Advances in Bioresearch
Adv. Biores., Vol4 (3) September 2013: 109-114
©2013 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr/abr.htm
CODEN: ABRDC3

ORIGINAL ARTICLE

Evaluating Tissue Cross section Method by Microwave Energy, using different Tissue organs of the Broiler and Sheep as a Modal

Mohammah Raza Hafazi Ahmadi [1], Ali Mohammad Bahrami [2*], Ehsan Hosseini[3]
1-Ilam University of Midical Sciences, Department of Pathology, Ilam Iran
2- Faculty of Para-Veterinary Medicine , Ilam University , Ilam, Iran and Azad university ,Ilam Unit, Ilam ,Iran.
3- Faculty of Para-Veterinary Medicine , Ilam university , Ilam, Iran
Corresponding author: Bahrami AM, para-veterinary Medicine. Ilam University ,Ilam ,Iran
Email : am.bahrami@ilam.ac.ir

ABSTRACT
The aim of this research is to introduce a new technique, economic and time consumption for proceeding tissue cross section microscopy. The reviewer report of microwave showed a histomorphologic quality of (% 74.35) comparable to the conventional method (% 85.5) and the result was not statistically significant (P<0.11). The final time and the energy wave length (w) was obtained in this experiment for microwave proceeding tissue specimens in formalin was 30 w x 60 min in 58 °c, for absolute alcohol was 30w x 60min in 54°c and for paraffin (wax) 90w x 60min in 65°c. Turn-around times (TAT) in general, were improved and the maximum specimens proceeding for microwave in this study was 3 and half hr. The shrinkage of the tissue proceeded in microwave method was %62.83 in comparison to conventional method how was %77.8, respectively. The percentages of preservation of tissue integrity for specimens in microwave proceeding tissues was %74.35 and in conventional methods %84.61. Total diagnostic quality of tissues in microwave specimens was %85.5 in comparison to conventional method that was %94.1. No significant differences (p<1) were observed in the result of specimens in the cytoplasmic components of the tissue in both methods (%61.4).
Key words: *Histology, Tissue, Sheep, Conventional, Microwave, Methods.*

Received 01/06/2013; Accepted 30/06/2013

INTRODUCTION

Microwaves are an integral part of our daily lives, and applications in the pathology laboratory are an inevitable Outcome. This form of non ionizing radiation produces alternating electromagnetic fields that result in the rotation of dipolar molecules such as water and the polar side chains of proteins through 180° at the rate of 2.45 billion cycles per second. The molecular kinetics so induced result in the generation of instantaneous heat that is proportional to the energy flux and continues until radiation ceases. In addition to most biologic material, inorganic molecules with an asymmetric electrical charge also can be rotated by this electromagnetic flux [1].

Microwave-assisted tissue processing offers a solution to these problems. The use of microwave technology in histologic procedures was introduced by Mayers in 1970 [2], but the technique did not gain broad acceptance until the 1990s [3,4,1,5]. The use of microwaves leads to a significant reduction in processing time, thus eliminating the 1-day delay of diagnosis. While conventional processing results in batch production, the microwave- assisted method offers a possibility for continuous throughput processing. The method enables a continuous high flow of histologic specimens through the processor with a total processing time of as low as 1 hr. Furthermore, xylem are excluded from tissue processing, thereby reducing the potential health risk [6].

In this article, we describe our experiences with the microwave-assisted tissue processor in comparison with conventional routine tissue proceeding method, as well as its possible impact on turn-around time (TAT), The final time and the energy wave length (w) requirement for the specimen . The shrinkage of the tissue, the percentages of preservation of tissue integrity for specimens, percentages of total diagnostic quality of the tissues, the percentages of tissue color absorbance, nucleic resolution of specimens and the percentages of cytoplasmic components of the different tissues of sheep as a modal in microwave. In this

study, we present the histomorphologic quality and discuss the advantages and challenges of this new technique.

METHODS AND MATERIALS

In order to assess the histomorphologic quality of slides following tissue processing by the microwave-assisted method, a blind comparative study was made in Qaem laboratory private hospital Ilam southwest Iran, December 2012. In this study, the quality of microwave-processed and conventionally processed tissues from the same specimen was compared. One of the aims of the study was to examine whether all tissue types were suitable to undergo microwave-assisted tissue processing. Tissue samples from a variety of organs included lymphatic glands, liver, small and large intestine, eye, brain, heart, cartilage, gallbladder, muscle, salivary glands, breast, spleen etc… of the broiler and sheep were randomly collected from industrial slaughter houses of Ilam southwest of Iran selected for both methods. From each specimen, two adjacent slices of appropriate thickness were taken. One slice was processed routinely overnight conventional method (table 1), the other was processed by the microwave-assisted method (table 2). The paired tissue sections were mounted on the same glass slide in a random manner. The slides were H&E-stained and subsequently presented for evaluation by the 4 PhD pathologists. The pathologists were without knowledge of the type of processing used in either of the two tissue sections on the glass slide. In each case, the observer was asked to assess which of the two tissue sections on the glass slide was the best regarding the histomorphologic quality. The pathologist could also assess the histologic quality of the two tissue sections as equal.

Turn-around time

The TAT for histologic specimens is the time spent between receipt of the specimen in the pathology laboratory and signing out the completed histopathology report. In order to examine whether the introduction of the microwave assisted processor had an impact on TATs, are retrospective review of TATs for histologic specimens was made.

Table 1. Traditional Processing

Fixation	**Times (min)**
40°C	
Formalin %5	50 min
Specimens slice in 4-5mm and keep in tissue processor basket (tissue processor were compound of 12 basket and the cycle complete within 21-24 h)	
Formalin %5	100 min
Formalin %5	100 min
70% alcohol methanol	80 min
80% alcohol methanol	80 min
90% alcohol	80 min
95% alcohol	80 min
100% alcohol	80 min
100% alcohol	60 min
100% alcohol	60 min
In the next step to avoid tissue dryness, instate of xylene we had use chloroform	
Chloroform	50 min
Chloroform	50 min
Liquid Paraffin (50-60°c)	100 min
Liquid Paraffin (50-60°c)	100 min
After this step tissue samples were embedded in liquid paraffin, block prepared and transfer in freezer for 1 hr, microtome-sectioned (4µ), using BenMary bath with 45°c, mounted on glass slides, and stained with hematoxylin-eosin (H&E) procedure.	
Total time* 21- 24hr	

Table 2. End procedure timing obtain by Microwave proceeding for spacemen's

Fixation	**Times (min)**
40°C	
Formalin %5	30 min
Formalin %5	30 min
100% alcohol methanol	60 min
Izopropanol alcohol	60 min
Liquid Paraffin (50-55°c)	30min
After this step tissue samples were embedded in liquid paraffin, block prepared and transfer in freezer for 1 hr, microtome-sectioned (4µ), using BenMary bath with 45°c, mounted on glass slides, and stained with hematoxylin-eosin (H&E) procedure.	
Total time* 3 and half hr .	

RESULTS

The observer report of microwave-assisted processing method showed a histomorphologic quality of (%74.35) comparable to the conventional method (%85.5) and the result was not statistically significant (*P<0.11*). The final time and the energy wave lent (w) was obtained in this experiment for microwave proceeding tissue specimens in formalin 30 w x 60 min in 58 °c, for absolute alcohol was 30 w x 60min in 54°c and for paraffin (wax) 90 w x 60 min in 65°c. Turn-around times (TAT) in general, were improved and the maximum specimens proceeding for microwave in this study was 4 hr. The shrinkage of the tissue proceeds in microwave method was %62.83 in comparison to conventional method that was %77.8, respectively. The percentages of preservation of tissue integrity for specimens in microwave proceeding tissues was %74.35 and in conventional methods %84.61. Total diagnostic quality of tissues in microwave specimens was %85.5 in comparison to conventional method that was %94.1. The percentages color absorbance of tissue in the specimens proceeded by microwave was %75.64 and for sample proceed with conventional method was %78.5, respectively. No significant differences (*p<1*) were observed in the result of specimens in the cytoplasmic components of the tissue in both methods (%61.4). In case of pure fat tissues microwave did not respond good result.Results of preceding fatty tissues such as lipoma by microwave assisted method was poor but in %86 of the cases, the conventional processing method was rated best. Other tissue types, including specimens from the female genital tract and the gastrointestinal (GI) tract, were rated differently with equal quality in % 87 and % 82 of the cases, respectively. Specimens from the kidney, liver, pancreas, eye, brain, heart, cartilage, gallbladder, muscle, salivary glands, breast, and spleen were rated either best by microwave processing or equal in histologic quality in approximately most of the cases no significant differences were observed. As for tissue types such as the skin and the thyroid gland, the tissue section slides were rated as equal in histomorphologic quality in (%61) and %68 of the cases by using microwave and conventional method, respectively. Image 1 through image 5 shows the quality and comparison of the slide specimens prepared with microwave and conventional method.

We have since reduced the percentages of formalin for fixation of specimens to one half of what was required and was use in conventional method processing

DISCUSSION

Results of this experiment indicated that %86 the cases of specimens, the conventional processing method was rated best. Other tissue types, including specimens from the female genital tract and the gastrointestinal (GI) tract, were rated differently with equal quality in %87 and %82 of the cases, respectively. Specimens from the kidney, liver, pancreas, eye, brain, heart, cartilage, gallbladder, muscle, salivary glands, breast, and spleen were rated either okay by microwave processing or equal in histologic quality in approximately most of the cases no significant differences were observed. As for tissue types such as the skin and the thyroid gland, the tissue section slides were rated as good in histomorphologic quality in %61 and %68 of the cases by using microwave and conventional method, respectively. Microwave-assisted tissue processing has been studied for a variety of applications since 1970. It has achieved widespread acceptance as a processing technique for paraffin block of tissues placed on slides for immune histochemical staining. However, microwave technology was first used in the processing of tissue for routine histologic preparation. Despite this acceptance as the preferred method for antigen retrieval and slide preparation for immune histochemical staining, microwave processing has not become a widely accepted method for the routine processing of surgical pathology specimens. Since 1986, a number of articles have been published, describing the use of microwave ovens in various areas of tissue processing [4,7,8].

Because of the 21 hours or longer presently required to prepare tissues for histology, diagnosis of biopsy specimens on the day after receipt of the specimen is customary. The diagnosis of complex specimens may take even longer. Table 1 and 2 schematizes the various steps, relevant methods, and their approximate time requirements from receipt of tissues in the laboratory to availability of microscopic sections for diagnosis reported with microwave and conventional methods. The various steps required by traditional methods responsible for this long process, that is, incubation in separate solutions of formalin for fixation, a series of increasing concentrations of alcohol for dehydration, and xylene for clearing tissue of alcohol before impregnation. This 21-hour or longer process is usually performed overnight in automated instruments. The pathologist, therefore, is unable to render a diagnosis based on microscopic examination of tissue sections until the next day at the earliest, almost 24 hours after the specimen arrives in the laboratory [9]. In this study processing time of specimens for microwave method was 4 hour and for traditional methods were 21 hr. Attempts are being made to reduce the time for tissue processing, either by shortening the cycle of automated traditional processing method processors or by using microwave-assisted methods. They reported that it was possible to produce significant acceleration

of tissue processing by introducing microwaves to the procedure [10]. In 1998, Visinoni [11] and his coworker followed up with a description of the first microwave tissue processor that completed processing in between 30 and 120 minutes, depending on the thickness of the specimen block. The latest report by microwave assist was obtained by moghadam and his coworker for tissue of sheep was 4 hours, in comparison with present study that the TAT were 3 and half hour which shows the evoulotion of the method for half and hour [12].

It is evident from the foregoing review that microwaves have made a major impact in the pathology laboratory. This form of non ionizing radiation has been employed to accelerate numerous procedures embracing all aspects of the preparation of tissues for optical microscopy and ultrastructural examination. Furthermore, the application of microwaves has made significant contributions in the turn-around time of tissue for histologic specimens, economical point of view and faster analyses of the cross section report. Despite these extensive applications, several areas of controversy remain unresolved. These pertain to the mechanisms of action of MWs especially as a primary fixative, and its actions in antigen retrieval[13]. It could be concluded that we found positive impact after introducing microwave assisted methods for veterinary specimens.

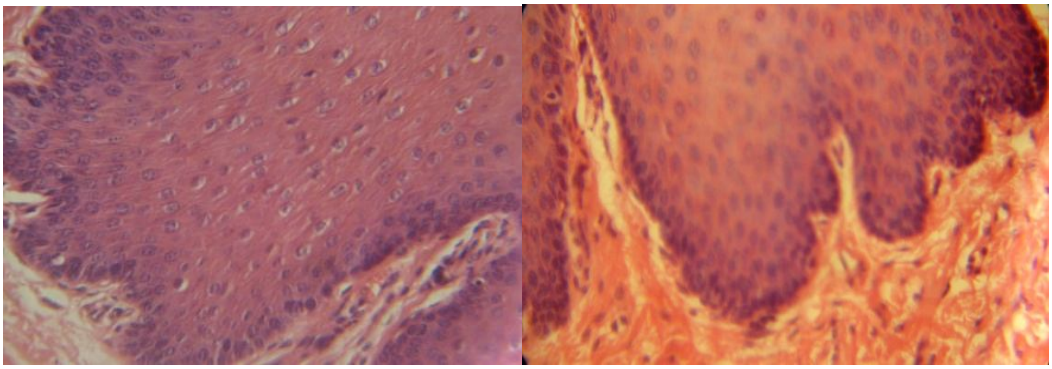

Image1. Squamous specimens of sheep proceed by microwave. A, Squamous specimens of sheep proceed by conventional method .B, (H&E, original magnification x400)

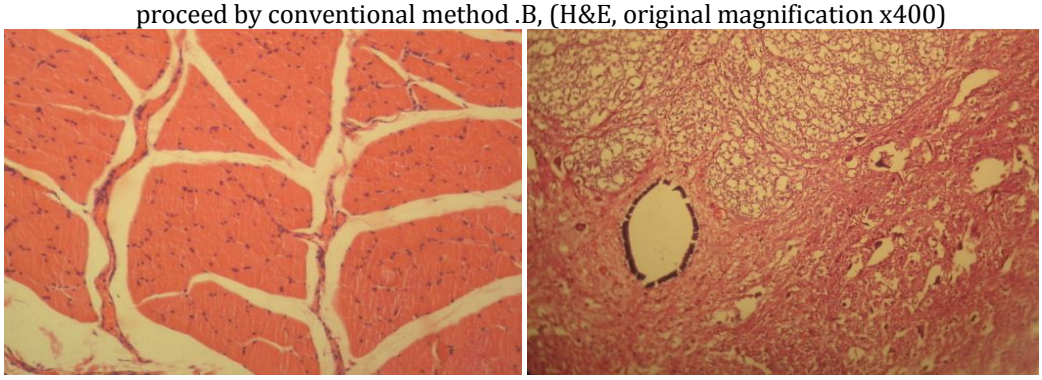

Image 2.Skeletal muscle specimens of sheep proceed by microwave. A, skeletal muscle specimens of sheep precede by conventional method. B, (H&E, original magnification x400)

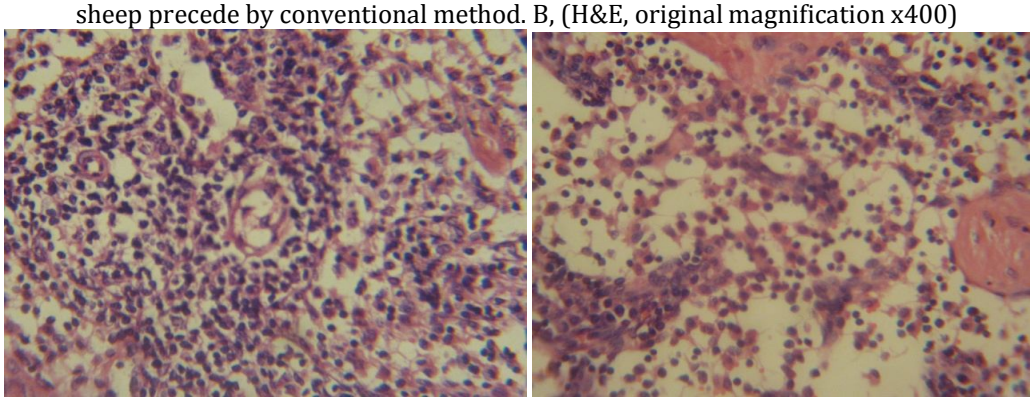

Image3. Lenphnods specimens of sheep proceed by microwave. A, Lenphnods specimens of sheep precede by conventional method. B, (H&E, original magnification x400)

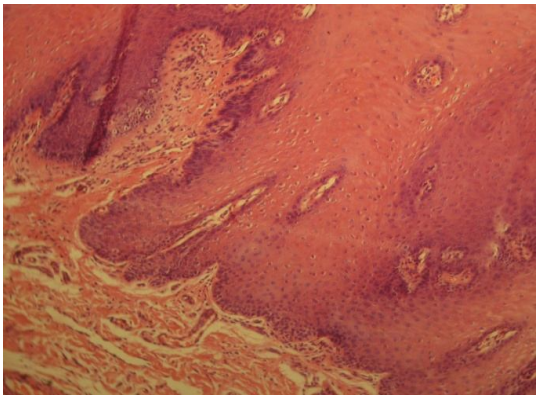

Image4.Heart specimens of sheep proceed by microwave. A, Heart specimens of sheep precede by conventional methods. B, (H&E, original magnification x400)

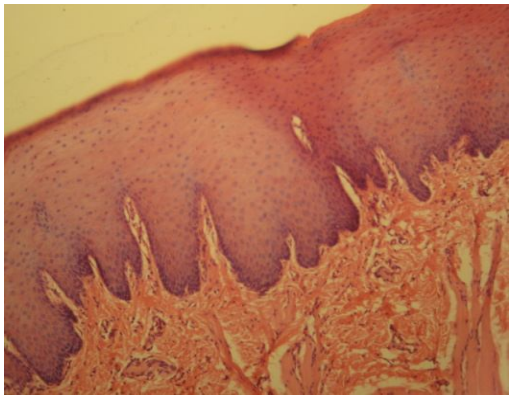

Image5.Esophagus specimens of sheep proceed by microwave. A, Esophagus specimens of sheep precede by conventional methods. B, (H&E, original magnification x400)

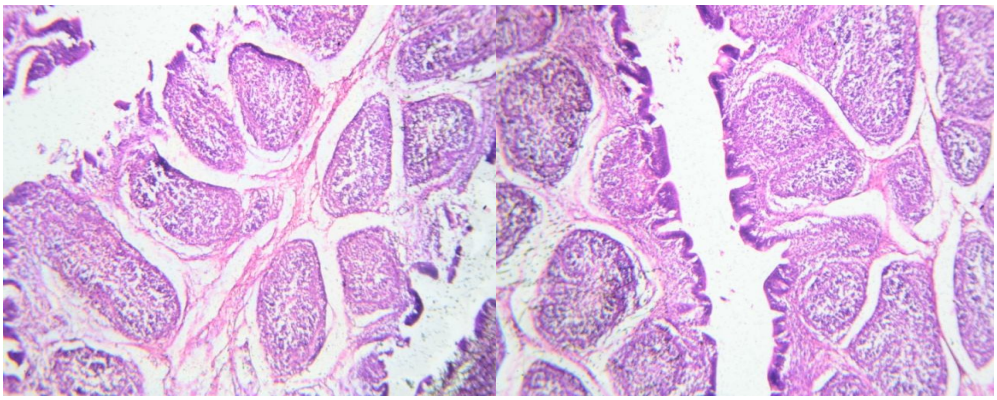

Image6.Iliume specimens of broiler proceed by microwave. A, Iliume specimens of broiler precede by conventional methods. B, (H&E, original magnification x400)

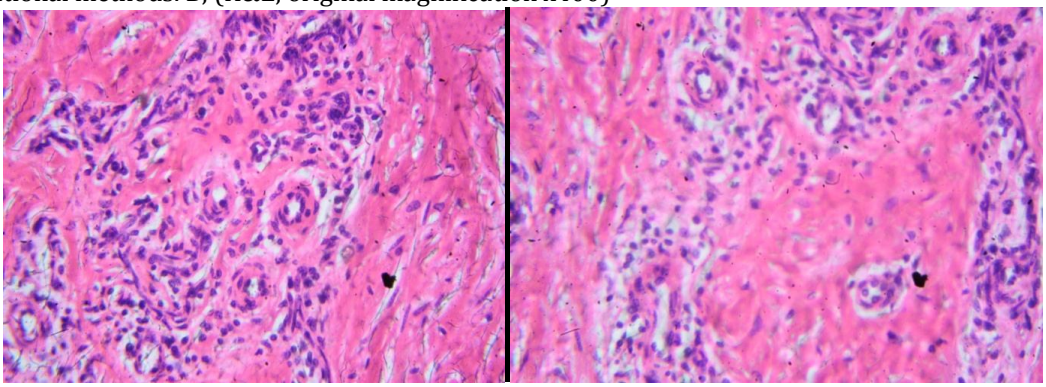

Image7.Lamina properia specimens of broiler proceed by microwave. A, Lamina properia specimens of broiler precede by conventional methods. B, (H&E, original magnification x400)

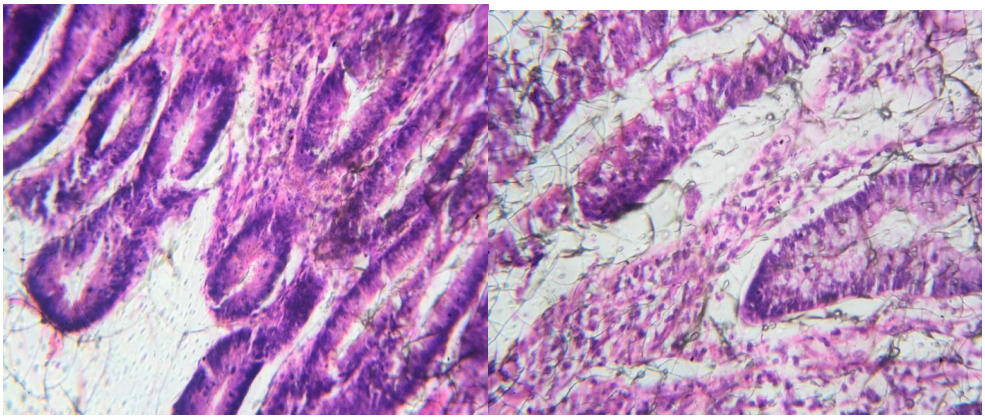

Image7.Intestinal vili specimens of broiler proceed by microwave. A, Intestinal vili specimens of broiler precede by conventional methods. B, (H&E, original magnification x400)

REFERENCES

1. Anthony S Y Leong and Raija T Sormunen . (1998). Microwave Procedures for Electron Microscopy and Resin-Embedded Sections. *Micron* ; Vol. 29, No. 5, pp. 397–409.
2. Mayers CP. (1970). Histological fixation by microwave heating. *Clin.Pathol*. 23: 273–275.
3. Emerson E, Tripp SR, Baird BC, Layfield LJ, Rohr LR. (2006). A comparison of immune histochemical stain quality in conventional and rapid microwave processed tissues. *Am.J.Clin.Pathol*. 125: 176–183.
4. Leong ASY. (1991). Microwave fixation and rapid processing in alargethrough put histo pathology laboratory. *Pathology*. 23:271–273.
5. Leong ASY, Sormunen RT. (1998). Microwave produces for electron microscopy and resist-embedded sections, *Micron*. 29:397–409.
6. Julie Munkholm, Maj-LisTalman, Thomas Hasselager . (2008). Implementation of a new rapid tissue processing method. *Advantages and challenges Julie Pathology Research and Practice* 204:899–904.
7. Ralph Rohr L, Lester J, Layfield , Deborah Wallin HT and Denise Hardy HT. (2001). A Comparison of Routine and Rapid Microwave Tissue Processing in a Surgical Pathology Laboratory Quality of Histologic Sections and Advantages of Microwave Processing .*Am. J. Clin. Pathol.* 115:703-708.
8. Leong ASY, Milios J. (1993). An assessment of the efficacy of the microwave antigen-retrieval procedure on a range of tissue antigens. *Appl Immunohistochem.* 1:267-274.
9. Morales Azorides R, Harold Essenfeld, Ervin Essenfeld, Maria Carmen Duboue, Vladimir Vincek, and Mehrdad Nadji. (2002). New Technology in Laboratory Medicine, Continuous-Specimen-Flow, High-Throughput, 1-Hour Tissue Processing. *Archives of Pathology & Laboratory Medicine*: Vol. 126, No. 5, pp. 583-590.
10. Boon ME, Wals-Paap C, Visinoni F . (1995). The two-step vacuum-microwave method of histoprocessing. *Eur. J. Morphol.* 1995; 33: 349 -358.
11. Visinoni F , Milios J, Leong ASY et al. (1998). Ultra-rapid microwave/variable pressure induced histoprocessing: description of a new tissue processor. *J. Histotechnol* ; 21: 219 - 224.
12. Moghadam M.N,Delpishe A,Hafezi Ahmadi M.R,. Comparative study of conventional and microwave energy method for tissue processing.MD Thesis .Medical University.Ilam.Iran.
13. Leong Anthony SY. (2004). Microwaves and Turnaround Times in Histo processing Is This a New Era in Histotechnology? . *Am. J. Clin. Pathol.* 121: 460- 462.

Citation of This Article

Mohammah Raza Hafazi Ahmadi, Ali Mohammad Bahrami , Ehsan Hosseini . Evaluating Tissue Cross section Method by Microwave Energy, using different Tissue organs of the Broiler and Sheep as a Modal. Adv. Biores., Vol 4 (3) September 2013: 109-114.