Section: Pathology

Original Research Article

# TISSUE MICROARRAY CONSTRUCTION: AN ALTERNATIVE, ECONOMICAL AND MANUAL METHOD: AN ONE YEAR EXPERIMENTAL STUDY

*Ashna Muhusin[1], K. Durga[2], M. Vijayalakshmi[3], N. Mohan Rao[3], G. Bhavana[3], B. Syam Sundara Rao[3], V. Thejaswini[4]*

[1]*Postgraduate, Department of Pathology, Narayana Medical College, Nellore, Andhra Pradesh, India*
[2]*Professor &HOD, Department of Pathology, Narayana Medical College, Nellore, Andhra Pradesh, India*
[3]*Professor, Department of Pathology, Narayana Medical College, Nellore, Andhra Pradesh, India*
[4]*Assistant Professor, Department of Pathology, Narayana Medical College, Nellore, Andhra Pradesh, India*

***Received*** *: 03/01/2026*
***Received in revised form*** *: 10/02/2026*
***Accepted*** *: 28/02/2026*

**Corresponding Author:**
**Dr. K. Durga,**
Professor &HOD, Department of Pathology, Narayana Medical College, Nellore, Andhra Pradesh, India.
Email: drkdurga.path@gmail.com

**DOI:** 10.70034/ijmedph.2026.1.546

**Source of Support:** Nil,
**Conflict of Interest:** None declared

**Int J Med Pub Health**
2026; 16 (1); 3189-3193

ABSTRACT

**Background:** Tissue Microarray (TMA) is a novel technique that is now integral in pre-clinical and translational research. However, the relatively high cost and construction may hamper many researchers from using this essential tool of modern pathology research. The aim is construction of manual tissue microarray kit from various formalin fixed paraffin blocks with punch biopsy needle in a cost-effective way with available resources and to study multiple IHC markers.

**Materials and Methods:** A one-year experimental study conducted in Department of Pathology at a tertiary healthcare center. 100 cases were taken including inflammatory, benign and malignant lesions. TMA blocks were made using skin punch biopsy needle of various sizes ranging from diameter of 1.5 mm, 2 mm, 2.5 mm and 18 gauge cannula as a paraffin core plunger. hematoxylin and eosin (H&E) staining done followed by immunohistochemical (IHC) studies were done. Pitfalls, solutions and evaluation of outcomes were documented.

**Results:** Out of 100 cases H and E results interpretation was improving as the size of the donor block increases. Benign and malignant tumors IHC uptake and interpretation improved as the diameter of biopsy needle increases. Moreover it was observed that different IHC markers can be stained on different tissues on same slide.

**Conclusion:** Manual TMAs can thus be used as an alternative to traditional paraffin-based techniques for research applications in resource-limited centers.

**Keywords:** Tissue Microarray, immunohistochemistry, fluorescence in situ hybridization, Lesion.

## INTRODUCTION

Tissue Microarray (TMA) is a novel technique that is now integral to pre-clinical and translational research.[1] TMA is an important tool for conserving precious tissue resources from increasingly smaller biopsies and for controlling experimental costs and variations across sample sets.[2] TMA has several advantages, such as simultaneous analysis of a large number of specimens, lower assay volume, conservating the valuable tissue, and time effectively. This technique enables pathologists to perform large-scale analyses using immunohistochemistry, fluorescence in situ hybridization (FISH), or RNA in situ hybridization (ISH). It also allows parallel molecular profiling of clinical samples at DNA, RNA, and protein levels.[3] However, the relatively high cost of TMA molds and custom designs may hamper many researchers from using this essential tool of modern pathology research.[4] TMAs have certain drawbacks, particularly in that preparation and construction on a larger scale is difficult.[5,6] A study found that the microarray core gave the same result for Estrogen Receptor (ER) status as that of the whole section in 96 % of cases, which proves that TMAs are

representative of the whole tumor.[1,7] However, few studies have explored the pitfalls, including breakage and cracking of TMA blocks and bulging of cores, encountered while constructing manual TMAs in resource-limited settings.[8]

**Aim**

Construction of manual tissue microarray kit from various formalin fixed paraffin blocks with punch biopsy needle in a cost effective way with available resources and to study multiple IHC markers.

## MATERIALS AND METHODS

A one-year experimental study was conducted in a tertiary health care centre.100 donor paraffin blocks and corresponding slides were selected from the Department of Pathology, including 45 benign tumors, 45 malignant tumors, and 10 inflammatory lesions. The tumor areas were marked on hematoxylin and eosin-stained slides using a marker pen. Each demarcated area was assigned a separate code comprising letters, numbers, and documented. The hematoxylin and eosin (H&E) glass slide was then overlaid on the block, and the corresponding desired area was identified on the block and demarcated using a marker pen.

**Donor block preparation:** Previously selected tissue areas were obtained by punching cores from the donor blocks using a skin biopsy punch needle, perpendicular to the optimum tissue sampling. The block was warmed to avoid cracking of the tissue. The biopsy needle was cut in half for easy removal of the core.

**Table 1: Distribution of lesions**

| Type of lesion | Frequency (n) |
|---|---|
| Inflamatory lesions | |
| Appendicitis | 5 |
| Cholecystitis | 5 |
| Benign tumors | |
| Neurofibroma | 10 |
| Glioma | 10 |
| Schwanoma | 10 |
| Leiomyoma – uterus | 15 |
| Malignant tumors | |
| Breast carcinoma | 10 |
| Neuroendocrine tumors of pancreas | 10 |
| Squamous cell carcinoma | 10 |
| Lymphoma | 15 |

**Figure 1: Donor block preparation and manual extraction of the core using skin biopsy punch needle.**

After pulling out the biopsy punch, the tissue core was extracted with the help of a 18 gauge cannula with its tip cut which was used as a plunger and transferred to the respective holes in the recipient block using forceps, arranged in the previously decided format in the recipient blocks.

**Figure 2: 18G cannula is used for extraction of the paraffin core from the donor block.**

**Figure 3: a. 1.5 mm tissue block, b. 2 mm tissue block, c. 2.5mm tissue block. d & e. integration of tissue cores to the block.**

Recepient block preparation. To prepare the recipient paraffin blocks for the paraffin cores, the holes were bored using a skin punch biopsy needle at 1.5 mm, 2 mm, and 2.5 mm. Each tissue core extracted is transferred to respective holes [Figure 3 B, C, D]. The biopsy number, patient details, and allotted codes were documented in an Excel sheet and photographs were taken to avoid interchanging of tissue [Figure 3A] Upon completion of the assembly of each TMA, a clean microscope slide was attached to the face of the TMA block to apply firm under gentle pressure to press down any protrusion from the surface of the block. To facilitate integration of the donor tissue cores into the recipient block, the block was placed in an oven at 60°C for 10 min on a microscope slide. The irregularity of the block surface was then flattened

by applying gentle and even pressure to the microscope slide. To remove the attached glass slide, another glass slide is heated and placed over it and gently removed from the recipient block. Recepient block is cooled. The sections of the TMA blocks were routinely processed for hematoxylin and eosin (H&E) stain and followed by IHC staining was done.

To study different IHC on different tissues in the same slide, four different tumors were chosen. Manual TMA blocks containing four tissues arranged in a zigzag manner [Figure 5 A].

**Figure 4: Block is cooled and glass slide is removed.**

The routine IHC procedure was followed, except for one time buffer in an oven at 98 °C for one min. After heating, the buffer was removed from the oven and the slides were kept in the preheated buffer to avoid tissue loss. After processing, instead of using calibrated pipettes, a 2 cc syringe was used to drop the antibodies, buffer, and washing. The slides were carefully tilted and washed with a syringe to avoid cross-reacting with the antibodies [Figure 5 B].

**Figure 5: a. Multiple Tissue cores arranged in Zig Zag Manner for IHC studies. b. calliberated needles are used instead of pippets.**

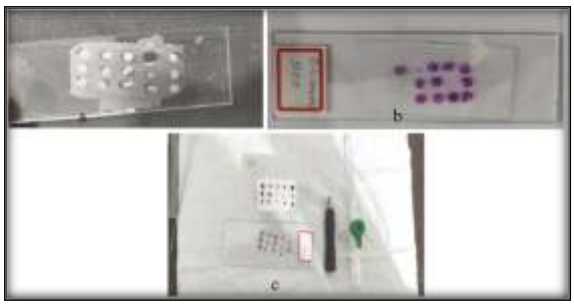

**Figure 6: a. sectioning of manual TMA block, b. Hematoxylin and eosin stained slides, c. manual tissue microarray kit.**

## RESULTS

By using self-made TMA kits and recipient paraffin blocks, successfully constructed self-made high density TMAs with varying core diameters. TMA kits made from disposable skin biopsy punches and were able to construct TMAs that had 15 cores, 13 cores and 10 cores, using 1.5 mm, 2 mm, 2.5 mm punch biopsy needle respectively. All tissue cores morphologically and immunologically represented the original tumor. The self-made manual TMA kits did not cause noticeable damage to the paraffin blocks when punched out. Out of 100 cases, H and E results improved as the size of the tissue core increased. Benign and malignant tumors IHC uptake and interpretation improved as the diameter of biopsy needle increases. Ten inflammatory cases were studied and was able to appreciate whether it is the acute, chronic or acute on chronic inflammation.

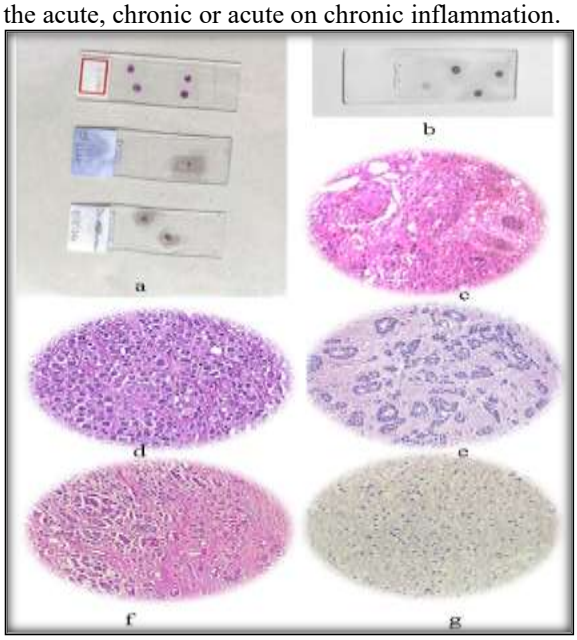

**Figure 7: a. Different IHC markers of different tumors in same slide. loss of tissue noted during ihc procedure (b) tissue cores arranged in zig- zag manner. c. TMA of squamous cell carcinoma of larynx (100x). d. diffuse large b cell lymphoma (100x) . Breast carcinoma (100x) . f. H and E image of neuroendocrine tumor of pancreas (100x). g. H and E microphotograph of oligodendroglioma.**

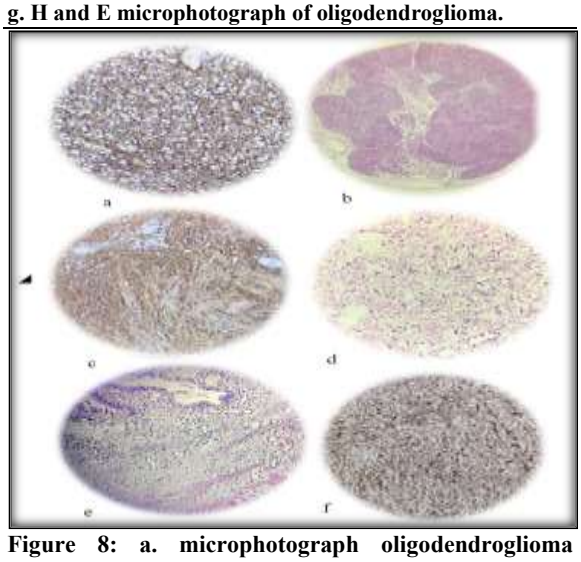

**Figure 8: a. microphotograph oligodendroglioma showing IHC with GFAP positivity. b. schwannoma - H and E microphotograph (100x). c. schwannoma-IHC with s100 positive (100x). d. neurofibroma TMA – hemaoxylin and eosin stained mcrophotograph (400x).e.micrograph of chronic cholecystitis (100x)f. neurofibroma TMA-IHC with s100 shows positivity (100x).**

## DISCUSSION

In the era of precision medicine, where tissue is an issue, using small amounts of tissue for ancillary techniques can be quite beneficial. TMA is an essential tool for biomarker expression using archived oncologic specimens for various molecular and histological studies.[9] Comprehensive TMA experiences add value to decision making for target identification, candidate drug selection, and translational activities for biomarker development. Due to the high cost of TMA kits, it is almost always impossible to make use of this novel technique in hospitals and laboratories with limited resources and lack of funds. Constructing of manual TMA kits with locally available resources in the hospital provides a unique way of improving the diagnostic quality and initiation of immediate treatment or referral of patients.

The advantages of this method for constructing TMAs are summarized as follows:

a) Economical and easy assembling of materials for constructing TMA blocks.

b) Different tissue core diameters are selected according to need.

c) This method is easy to operate in resource limited settings, with minimal or no automation.

Documentation of patient details corresponding to the tissue cores was a tedious task faced during the study. Each data point was documented in Microsoft Excel and cross-checked after completion of each recipient block. Array mapping with the subsequent TMA that was constructed based on it.

Based on recent studies done by Tanvi Jha et al,[1] and Palo s et al,[8] also came across similar challenges while making the TMA blocks such as extraction of tissue core, blockage of the punch core, cracking of the recipient block, handling delicate tissue, transfer of recipient block, adjusting the size of the core for accurately fitting in to the recipient block, challenges while sectioning and staining. Initial few blocks it was difficult to get the core out of the biopsy needle and tissue got damaged, on further efforts keeping empty paraffine core on the biopsy needle prior to punching the tissue block, to avoid breakage of the core while pushing from the cannula In addition unevenness of the recipient block made it difficult to section as few cores were got empty even tissue was present in the parent core ,this concern was overcame by making all tissues in same plane by adjusting the length of the core with microtome blade or decreasing the depth of the core hole by filling with wax followed by heating in oven for 60° Celsius. Staining of different tissues using different immunohistochemical stain was the difficult part of the study. Initially there was loss of tissue cores and cross reaction of different antibodies to each other noted, which was rectified by making a zig zag pattern of arrangement of tissue cores and using needles instead of calibrated pipettes. Pitfalls faced during the study are analyzed, documented and its solutions are effectively implicated in the study itself and summarized as follows.

| Pitfalls during constructing TMA Kit | Solutions |
|---|---|
| Breakage and cracking of donor and recipient paraffin blocks while making bores | Prior warming of the donor and recipient block |
| Damage to the tissue while extracting it from the punch biopsy needle by cannula | Double punching with initial plain paraffin block followed by donor tissue |
| Size of core is more that the recipient bore hole | Adjusted the length of core with microtome blade |
| Size of the core tissue is less than the recipient hole | Extra plane paraffin core is first inserted followed by transfer of tissue to avoid deep seating of core |
| Difficult in sectioning due to non-union core holes | Kept glass slide facing the block upside down warmed it in oven for filling the uneven gaps with paraffin |
| Loss or misplacement of tissue while handling with forceps | Direct insertion of core extracted form donor block into recipient block using plunger |
| Appearance of serrations and loss of cores during sectioning | Further cooling of the blocks followed by serial and deeper sections |

**Limitation:** Only the chronicity of inflammatory lesions can be assessed using TMA, additional finding like individual cellular details was difficult to appreciate as only few selected cores were studied. Few of the tissue sections were lost during sectioning and staining, which could not retrieved. Maximum core per slide was 15, compared with conventional TMA kit the sample size per slide was low. More than morphological studies using H and E. TMA is more beneficial for anxillary tests such as IHC, FISH and genetic analysis.

## CONCLUSION

Manual TMA construction is a cost-effective method of constructing TMAs with simple cheap and readily available resources. In resource limited setting manual construction is the main stay for diagnostic and prognostic utility. Morphological-based molecular profiling could be used as a general approach for mass-scale molecular profiling based on H&E-stained images, allowing quick, accurate, and inexpensive methods for simultaneous profiling of multiple biomarkers in cancer tissues. Hence tissue microarray is a practical and effective tool for digitalization. AI application for mass studies, anxillary tests, high-throughput molecular analysis of tissues that is helping identify diagnostic and prognostic markers.

# REFERENCES


1. Jha T, Mahapatra S, Diwaker P, Arora VK, Sharma S. Navigating Tissue Microarray Construction: A Guide for Avoiding Pitfalls and Mastering Key Technical Aspects. Journal of Clinical & Diagnostic Research. 2024 Apr 1;18(4). DOI: https://doi.org/10.7860/JCDR/2024/68591.19288
2. Qin P, Li L, Zhao L, Bian P, Xiong Z. Constructing high-density tissue microarrays with a novel method and a self-made tissue-arraying instrument. Pathology-Research and Practice. 2023 May 1;245:154430. DOI: 10.1016/j.prp.2023.154430
3. Jawhar NM. Tissue Microarray: A rapidly evolving diagnostic and research tool. Annals of Saudi medicine. 2009 Mar; 29(2):123-7. DOI: 10.4103/0256-4947.51806
4. Choi CH, Kim KH, Song JY, Choi SJ, Kim L, Park IS, Han JY, Kim JM, Chu YC. Construction of high-density tissue microarrays at low cost by using self-made manual microarray kits and recipient paraffin blocks. Korean Journal of Pathology. 2012 Dec;46(6):562. DOI: 10.4132/KoreanJPathol.2012.46.6.562
5. Glinsmann-Gibson B, Wisner L, Stanton M, Larsen B, Rimsza L, Maguire A. Recommendations for tissue microarray construction and quality assurance. Applied Immunohistochemistry & Molecular Morphology. 2020 Apr 1;28(4):325-30. DOI: 10.1097/PAI.0000000000000739
6. Gollapudi S, Singh K, Small C, Mukherjee S, Ohgami RS. Creation of efficient pathology research pipelines for discovery: tissue microarray construction coupled with digital image analysis. Journal of Clinical and Translational Pathology. 2021 Dec 15;1(1):28-31. doi: 10.14218/JCTP.2021.00012
7. Parker RL, Huntsman DG, Lesack DW, Cupples JB, Grant DR, Akbari M, et al. Assessment of interlaboratory variation in the immunohistochemical determination of estrogen receptor status using a breast cancer tissue microarray. Am J Clin Pathol. 2002;117(5):723-28. DOI: 10.1309/PEF8-GL6F-YWMC-AG56
8. Palo S. A simplified method of manually constructing small format tissue microarray for use in resource-constrained settings. Iran J Pathol. 2023;18(1):210-16. doi: 10.30699/IJP.2023.562055.2972
9. Hewitt SM. Tissue microarrays as a tool in the discovery and validation of predictive biomarkers. Methods Mol Biol. 2012;823:201-14. DOI: 10.1007/978-1-60327-216-2_13.