The Pharma Innovation Journal 2023; 12(3): 2075-2078

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(3): 2075-2078

www.thepharmajournal.com
Received: 18-01-2023
Accepted: 20-02-2023

Ramya MP
Department of Veterinary Anatomy and Histology. Veterinary College, Bangalore, Karnataka, India

Jamuna KV
Department of Veterinary Anatomy and Histology. Veterinary College, Bangalore, Karnataka, India

Ramkrishna V
Department of Veterinary Anatomy and Histology. Veterinary College, Bangalore, Karnataka, India

Corresponding Author:
Ramya MP
Department of Veterinary Anatomy and Histology. Veterinary College, Bangalore, Karnataka, India

Innovative method of double infiltration in comparison with other infiltrating media for histological study of tissues

Ramya MP, Jamuna KV and Ramkrishna V

Abstract
Double infiltration is also one of the technique along with paraffin, paraffin with ceresin and celloidin method. Which has been described in many staining technique text books. The concept of double infiltration by different authors have given a positive method for staining different tissue components as well as better histological sections, however the use of jelly wax along with paraffin as a double infiltration, has not been described in the literature reviewed. In the present study jelly wax was used as a first infiltrating media and paraffin as a second infiltrating media.

Jelly wax is also a type of paraffin with low viscosity. Being lower viscosity it has got a better infiltration property. There by an attempt has been made to study the effect of double infiltration in different organs. Landes *et al*. (2005) has used double infiltration in plastinated specimens with celloidin infiltrating media and embed in cedax.

Keywords: Tissue, infiltrating, jelly wax and resin

Introduction

Material and Methods

Tissues such as spinal cord, cardiac muscle and lymph node were collected directly from the slaughter house and preserved in 10% formal saline for 24 hours. Tissues were processed in a routine steps. For infiltration 15% jelly wax was taken and three changes with one hour interval at room temperature was done. 2nd infiltration was done using paraffin under 58 ºC in oven with 3 changes at the interval of one hour. Blocks were prepared using pure paraffin wax. Sections cut at 6 µ were stained using Haematoxylin and Eosin Phloxine method and also by special stains such as Weigert's Resorcin Fuschin, Masson's trichrome and gomori's reticulum stain.

The criteria to evaluate the slides were calculated as per the procedure described by Judy *et al* (2002), who have mentioned the scale of slide reading from one to five 1= worst, 5= best depending upon the staining reaction. Under the light microscopic assessment, staining of the cell component, affinity for stain, affinity for collagen, reticular, striated muscle fibre and neuronal elements have been taken as described by Keklikoglu and Akinci (2013) and the rating was given from (-) to (++++).

Results and Discussion

Comparative study using infiltrating media as paraffin, paraffin with ceresin and double infiltration using jelly wax as first infiltrator method has been made. The detailed staining affinity, stain compatibility and distinct characteristic of connective tissue fibre, muscular tissue and nervous tissue were identified in all the three methods of infiltration. The introduction of jelly wax in the double infiltration technique as first infiltrator was a new method of double infiltration, which has not been identified in the literature reviewed.

The average slide reading of different tissues such as spinal cord, cardiac muscle and lymph node using all the three infiltration method is shown in table No. 1, as described by Judy *et al.* (2002).

Table 1: Showing average slide reading of different tissues under different infiltrating media as per Judy *et al.* (2002)

Set 1-Paraffin infiltration	Average slide reading
Spinal cord	3
Cardiac muscle	3
Lymph node	4
Set 2-Paraffin with ceresin infiltration	**Average slide reading**
Spinal cord	2.5
Cardiac muscle	3.5
Lymph node	3.5
Set 3-Double infiltration	**Average slide reading**
Spinal cord	4
Cardiac muscle	4
Lymph node	4.5

Table 2: Showing results of general staining, brightness, density, and density of the base stain under 100X magnification

Sl. No.	Routine stains			
	Spinal Cord	**P**	**P with C**	**D I**
1.	H & E phloxine	++Fig. 1	+++Fig. 2	++Fig. 3
2.	Thionin stain	++Fig. 4	++Fig. 5	++Fig. 6
	Cardiac Muscle			
1.	H & E phloxine	++Fig. 7	++++Fig. 8	++++ Fig. 9
2.	Gomori's reticulum	++Fig. 10	+Fig. 11	++++Fig. 12
	Lymph Node			
1.	H & E phloxine	+++Fig. 13	++++Fig. 14	++++Fig. 15
2.	Gomori's reticulum	++++Fig. 16	++Fig. 17	++++Fig. 18

Spinal Cord-H & E phloxine

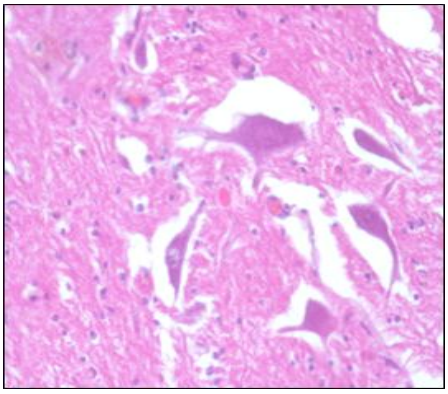

Fig 1: Paraffin infiltration

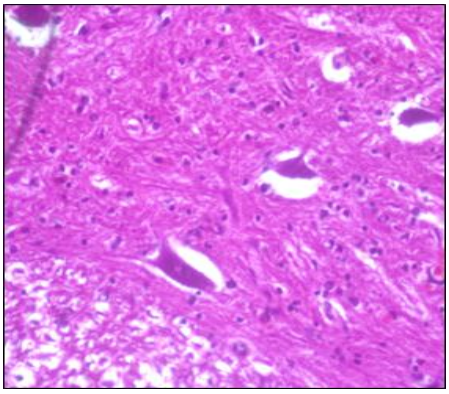

Fig 2: Paraffin with ceresin infiltration

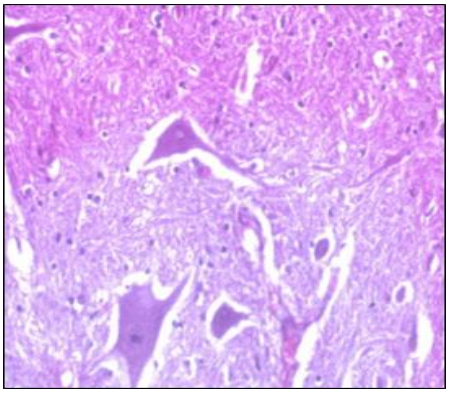

Fig 3: Double infiltration

Spinal Cord-Thionin stain (X100)

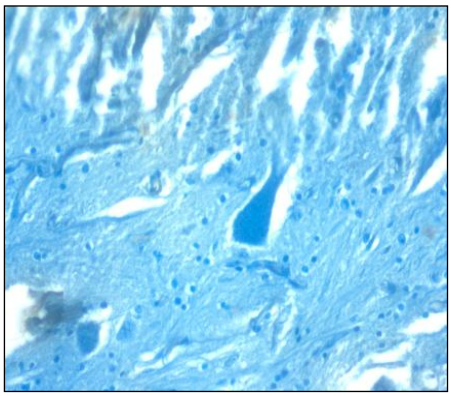

Fig 4: Paraffin infiltration

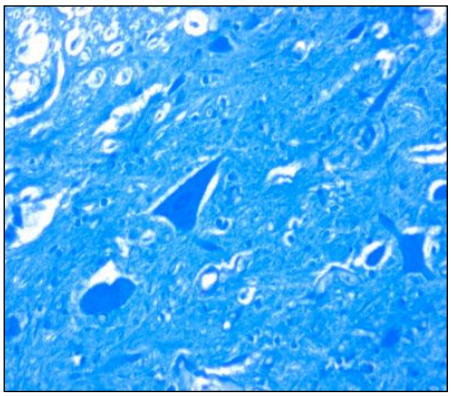

Fig 5: Paraffin with ceresin infiltration

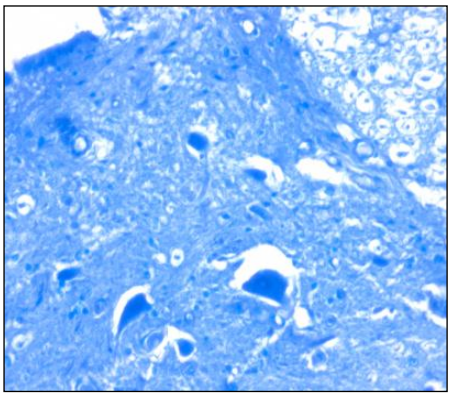

Fig 6: Double infiltration

Cardiac Muscle-H & E phloxine

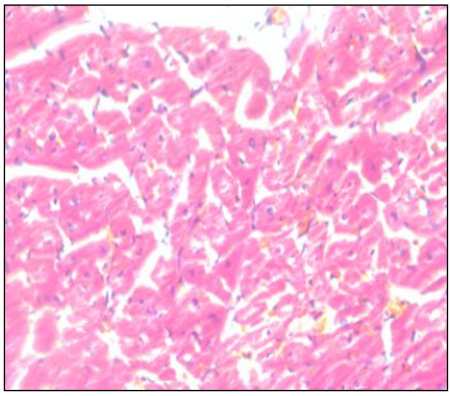

Fig 7: Paraffin infiltration

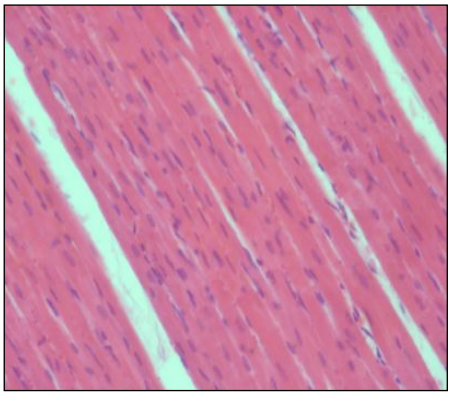

Fig 8: Paraffin with ceresin infiltration

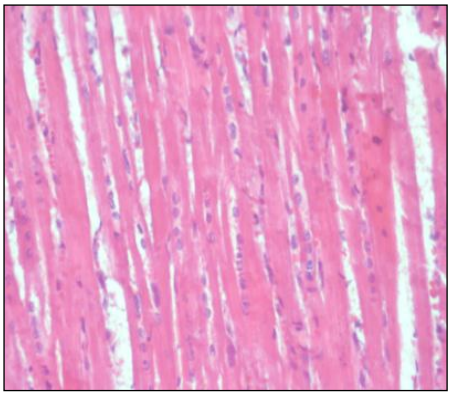

Fig 9: Double infiltration

Cardiac Muscle-Gomori's reticulum

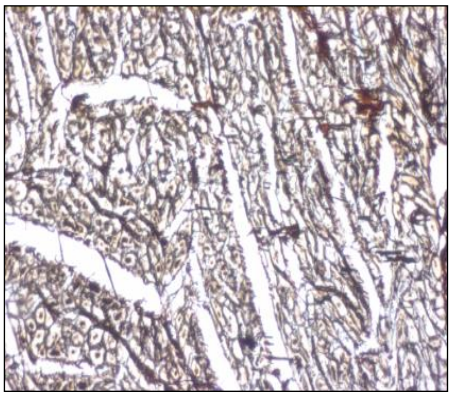

Fig 10: Paraffin infiltration

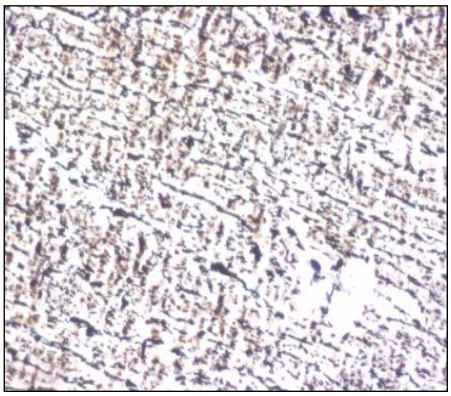

Fig 11: Paraffin with ceresin infiltration

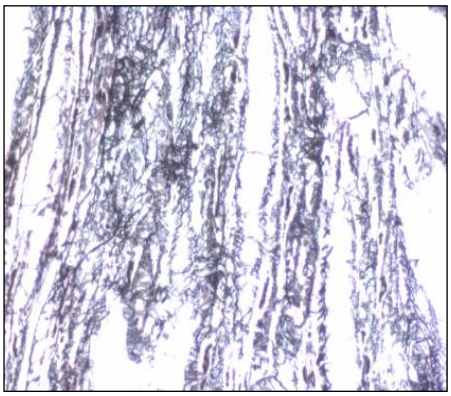

Fig 12: Double infiltration

Lymph Node-H & E phloxine

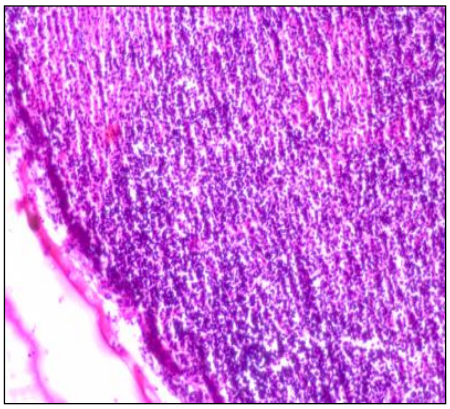

Fig 13: Paraffin infiltration

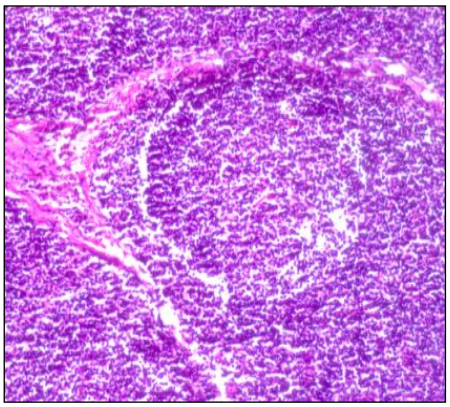

Fig 14: Paraffin with ceresin infiltration

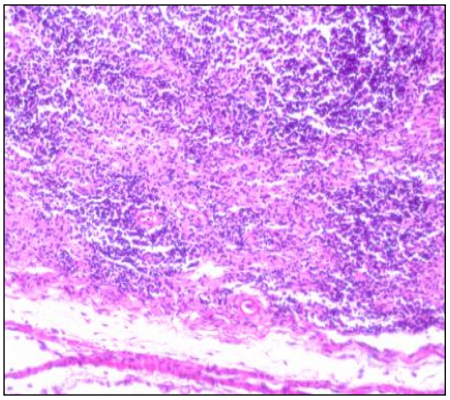

Fig 15: Double infiltration

Lymph Node-Gomori's Reticulum

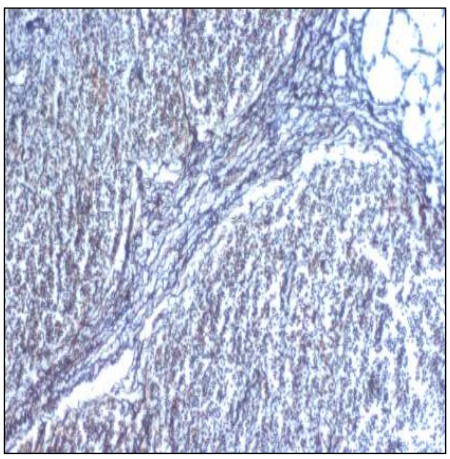

Fig 16: Paraffin infiltration

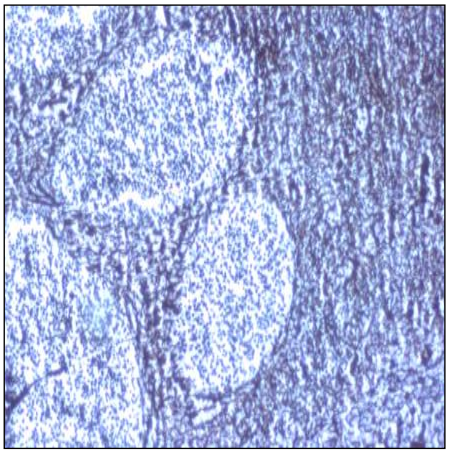

Fig 17: Paraffin with ceresin infiltration

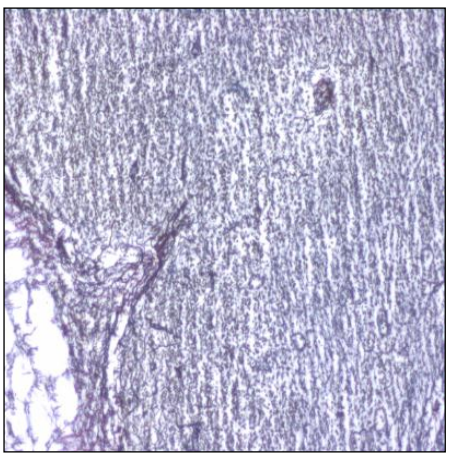

Fig 18: Double infiltration

Table 3: Showing under criteria 2. I.e. general morphology of cells, nuclear or cytoplasmic contrast under 1000X magnification

Sl. No.	Organ	Stain	Infiltrating medias			
			P	P with C	D I	R
1.	Spinal cord	Thionin	++++	+++	++	++
2.	Cardiac muscle	H & E phloxin	++	++	+++	++++
3.	Lymph node	H & E phloxin	++	++	++++	+++

Table 4: Showing under criteria 3. The general staining of tissue components such as collagen fibres, elastic, reticular fibres, striated muscle fibres and neuronal elements under 400X magnification

Sl. No.		Infiltrating medias			
		P	P with C	D I	R
	Spinal cord				
1.	Neuronal elements	++	++	++++	+++
	Cardiac muscle				
1.	Collagen fibres	++	++++	++	-
2.	Reticular fibres	+++	-	++++	++
3.	Striated muscle fibre	++	++++	+++	-
	Lymph node				
1.	Collagen fibres	+++	++	+++	+++
2.	Elastic fibres	+++	++++	++++	++++
3.	Reticular fibres	++++	++++	++++	++

Double infiltration has also been studied by Landes *et al.* (2005) [1] using plastinated and celloidin infiltration. They found that sufficient micro anatomical details and the muscle distribution were better differentiated.

Melina and Peter (2007) [3] used agar gelatin for double embedding. They suggested that use of agar alone for pre embedding tissues cause shrinkage during processing but the addition of gelatin for the double embedding process allowed the flexibility of the tissue and infiltrating mixture and reduced shrinkage and folds in the sections.

Zozumi *et al.* (2010) [7] adopted new method of double embedding technique for endoscopic submucosal dissection specimens by using Agar and Gelatin. They found that the present method was better than the usual tissue processing method and suggested that each large tissue specimens can also be used for double embedding technique.

Yadav *et al.* (2015) [6] improvised double embedding technique for minute biopsy and suggested that agar paraffin embedding technique was simple, reliable and user friendly. It also gave better histological and histochemical staining properties.

However, in the present study the use of jelly wax as a double infiltrating media showed much better results by using different stains and at the different magnification which were almost at far with the criteria described and evaluated by the above scientist using double infiltration technique.

The literature showing the double infiltration technique in the western world by various authors have used different infiltrating media which were much more costly and the technology involved is also more sophisticated as compared to the present technique using jelly wax and paraffin for the double infiltration method. Such technology will be highly valuable for the developing countries as it is very cheap and easily available in the market.

References

1. Landes AC, Weichert F, Geis P, Wernstedt K, Wilde A, Fritsch H, *et al*. Tissue plastinated vs celloidin embedded large serial section in video, analog and digital photography on screen reproduction: a preliminary step to exact virtual 3D modelling, exemplified in the normal mid face and clef-lip and palate J Anat. 2005;207:175-191.
2. Madhu D. Morphological studies on the visceral organs of wild ruminants. M.V.Sc. thesis Submitted to The Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar; c2013.
3. Melina VJ, Peter AC. Agar-gelatin for embedding tissues prior to paraffin processing. John Hopkins University, Baltimore, USA Bio Techniques. 2007;42:569-570.
4. Palmer LS, Guy HG, Montogmery, Moore MH. Studies the new paraffin-Resin infiltrating and embedding media for micro technique. J Am. Chem. Soc. 1941;44:1527-1538.
5. Shambhulingappa Y Baddi. Morphological studies on the skin of carnivorous wild animals. M.V.Sc. Thesis submitted to Karnataka Veterinary and Fisheries Sciences University, Bidar, Karnataka, India; c2009.
6. Yadav L, Thonmas S, Kini U. Improvised double-embedding technique of minute biopsies: A mega boon to histopathology laboratory Indian Journal of Pathology and Microbiology. 2015;58(1):12-16.
7. Zozumi M, Nakai M, Takashi I, Ikuo M, Hiroyuki H, Yoshitane T, *et al*. New double embedding technique for specimens of endoscopic submucosal dissection using agarose: comparison with other media. J Clin. Pathol. 2010;63:904-909.