Short communication

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# Optimization of tissue microarray technique for breast cancer patients: a short communication

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**Background:** Tissue microarray (TMA) is a novel technique for studying different types of cancer tissues in one block. TMA is not yet established in Syria, so we aimed in this project to apply and set the most optimal conditions of TMA creation of breast cancer tissues at the Pathology Department of our institute.

**Materials and Methods:** Eighty-eight blocks of breast cancer tissues were selected, considering the inclusion criteria. The tissue specimens of breast cancer patients were manually placed in the block by punching a core from a paraffin block, which was then released into a recipient block using a small trocar. Three different conditions were tested on the constructed TMA block.

**Results:** We determined the most effective parameters that proved high quality: incubating the newly constructed block at a temperature of 43°C for 24 h in the oven and then cutting it the next day after cooling it to room temperature; also, cutting with a 5 μm thickness created the preferable stained slides later. CD3 staining showed high expression of tumor-infiltrating lymphocytes among triple-negative breast cancer patients and high expression of CD3 in triple-negative cancer patients.

**Conclusion:** The optimization of parameters presented in our study resulted in perfect TMA generation and successful immunohistochemistry staining for cancer research at our institution.

**Keywords:** Tissue microarray, optimization, temperature, breast cancer

## Introduction

Tissue microarray (TMA) is a useful tool for studying and evaluating different cancer types[1]. Hundreds of tissue samples can be studied simultaneously for molecular marker status at the nucleic acid or protein level[2]. Although semi-automated or fully-automated technologies for TMA creation give excellent precision in the core transfer, they do not avoid the need for technical expertise to successfully generate good-quality TMA blocks and sections. TMA allows synchronized analysis of molecular targets at the DNA, mRNA, and protein levels under standardized conditions on a single slide and also provides maximal use and preservation of archived tissue samples[3].

**HIGHLIGHTS:**

- Tissue microarray (TMA) is a cost-effective technique to detect cancer biomarkers.
- Conditions of construction should be optimized before applying this technique.
- Temperature was the most important variable during TMA construction.
- Organizing a bank of TMA blocks for each kind of cancer is a great step toward studying new proteins and detecting new cancer biomarkers.

A large number of archival specimens can be analyzed in parallel by applying modern TMAs using tissue-based techniques such as immunohistochemistry (IHC), multiplex immunofluorescence, and fluorescent in situ hybridization (FISH)[4].

This technology is a cost-effective method for analyzing multiple tissue samples. TMA block can be performed by punching tissue cores from selected regions from each block and arranging the punched cores into a recipient block instead of cutting each block individually, which is a time-consuming process. TMAs are flexible concerning input, design, and arrangement of tissue. This technology has gained momentum in the past two decades, contributing to advances in cancer research and biomarker discovery in a range of tumor types[5,6].

TMA is not yet performed in Syria, which is a third-world country, so the goal of this project was to first administer and optimize this technique at the Pathology Department of Tishreen University Hospital, Latakia, Syria. And to apply a new technique that allows the analysis of several hundreds of tumor samples of a large number of patients on one slide, and finally, to study the tumor-infiltrating lymphocytes (TILs) and expression of CD3 in breast cancer tissues.

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## Materials and methods

### *Formalin-fixed paraffin-embedded (FFPE) tissue*

Eighty-eight blocks of breast cancer patients (age: 22–79 years old) were selected from the Department of Pathology of our institutional hospital. These cases were diagnosed and archived between 2020 and 2021. Inclusion criteria were hospitalized patients with IHC results of estrogen receptor (ER), progesterone receptor (PR), and human epithelial receptor neu 2 (HER2). The blocks contain FFPE tissue preserved and archived numerically; each number refers to the patient data.

Ethical approval for this study was provided by the Ethical Committee of the Institutional Review Board of our university in accordance with the Declaration of Helsinki (Number 121 s.s Date: 18/01/2021).

Blocks were selected from the Department of Pathology according to the tumor pattern and subtypes of invasive ductal carcinoma and invasive lobular carcinoma and the criterion of being newly diagnosed patients (2019 onward).

Also, samples were selected with the inclusion criterion that the patient had a diagnosed type based on the expression of hormonal receptors (ER, PR, and HER2 neu) and that she had not started chemotherapy or immunotherapy yet. The spot to be taken for the TMA technique was chosen with the help of a pathologist to determine the spot containing most tumor cells and immune cells together from every single block.

Once appropriate blocks were selected for generating TMA, slides stained with hematoxylin and eosin (H&E) were reviewed by two pathologists to mark the exact areas to punch for TMA creation.

### *Preparation of TMA*

The samples of breast cancer tissue were placed in the block. We utilized the tissue microarray 1S (EZ-TMA) kit in which blocks contain 170 cores, and the diameter of each core is 1 mm.

Constructing TMA was achieved manually by punching a core from a donor paraffin block, which is then deposited into a recipient block; three punched tissues were placed in three successive cores for each donor. This process was achieved using a small trocar.

The first TMA block included 42 breast cancer cases; each block was punched and placed in three to four cores, and the other block contained 46 breast cancer cases also placed in a triplex.

### *Tempering to merge tissues with the block*

After inserting all cores into the recipient block, the constructed TMA block was placed on a slide and tempered. Three different conditions were examined:

Protocol 1 (43°C for 2 h): The TMA block was incubated for 2 h at 43°C in the oven and then cooled for 10 min to room temperature.

Protocol 2 (46°C for 2 h): The TMA block was incubated for 2 h in a 46°C temperature using the oven and then cooled for 10 min to room temperature.

Protocol 3 (43°C for 24 h): The block was left at a temperature of 43°C for 24 h in the oven and cut the next day after cooling for 24 h to room temperature.

### *Cutting with microtome*

Our constructed TMA blocks were sectioned using a Manual Rotary Microtome M380 (Medite) with a clearance angle of 10° and setting the *x*-axis and *y*-axis to prevent tissue loss until achieving a parallel surface for continual optimal sectioning. Three different thicknesses of the sections were 3, 5, and 7 μm. About 50–60 sections were cut to assess quality and ease of cutting. We performed the experiment of block cutting twice: once on the same day of staining and the other cutting on a day and staining the next day.

### *H&E staining and IHC*

Prepared sections were stained with H&E stain using the programmed automated slide stainer (Medite TST44C) in the Department of Pathology of our university hospital. IHC was performed to detect TILs using anti-CD3 antibody from Bio SB Inc. The IHC was applied according to the IHC protocol of the Pathology Department. The slides were examined and reviewed by two pathologists to determine CD3 expression and intensity on infiltrating lymphocytes.

## Results

After examining several variables in insertion, merging, and slicing, we determined the optimal parameters for effective and high-quality TMA. The third protocol (incubating the newly constructed block at a temperature of 43°C for 24 h in the oven and then cutting it the next day after cooling to room temperature), cutting with a 5 μm slide thickness, created the most preferable stained slides later.

In addition, cutting the block and staining the slides on the same day gave better results in terms of reducing tissue loss from the slide after staining. Optimization of merging temperature is one of the most important steps in ensuring a well-formed TMA block that integrates the wax from the recipient block with the surrounding wax for biopsies from donor patients.

The most important factor for optimal and felicitous TMA generation is the step of tempering the TMA block after core punching and insertion to facilitate the integration of recipient block wax with the donor core wax to ensure donor core merging within the TMA block.

Testing of Protocol 1 (TMA block incubated for 2 h at 43°C in the oven and then cooled for 10 min to room temperature) and Protocol 2 (TMA block was incubated for 2 h at 46°C temperature using the oven and then cooled for 10 min to room temperature) showed that the temperature and duration were not enough for the cores to merge with the recipient block and led to dislodging of tissues from the block during sectioning. The most successful tempering protocol was Protocol 3, in which the constructed TMA block is incubated at 43°C for 24 h with cores facing down on a glass slide. Then, the next day, the block was cooled to 4°C, and the slide was removed, preparing to cut the block.

*Cutting with microtome*: After cutting the block with three different thicknesses 3, 5, and 7 μm, we found that cutting with 5 μm thickness was the optimal condition to have the best stained slide later. This judgment was based on the results we got when staining slides sectioned with the three different thicknesses. Staining of 3 μm slides resulted in the loss and precipitation of

many spots after the end of the staining stages, which led to the inability to study biopsies and determine the expression of the required antigen. On the other hand, the staining of 7 µm slides led to confusion in the results because of the existence of artificial staining in the background after the end of the staining process, which may be interpreted as overexpression or false positivity.

The applied protocol with the parameters and results obtained are explained in the following chart (Fig. 1)

CD3 staining using anti-CD3 antibodies showed high expression of CD3+ on TILs (TILs CD3+) among 11% of breast cancer patients (Fig. 2), medium expression of TILs CD3+ among 19.5% of patients, and low or no expression among 69.5% of patients (Fig. 3).

The highest expression of TILs was detected in triple-negative breast cancer, and the high expression of CD3 in triple-negative cancer patients is related to the aggressiveness of this kind of cancer. These results are compatible with Rashed *et al.*'s findings[7].

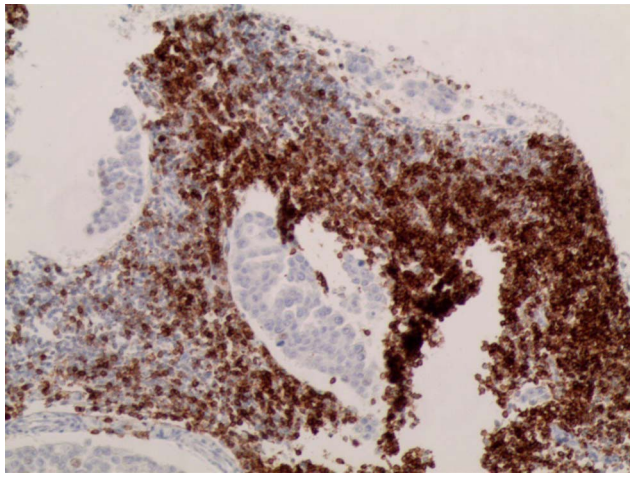

**Figure 2.** Tissue microarray biopsies stained with anti-CD3 antibodies for tumor-infiltrating lymphocytes in breast cancer tissue (CD3 expression is 60%).

## Discussion

TMA is a useful technique for studying tumor biopsies. It is a very cost-effective method that allows analyzing several protein targets under standardized conditions on a single slide, but this technique should be optimized before using it to detect different molecules.

TMAs are considered a powerful technique for biomarker detection and evaluation in cancer research. TMA is an excellent choice when testing hundreds of tissue samples with less steps and reagents than IHC the conventional way; you can use a minimal amount of primary antibody to perform IHC staining[8]. Numerous tissue samples from several types of cancer can be examined simultaneously on one slide. This process preserves tissue as well as reduces the reagent cost of detecting a single biomarker[9]. TMAs can be used in many types of human cancer investigations, including breast cancer[1], prostate cancer[10], and colon cancer[11].

This technique allows pathologists to perform large-scale analyses using IHC, RNA in situ hybridization (ISH), or FISH at markedly lower costs and at substantially faster duration compared with conventional histopathology[12].

The gold standard methodology for protein expression analysis in tissue specimens is IHC. The integration of conventional IHC and TMA technology allows for the synchronized analysis of hundreds of tissue samples with a high degree of experimental standardization. The same immune-staining protocols used for large sections can be used for one TMA section, including the antigen retrieval process for staining routinely archived formalin-fixed tissue samples. The development of optimal IHC protocols is highly important for TMA studies because minor protocol variations often have a marked impact on the outcome of the staining[13].

Temperature was the most important variable during TMA construction. After the finishing of core insertion from desirable breast cancer archived blocks, the tempering process was sensitive to ensure the core merged into the recipient block. Three tempering protocols with different temperatures and incubation periods were tested in our study. After testing three sets of conditions, we determined that the optimal protocol involved 24 h of

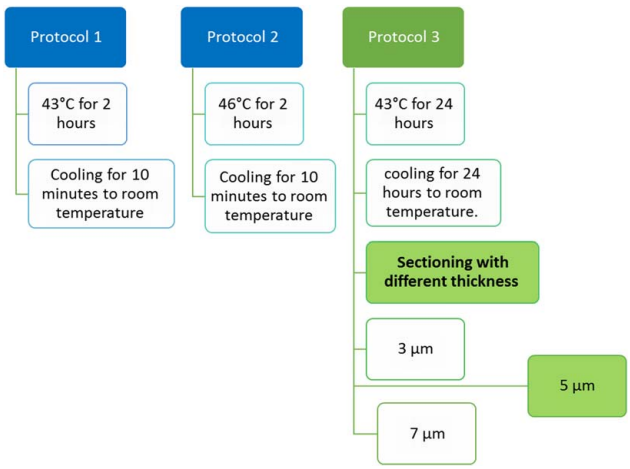


**Figure 1.** The applied protocol with the parameters and results obtained for tissue microarray optimization.

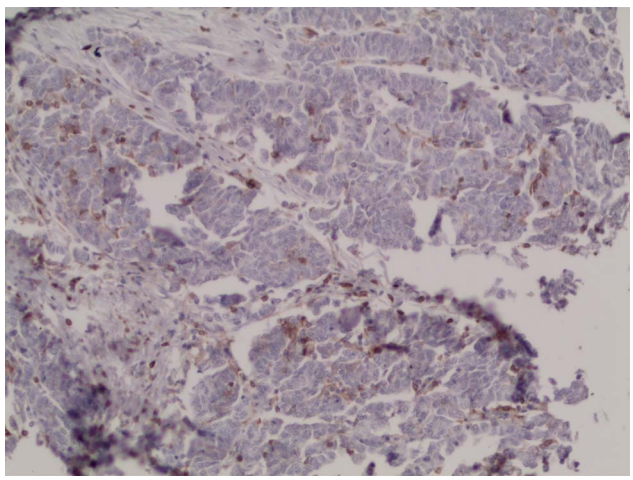

**Figure 3.** Tissue microarray biopsies stained with anti-CD3 antibodies for tumor-infiltrating lymphocytes in breast cancer tissue (CD3 expression is 20%).

incubation at 43°C, then cooling down to 4°C for 1 h. Our optimal temperature and duration were different from a previous study; Sexton *et al*.[9] showed that the optimal protocol for core integration with the template block is performing repetitive cycles of 1-h incubations at 37°C and 4°C, which was considered a time-consuming process[9]. This step is very necessary for the block preparation and successful sectioning. In another previous study, overnight incubation of the newly formed TMA block at 37°C or at 50°C for 1 h was important to facilitate the merge of donor cores with the recipient block, although the second protocol was preferable for larger diameter cores[14]. We can consider that our results are in concordance with the two previous studies.

Another important step toward good TMA generation is the sectioning thickness to obtain the best staining, especially IHC. Our experiments show that sectioning with 5 μm gave slides with less loss of spots and better IHC staining, and this was similar to previous studies where 5 μm sections generated the best TMA slides for molecular and immunohistochemical analyses[15]. Also, in another study, 5 μm slides were preferable for TMA sectioning and IHC staining[14].

We did the cutting twice: once on the same day cutting and staining, and once cutting on a day and staining on the next day. The reason for this experiment is that there is some evidence to suggest that such tissues remain antigenically intact after cutting[16].

On the other hand, there is another suggestion that although paraffin should protect the tissue from oxidation or other damage, there is evidence that once tissues are sectioned, they are subject to rapid loss of antigenicity[17]. Our results showed similarity in the quality of the slides and positivity of the studied antigen (CD3).

There are several strength points in this study, including the study of multiple parameters affecting the TMA construction, such as incubation duration, temperature, and sectioning, as well as performing H&E and IHC. Furthermore, this is the first project in Syria to study cancer using the TMA technique besides conventional histopathology. However, there are also some limitations to consider. Firstly, the number of samples is relatively low and were all gathered in two blocks, so we could not apply statistical analysis to the limited number of blocks. Secondly, there are other conditions that affect the construction of TMA block, for example, wax type, which was not studied in our research. Finally, the IHC was limited to one biomarker (CD3), which is not enough to assess the quality of the constructed TMA block. Other conditions are recommended to be included, and numerous biomarkers are recommended to be assessed by IHC on the newly constructed blocks.

## Conclusions

In summary, the evaluated parameters discussed in this study resulted in successful TMA generation for cancer research at our institution. Organizing a bank of cancer tissue and preparing TMA blocks for each kind of cancer is a great step toward studying new proteins and detecting new cancer biomarkers in a cost-effective and standardized way. TMA sections prepared this way can be used for different assays with manual and automated platforms. TMA technique enables the study of hundreds of cases by analyzing just one master slide. Microarray analysis has the advantage of processing all samples at one time using identical conditions and reducing the amount of archival tissue required for the study.

## Ethical approval

Ethical approval for this study was provided by the Ethical Committee of the Institutional Review Board at Tishreen University in accordance with the Declaration of Helsinki (Number 121 s.s Date: 18/01/2021).

## Consent

Written informed consent was obtained from the patient for publication. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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No funding was received for this study.

## Author contribution

R.M.H.: principal investigator and writing the draft; Z.A.-S.: performed histopathological examination and supervision; R.A.: participated in article drafting and supervision; M.A.: methodology; R.I.: reviewing and editing; Y.E. and J.V.: resources and validation.

## Conflicts of interest disclosure

The authors declare that they have no conflicts of interest.

## Research registration unique identifying number (UIN)

The research is a short communication study on archival paraffin blocks that are stored at the Department of Pathology at Tishreen University for research purposes and does not involve human participants or direct interventions on humans. The protocol was approved and registered by the Research Ethics Committee at Tishreen University. The protocol was approved and registered by the Research Ethics Committee at Tishreen University.

## Guarantor

Zuheir Al-Shehabi.

## Data availability statement

Not applicable.

## Provenance and peer review

Not commissioned, externally peer-reviewed.

## Presentation

Not applicable.

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