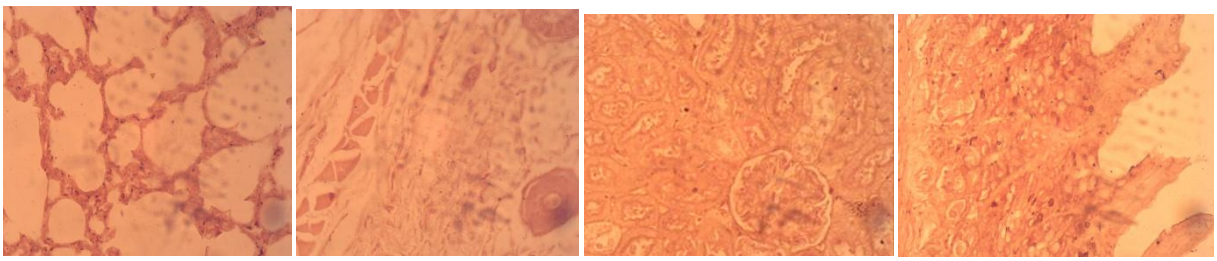
A: LUNG X200 B: KIDNEY X400 C: SKIN X400 D: INTESTINE X200

FIGURE 5: COUNTERSTAINING WITH 5% HENNA SOLUTION WITH MORDANT.



A: SKIN X200 B: LUNG X200 C: KIDNEY X200 D: INTESTINEX200

FIGURE 6: COUNTERSTAINING WITH 5% ROSELLE SOLUTION WITHOUT MORDANT.



A: LUNG X400 B: SKIN X400 C: KIDNEY(X400) D: INTESTINE(X400)

FIGURE 7: COUNTERSTAINING WITH 5% ROSELLE SOLUTION WITH MORDANT.

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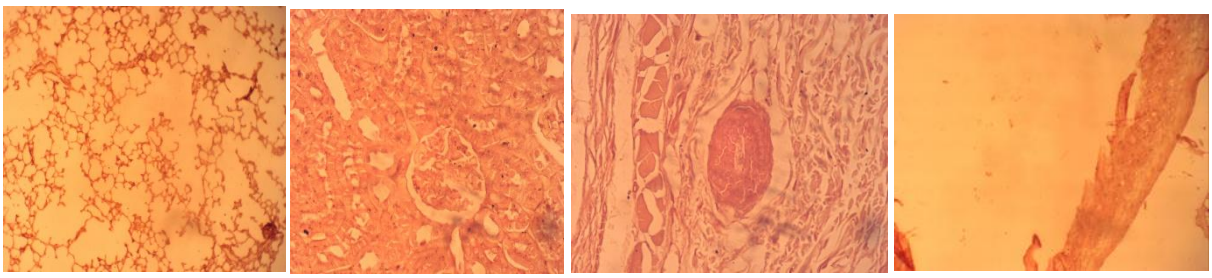
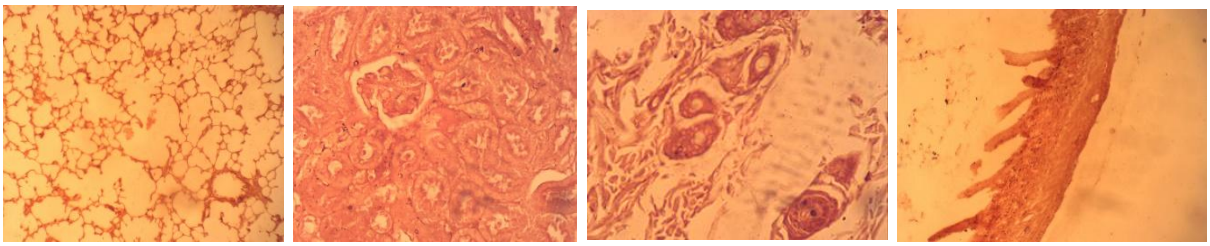


A: LUNG X200 **B: KIDNEY X400** **C: SKIN X400** **D: INTESTINE X200**

FIGURE 8: COUNTERSTAINING WITH 10% ROSELLE SOLUTION WITHOUT MORDANT.



A: LUNG X200 **B- KIDNEY X400** **C: SKIN X400** **D: INTESTINE X200**

FIGURE 9: COUNTERSTAINING WITH 10% ROSELLE SOLUTION WITH MORDANT.

DISCUSSION

Staining has become a vital part of histology. However, it involves the use of synthetic stains constituting serious threats to the ecosystem. Hence, the idea of exploring naturally found stain as substitutes of eosin was conceived. Henna is a widely used ornamental stain for hair, skin, and nails giving a reddish-orange to brown color (Hani et al., 2015; Raju et al., 2018; Kaniz, 2020). Ihuma et al., (2012) reported that methanol extracts from H. sabdariffa has been used as a staining agent for some fungi and thereby reduced the problems associated with over-dependence on toxic, expensive, and scarcely available exotic stains.

The results of this study show that 2% and 5% for henna, and also 5% and 10% roselle stain the cytoplasm brownish while in the control H and E, eosin-stained the cytoplasm pink-red. It was observed that the pH of the extract solution of roselle was more acidic than that of henna. Also, the solutions constituted with mordant (aluminum sulfate) were more acidic. Since the acidity of eosin is what makes it capable of staining the cytoplasm of cells, it was inferred that the acidic nature of the extracts made them capable of staining the cytoplasm of the various cells.

In spite of the fact that the mordant used made the solutions more acidic, no increase in clarity of the cells was observed in the photomicrographs obtained with them. Finally, the solutions took a long time (about 30 minutes) to stain the cells, unlike eosin which can counter stain in 1 minute.

CONCLUSION

With the rising attention given to the ecosystem, the need to replace synthetic dyes with naturally available dyes due to their eco-friendly nature is pertinent. Premised on the result of this study it was concluded that henna (Lawsonia inermis) and roselle (Hibiscus sabdariffa) can be used as substitutes of eosin stain. The naturally acidic nature of their aqueous solutions at the tested concentrations enabled their staining of cytoplasm.

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Recommendation

Further studies for suitable mordant and/or accentuators that would increase the staining intensity as well as reduce the staining time, thus making henna and roselle a potent stain are recommended. Aqueous extracts of henna and roselle should be considered for use as an alternative when there is a shortage or absence of eosin in histopathology laboratories.

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