THE SCIENCE WORLD
A Monthly e Magazine
ISSN:2583-2212
April 2024 Vol.4(4), 1309-1319
Popular Article

Histopathological Evaluation of Tissue Biopsy Specimens: An Overview

Neeteesh Kumar[1], Surya Kant*[1], Akshit Tyagi[2], K. D. Singh[3], A. K. Verma[3]
[1]PhD Scholar, Department of Veterinary Pathology
*[1]PG Scholar, Department of Livestock Production Management
[2]PG Scholar, Department of Veterinary Pathology
[3]Assistant Professor, Department of Livestock Farm Complex
College of Veterinary Science & Animal Husbandry, A.N.D.U.A.T., Kumarganj, Ayodhya, U.P. - 224229
https://doi.org/10.5281/zenodo.10933554

Abstract

In pathology, histopathology is a discipline of expertise that deals with recognizing minute structural alterations marked by ailments in tissue layers. Most of histopathological techniques simulating those of applied for study the normal histological structures. For the demonstration of minute histological changes, the tissue must be processed in such a manner that it will provide maximum information. Particularly in the veterinary sciences, histological diagnosis is a disregarded field. Many times, it has been observed that the procedures are not properly followed or the qualified person trained for histopathology is not available, which in turn affects the interpretation and /or diagnosis.

Keywords: Histopathology, Biopsy, Xylene, Ethanol, Eosin.

Introduction

The histopathological examination of testicular tissue offers suitable diagnostic method for investigating possible testicular causes of diseases. Pathology is the review and finding of sickness through the assessment of body tissue, which is commonly fixed on glass slides and saw under a magnifying instrument (Slaoui and Fiette, 2011; Johnson, 2022). A valuable diagnostic approach is the histopathological evaluation of tissue biopsies for recognizing evidence of infectious organisms. Conventional culture affirmation of tissue biopsies frequently neglects to recognize any disease-causing organism (Johnson, 2022)

Histopathological procedures

The microscopic examination of tissue or organs can be achieved by their smears or using

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
Published 05.04.2024

vitals staining or by sectioning; the latter method being more commonly used in histopathological laboratories. (Lillie, R.D, 1965)

Smears

The microscopic examination using smears of any organ/tissue /cells is very rapid method which gives the results within hrs. A drop of blood is placed on clean glass slide and with the help of another slide; the smear is prepared:" The tissue pieces from organs are cut using a sharp knife and the cut surface is mildly touched with clean glass. slides with some gentle pressure which gives an impression on the slide. This is also known as impression smear; generally, 2-5 smears are prepared on a slide. If the collected tissue material is too less than it is being pressed between two slides and the impression thus obtained on both the slides are used for study. The wet smears are fixed with methanol and can be stored or transported to laboratory for examination. The impression smears of hippocampus, cerebellum and cerebrum of brain are very useful for demonstration of Negri bodies in rabid animals for diagnosis of rabies. The impression smears are stained with Seller's stain for few seconds, washed, air dried and examined under oil immersion microscope for the presence of inclusion bodies also known as Negri bodies. These inclusions are characterized by intracytoplasmic, eosinophilic appearance with basophilic granules and round to oval in shape with a clear hallo (Windsor, 1994)

In case of pox infection in animals, the impression smears are prepared from scab or pustule for demonstration of intracytoplasmic inclusions Sometimes the viral inoculums is inoculated on chorioallantoic: membrane (CAM) of embryonated eggs; the impression smears of CAM may yield the viral inclusions. In certain bacterial diseases like hemorrhagic septicemia and enterotoxaemia, it becomes very difficult to demonstrate the organism in blood or in tissues for confirmatory diagnosis. The material is inoculated in laboratory animals like mice, Guinea pigs etc. The impression smears are then prepared from liver, spleen and other relevant organs of laboratory animals for demonstration of the organism. (Latendresse, 2002)

Vital staining

Vital staining procedures are not much in use directly in the diagnosis. However, for detection of phagocytic cells in body the vital stains are used. In the living animals when vital straining procedures are used for localization of phagocytic cells, these are known as intravital. In vitro use of vital stains is called as supravital staining which is being done for the live and dead lymphocyte count in leucocyte migration inhibition test (LMIT), lymphocyte stimulation test (LST), macrophage migration inhibition test (MMIT) and macrophage function tests (MET). (H.Fatakdawala, 2009).

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
Published 05.04.2024

Routine histopathological techniques of sectioning

The tissue pieces from morbid animals should be collected properly and fixed in a suitable fixative. Then these are processed and sections of 4-5 micron are cut and taken on slides (Paget and Thomson, 1979). These sections are stained and mounted to make the permanent preparations of slides. The different steps required for making the tissue slides are described briefly as follows:

1. Collection of tissue:

The collection of tissues is an important step which is many times not given proper attention. The whole diagnostic process depends upon the collection of tissue pieces. A representative tissue should have been collected carefully and should have the normal as well as abnormal (lesion) part. The tissues must be collected by qualified person after a thorough examination of each organ/system. Sometimes it has been observed that the collection of tissues is performed by attendants or rudely by the qualified persons and proper attention is not paid. It should be kept in mind that a representative tissue sample will only give the correct diagnosis which cannot be altered afterwards. At the time of tissue collection following points must be kept in mind which will be beneficial for making a correct diagnosis. (Kittel *et al.,* 2004; Morawietz *et al.,* 2004)

- The tissue, pieces from morbid animal should be collected as early as possible after the death of animal. Once the autolytic changes started in the dead body; it will not give true picture of microscopic lesions due to autolysis.
- At the time of tissue collection, it should be kept in mind that the representative tissue piece should include the part of lesion and a part of normal tissue, which facilitates the identification of organ/tissue at the time of microscopic examination.
- The tissue pieces should be cut with sharp knife and using only one stroke Blunt edge knife may require many attempts for cutting, which destroys the normal architecture of tissue.
- Tissue pieces for histopathological examination should be collected from all the organs. Sometimes it has been noticed that the tissue sample is taken from those part of body which shows gross lesions; merely absence of gross lesion does not mean that there will not, be microscopic alteration. In many disease conditions only microscopic changes occur which do not exhibited grossly. Such selective collection of tissues gives a biased interpretation, so it is better to have tissues from all the organs for proper interpretation and unbiased conclusions of histopathological studies.
- Tissues should be collected directly in the fixative and not in any other pot or water. Sometimes it has been observed that at the time of post mortem examination, the tissue samples are

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
Published 05.04.2024

collected in Petri dishes or in bottle and bring to the laboratory, then fixative is added. This seems to be a wrong practice. The tissue bottles filled with 2/3 fixatives must be available at the time of necropsy and tissue pieces should be collected directly in the fixative.

- The size of tissue piece should not be more than 5 to 10 mm as it facilitates the homogenous and smooth fixation. Large size tissues do not get fixed properly and in the middle, the tissue gets autolyzed.
- The tissue pieces from hollow organs like intestines, oviduct etc. should be cut transversely and placed on a hard paper, then it should be cut longitudinally in such a way that the serosal layer sticks to paper and mucosal layer gets free. Thereafter, it should be placed in fixative along with paper. This allows a good fixation and avoids the shrinkage and folding of tissue.

- At the time of postmortem examination, it has been noticed that the faecal matter is removed from the intestines by pressing/squeezing them or after opening the lumen by sharp objects like knife, slides etc.; which causes damage in the mucosal layer. The representative tissue should not be collected from such damaged portions.
- The tissues from encapsulated organs should be collected along with capsule or covering like brain should be collected along with meninges, kidneys and liver should be collected with their capsules. The coverings of such organs also yield useful information on histopathological examination.

2. Fixation:

The fixation of tissues is required for preventing the postmortem changes like autolysis and putrefaction by saprophytes, preservation of cellular constituents in life like manner and for hardening of tissues by way of conversion of semisolids to solid material for a proper histopathological preparation and their interpretation, the role of fixative is very crucial. Any faulty fixation cannot be remedied at any later stage an ideal fixative should be one that tissues quickly and should not interfere with the refractive index tissue components. (Va Dent J. 1972)

The choice of fixative depends on the type of investigation required. The formal saline (10% formaldehyde in 0.85% sodium chloride solution) is considered best fixative for routine histopathological studies (Lcong, 1994; Titford and Horenstein, 2005). The buffered formalin has certain advantages over formal saline and now-a-days it is recommended for routine use in histopathological laboratories. The buffered formalin can also be used for immunopathological studies. Buffered formalin is widely used and preferred because of its tolerance; tissues can be left for longer period without excessive hardening or damage and sectioned easily. Since it has neutral pH,

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
Published 05.04.2024

the formalin pigment is also not formed in the tissues. However, for immunopathological studies like immunoperoxidase staining techniques, the fixative for choice is formol sublimate. But in the absence of that buffered formalin may also be sued. The time required for proper fixation is 6-12 hrs for 5 mm thick block tissue (Am J Pathol. 2002).

3. Washing:

The tissue pieces after 6-12 hr fixation are taken out from fixative and cut into 2-3 small pieces of 2-3 mm size blocks. These blocks are, then kept in tissue capsules or in a gauge tide off with the help of thread. The identification marks written by copying pencil are also kept along with tissues. These capsules/gauge-containing tissues should be kept in running tape water overnight for at least 12 hrs. (Olcay Sertel,1007)

4. Dehydration:

In routine practice, the dehydration is done in ascending series of graded ethanol. The tissue blocks are kept in 50% ethanol of and then in 70%, 80%, 90%, 95%, absolute ethanol I and absolute ethanol II for one hour each. These ethanol-graded series should be kept in right glass stopper bottle or in screw cap jars to prevent the evaporation. In last bottle of ethanol II sometimes the copper sulfate is layered in the bottom, covered with filter paper, which increases the life of ethanol as it absorbs the water from alcohol. But the care should be taken, as soon as the copper sulfate begins blush due to absorption of water, the ethanol should be changed.

To increase the process of dehydration, the tissue blocks should be agitated either mechanically in an automatic tissue processor or by shaking the container periodically. The volume of alcohol should be at least 50 times more than the tissue placed for dehydration.

5. Clearing:

Usually, the clearing of tissue blocks is done in xylene. Like ethanol, xylene should also be kept in tightly stoppered bottle to prevent the evaporation. After dehydration the tissue blocks should be kept in ethanol and xylene (1:1) mixture for one hr, then the blocks are transferred to xylene I and xylene II for one hr each. If xylene is not available then benzene may be used for 3 hr as its action of clearing is slower than xylene. On complete clearing, the tissue becomes transparent, then they should be transferred in paraffin wax for impregnation.

6. Impregnation:

For the impregnation of tissue blocks, the paraffin wax in used either in paraffin embedding bath or in oven fixed at 60-62°C Temperatures. Both the oven and embedding bath are electrically operated with thermostat to adjust the desired temperature (Gormley and Fllis, 1994). At the time of

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
Published 05.04.2024

transfer of tissue blocks from xylene II, the paraffin wax must be kept at 60-62°C in liquid form for impregnation. Three changes are given in paraffin wax; each of one hr duration. The paraffin wax should be free from dust or other gross impurities; which can be removed by filtration through muslin cloth.

7. Casting of blocks:

After 3 hr impregnation of tissue blocks in paraffin wax, the blocks are formed in moulds using molten wax. The tissues are placed in moulds in such a way that desired surface should remain down ward, which should be on the base of mould. The sections are cut from this surface, so care must be taken to keep the tissue in a proper manner which should be cut into sections homogenously. The mould is then filled with molten paraffin wax and then the blocks are pled either at room temperature or in cold water. Various types of moulds like 'L' shaped or ring shaped can be used. If the moulds are not available, the blocks can be prepared in glass petridishes or in empty slide boxes. But care should be taken to lubricate the surface of such Petri dishes and other moulds with liquid paraffin or glycerin which facilitates the easy removal of blocks after cooling and hardening of paraffin wax (Rolls GO, Farmer NJ, Hall JB, 2012).

8. Trimming:

The blocks are removed from the moulds and are cut so as to give the one tissue per block and the wax is trimmed by knife or by rubbing on a hot plate in order to remove the extra wax on the either side of tissue. The tissue is exposed, which facilitate the side determination on which the section is to be cut. The identification of tissue should be fixed on one side of the block by touching the block with the small paper kept on it with hot forceps or knife, which bears the number. Then the blocks are fixed on block holder. Care should be taken that the number of markings of block should be kept on upper side at the time of trimming of the block on microtone to remove the extra wax and expose the whole surface of tissue. The trimming of blocks is done at 10-15 micron and a separate knife should be used for trimming and section cutting. Revised guides for organ sampling and trimming in rats and mice.

9. Section cutting:

Before the sectioning, the tissue blocks are cooled on ice or by keeping them in refrigerator. The tissue floatation bath should be cleaned and filled with water having a temperature of about 60-70°C. The blocks along with block holders are fixed in the microtome in such a way that the marking number will be on upper side giving the similar position to the blocks as it was during trimming. (J Oral Surg. 1978) Usually the sections are cut at 4–6-micron thickness on rotary microtome using a plain edge knife. The knife should be sharp enough that it should cut the desired thickness sections in

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
Published 05.04.2024

the form of a ribbon and will not cause damage to the tissue. By using a brush and forceps, ribbon of tissue sections is placed in tissue floatation bath. The tissue sections will spread here due to melting of paraffin wax and will take the shape similar to the tissue of that block. One can make out the selection here; the best-looking sections 1-5 can be lifted on a sticky glass slide, which should be kept in a tray at an angle so that the water is rem Michigan: Oxford University Press; 1967) oved. (The glass slides are made sticky by applying a sticky material on clean glass slides, which consists egg white and glycerine in 1:1 (V/V) ratio. The sticky material facilitates the sticking of sections on slides, which will not be damaged or removed during further processing of staining. Generally, 4-5 slides are made from each block and air dried in incubator or at room temperature. The following precautions should be taken at the time of section cutting, (Culing CF, 1985)

i. Adjust the microtone gauge at right place. Generally, it is adjusted at 4-6 micron for routine histopathological examinations.
ii. Knife should be properly fixed with the help of screws at an angle of about 45 degrees. Ensure that all the fittings are tightly fixed.
iii. The knife should be sharp enough to cut sections free from nicks. if the nicks are present on sections, the position Of knife should be changed or the knife should be properly stropped.
iv. The temperature of tissue floatation bath should neither be low nor it should be high than the prescribed. In low temperature, the tissue will not spread properly and its compression and crease will not be removed, while at high temperature the paraffin wax of tissue will melt quickly making the tissue fragments destroying the original shape of section.
v. Lift the tissue sections on slide at an angle (45^0) of slide so that the air bubbles should not appear in between the slide and section.
vi. Use little sticky material on slide: if it is more than drying process will take more time.
vii. If the ribbon of sections is large then it should be cut at the Junction of two sections with a sharp knife or blade and small pieces are made.
viii. During summer when temperature is above 40°C, the tissue sections should be cut either in a room or laboratory haying\ air conditioner or desert cooler. If such facilities are not available then make moisture in the environment by sprinkling of water on ground. It is necessary because at high environmental temperature the tissue sections stick to the knife and the ribbon is not properly formed.
ix. Drive the microtome smoothly in a regular speed, jerks should not be given.

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
Published 05.04.2024

x. For making the slides, use the diamond pencil and marking shoulder done at the time of section cutting itself.

10. Staining

After drying, the slides are kept in slide cabinets. One slide of each block is selected for staining using the following procedures: (Alturkistani, 2016)

a. **Removal of paraffin:** The slides are slightly warmed either in incubator or at the flame of a spirit lamp and placed in jar having xylene. Replace the xylene after 10-15 min with fresh xylene for another 10-15 min. This removes the paraffin from the tissue sections, (J Histochem Cytochem. 2005)

b. **Rehydration:** After removal of paraffin, the slides are kept in descending series of alcohol. For this, first they should be kept in absolute ethanol and xylene (1:1) mixture for 5 min; then in absolute ethanol, 95%, 90%, 80%. 70%, 50% ethanol for 5-6 min in each dilution. After that the slides are taken in water.

c. **Cleaning of slides:** With the help of muslin cloth, clean the slides from both the sides. Leave only 1 or 2 section on a slide and remove the extra sections and /or paraffin wax. Wash the slides in running tap water.(London, Boston: Butterworths; 1985)

d. **Staining in hematoxylin:** Place the slides in Harris hematoxylin or Meyer's hematoxylin for 10-15 min. Shake the slides'2-3 times for proper staining. Remove the hematoxylin solution and wash the slides in running tap water, then dip in acid alcohol for few seconds, which helps in differentiation. Wash in tap water and place the slides in ammonia water for few seconds for bluing and place in running tap water in order to remove the ammonia, H .J. Conn's Biological Stains (1969)

e. **Staining in eosin:** Place the slides in 2% aqueous eosin or alcoholic eosin for 2-5 min. After staining in eosin, quickly proceed for dehydration.

f. The slides are placed in 70%. 80%, 90%, 95% absolute ethanol for dehydration at least for 5 min in each solution, then place them in absolute ethanol: xylene mixture (1:1) for 5 min.

g. **Clearing:** Clear the sections in xylene and give 2 changes at least for 10-15 min each. The clearing in xyiene II can be extended for even up to one hour.

h. **Mounting:** Mount the slides with coverslip using Canada balsam or DPX mountant. For this the cover slips of desired size and shape are kept on filter paper and one or two drop of mountant is placed on coverslip. Takeout the slides from xylene and place on coverslip in such a way that the section is touched with mountant press gently and lift the slide. Remove air

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
Published 05.04.2024

bubble, if any, by pressing the coverslip with fine forceps and keep the slides in horizontal position in a tray for drying.

i. **Cleaning and labelling:** After drying, clean the slides with muslin cloth and xylene. Remove the extra mountant using a blade. Label the slide with a piece of paper and stick it on one corner of slide using gum or another adhesive. At the time of examination, the histopathologist should put the name of organ, main changes in sections/disease condition with other remarks on this label for future identification of the slide. (Am J Med Technol. 1976)

j. **Examination:** On hematoxylin and eosin staining, the nuclei of the cells take blue stain while the cytoplasm is pink or red. Examine the tissue section using 10X objective and if required then in high power or oil immersion. Precautions and important tips which should be considered at the time of staining are as under:

   i. Check the sections for staining after bluing in ammonia water for hematoxylin stain and after dehydration for eosin stain. If under stained then repeat the process or in case of overstrained, the sections can be differentiated for some more time in acid alcohol to remove the excess hematoxylin and in ethanol for removing the excess eosin.

   ii. Clean the slides thoroughly in water and remove all patches/spots of paraffin; which gives a good look to slides.

   iii. If on clearing in xylene, the cloudiness appears then repeat the dehydration process in absolute ethanol for 10-15 min. The cloudiness appears due to presence of water in the sections which reacts with xylene.

   iv. At the time of mounting, ensure that the tissue section is not-get dried. So to eliminate the chance of drying, proceed fast. Ensure the proper mounting of section on slides. Sometimes, the opposite side of the section is mounted and section becomes dry. To ensure the proper mounting, one should feel/touch the diamond pencil marking present on the same surface, then mount the sections. This can also be checked by touching the slide on reverse side for the presence/absence of tissue sections.

v. Labeling with paper should be done on same side, at which the section is present; which will be helpful at the time of examination.

Conclusion

In practically all areas of the biological sciences, histopathology is a potent diagnostic instrument with a broad range of purposes. It results from the amalgamation of pathology and

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
Published 05.04.2024

histology, the two fundamental branches of study. Histopathology makes it possible to identify alterations in the normal state of live tissues and maybe identify the causative agent, which may not be visible to the untrained eye. Pathologists demand various specific stains that are applied to the slides and feature just explicit microbes or lesions to assist with distinguishing reason for death or illness in an organism. Histopathology labs ordinarily use normalized fixation and staining conventions with cautious reagent to limit artifacts, high background, and false positives. Further, examination of the tissue by ensuring staining, immunoprobing, or molecular methods relies upon the initial preservation and treatment of the samples with protocol.

References

Abbey, L.M. and Sweeney, W.T.(1972). Fixation artifacts in oral biopsy specimens. *Va Dent J.* 49:31–4.

Akif Burak, T., Melih, K., Cenk, S. and Cigdem, G. (2009). "Object-oriented texture analysis for the unsupervised segmentation of biopsy images for cancer detection", Pattern Recognition, 42. 1104-1112,doi:10.1016/j.patcog.2008.07.007.

Alturkistani, H.A.; Tashkandi, F.M.; Mohammedsaleh, Z.M. Histological stains: a literature review and case study. Glob. J. Health Sci. **2016**, 8, 72.

Baochuan, P., Yi, Z., Qianqing, C., Zhifan, G., Qinmu, P. and Xinge, Y. (2010). "Cell Nucleus Segmentation in Color Histopathological Imagery Using Convolutional Networks", IEEE. 978-1-4244-7210-9/10.

Carleton, H.M., Drury, R.A. and Wallington, E.A.(1967). Carleton's Text Book of Histological Techniques. 5th ed. Vol. 41. The University of Michigan: Oxford University Press. 269.

Culing, C.F., Allison, R.T., Barr, W.T. and Culling, C.A. (1985). Cellular Pathology Techniques. 4th ed. United Kingdom: Oxford University Press;. In microtome and microtomy.

Culling, C.F., Allison, R.T. and Barr ,W.T.(1985). Cellular Pathology Technique. 4th ed. Vol. 78. London, Boston: Butterworths.(18). 78, 611.

Faoláin, E.O., Hunter, M.B., Byrne, J.M., Kelehan, P., Lambkin, H.A. and Byrne, H.J. (2005). Raman spectroscopic evaluation of efficacy of current paraffin wax section dewaxing agents. *J Histochem Cytochem.* 53: 121–9.

Fatakdawala, H., Xu, J., Basavanhally, A. and Madabhushi (2009). Expectation maximization driven geodesic active contour : application to lymphocyte segmentation on breast cancer histopathology,"Biomedical Engineering, IEEE Transactions on , In Press

Gormley, B., and Ellis, R.C. (1994) Resin embedding for light microscopy, in *Laboratory histopathology, a complete reference* (Woods, A.E., and Ellis, R.C., eds), Churchill Livingstone, New York, Vol. 1, pp 4.3-1/4.3-13.

Gurcan, M., Boucheron,L., Can, A., Madabhushi, A., Rajpoot, N. and Yener, B. (2009) ."Histopathological image analysis: A *review", IEEE Reviews in Biomedical Engineering*. 2.

Histomorphologic assessment of formalin substitute fixatives for diagnostic surgical pathology. *Arch. Pathol. Lab. Med.* **129**, (4), 502–506.

J Oral Maxillofac Pathol,v.22(2); May-Aug 2018.

Johnson, B. (2022). Histopathology as a diagnostic tool. *Journal of Interdisciplinary Histopathology*, 10 (1): 1

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
Published 05.04.2024

Kittel, B., Ruehl-Fehlert, C., Morawietz, G., Klapwijk, J., Elwell, M.R., Lenz, B., O'Sullivan, M.G., Roth, D.R., and Wadsworth, P.F. (2004) Revised guides for organ sampling and trimming in rats and mice – Part 2. *Exp. Toxic. Pathol.* **55**, 413–431.

Latendresse, J.R., Warbrittion, A.R., Jonassen,H., and Creasy, D.M. (2002) Fixation of testes and eyes using a modified Davidson's fluid:Comparison with Bouin's fluid and conventional Davidson's fluid. *Toxicol. Pathol.* **30**, 524–533.

Leong, A.S.-Y. (1994) Fixation and fixatives, in *Laboratory histopathology, a complete reference* (Woods, A.E., and Ellis, R.C., eds), Churchill Livingstone, New York, Vol. 1, pp 4.1-1/4.2-26.

Lille, R.D. and H.J. Conn's Biological Stains (1969). 8th edn, Baltimore;Williams and Wilkins.

Lillie, R.D. (1965). Histopathologic technique and practice histochemistry ed.3, New York, McGraw Hill Book co.

Loukas, C. G. and Linney, A.(2004). "A survey on histological image analysis-based assessment of three major biological factors influencing radiotherapy: proliferation, hypoxia and vasculature", Computer Methods and Programs in Biomedicine. 74(3), 183-199.

Morawietz, G., Ruehl-Fehlert, C., Kittel, B., Bube, A., Keane, K., Halm, S., Heuser, A., and Hellman, J. (2004) Revised guides for organ sampling and trimming in rats and mice – Part 3. *Exp. Toxic. Pathol.* **55**, 433–449.

Omar S. A. (2010). "Texture measures combination for improved meningioma classification of histopathological images", Pattern Recognition. 43. 2043-2053.

Paget, E.G., and Thompson, R. (1979). Standard operating procedures, in *Pathology*, MTP Press, Lancaster, pp 134–139.

Pizzolato, P.(1976). Formalin pigment (acid hematin) and related pigments. Am *J Med Technol.* 42:436–40.

Revised Guides for Organ Sampling andTrimming in Rats and Mice (2003).

Rohlander, M., Otzen, H., Rode, K., Jung, K., Schmicke, M., Harborth, T et al. (2020). Histological comparison of testicular needle biopsy and En Bloc samples in abattoir calves. Animals, 10: 1-12

Rolls, G.O., Farmer, N.J. and Hall, J.B.(2012). Artefacts in Histological and Cytological Preparations. 1st ed. Melbourne (Australia): Leica Biosystems Pty Ltd.106.

Sertel, O., Kong, J., Umit, V. and Catalyurek ( )Process Syst , "Histopathological Image Analysis Using Model-Based Intermediate Representations and Color Texture: Follicular Lymphom Grading",10.1007/s11265-008-0201

Slaoui, M and Fiette, L. (2011). Histopathology procedures: from tissues sampling to histopathological evaluation. *Methods in Molecular Biology*, 691: 69-82.Titford, M., and Horenstein, M. (2005).

Srinivasan, M., Sedmak, D. and Jewell, S. (2002). Effect of fixatives and tissue processing on the content and integrity of nucleic acids. *Am J Pathol*.; 161: 1961–71.

Tie Qi C. and Yi, L. (2002). "Color image segmentation-an innovative approach", Pattern Recognition. 35. 395-405.

Windsor, L. (1994) Tissue processing, in *Laboratory histopathology, a complete reference* (Woods, A.E., and Ellis, R.C., eds), Churchill Livingstone, New York, 1, pp 4.2-1/4.2-42.

Zegarelli, D.J.(1978). Common problems in biopsy procedure. J Oral Surg. 36:644–7.

AA3EADQ

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com

Published 05.04.2024