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A Simple Method for Generating Multitissue Blocks Without Special Equipment

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Abstract

The idea of multitumor block to expedite simultaneous analysis of multiple tissue specimens was pioneered by Battifora, and several variations have been published since then. More recently, microarray technology has been introduced to allow placement of up to several hundreds specimens in 1 block using manual or automated sampling devices. This paper reports a manual technique for preparation of a multitissue block. Generation of such blocks requires no special equipment, and flexible block design is possible depending on nature of available material and desired sample size. The first step is dissection of cubical or rectangular samples from paraffin blocks or processed tissue with a razor blade or scalpel. The tissue pieces can be tattooed on cut surface with a permanent marker to facilitate orientation and identification. This marking is preserved during embedding until the block is cut. If a “deep” block is desired, the tissue can be turned 90 degree to provide a greater vertical depth. For embedding, the pieces are laid in paraffin bath in desired order, and when completely melted, they are placed into a deep embedding mold and organized in multiple rows (5 to 10 pieces/row). Scaffolding and control tissue pieces (eg, placental liver or intestinal tissue) can be added as desired. Horizontal or vertical empty space should be preserved to allow for more effective separation of ribbons upon cutting, preventing unnecessary sacrifice of sections. Such blocks can accommodate 30 to 60 cases depending on the tissue size, and they can potentially generate up to several hundreds of sections. This technique is especially suitable when abundant tissue is available, for example, generating blocks containing libraries of normal tissues or defined tumors for antibody screening or tumor immunophenotyping.

Keywords

multitissue block; tissue array; immunohistochemistry; manual; technique

Multitumor blocks were introduced by Battifora in 1986 to enable more efficient immunohistochemical screening of antibodies and tissues.[1] The initial design was based on encasing tissue pieces by amniotic membrane to create a sausage-like block. Battifora further developed the multitissue block to be geographically configured in a “checker-board” manner, to include precise identification of individual pieces thus allowing efficient use in clinical research of tumors and antibody development.[2] Various applications of the same

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theme have been subsequently described.[3,4] More recently, Kononen et al[5] have developed a tissue array based on needle core sampling of donor blocks inserted in customized holes made in recipient blocks. This technique, either manually or automatically performed, can generate blocks that potentially contain several hundreds of specimens. However, preparation of this block requires special equipment, a manual or automatic arrayer. Wide popularity of tissue arrays is reflected by the over 25,000 articles retrievable from the PubMed database by the keyword.

This report describes creation of a multitissue block using very simple equipment and an embedding center. The resulting blocks can accommodate up to 50 to 60 tissue samples and design of deep blocks for a long life span is possible.

DESCRIPTION OF THE PROCEDURE

Equipment Needed

One-sided razor blades, permanent ink markers, embedding center, paraffin, embedding molds (various depths), forceps (multiple, nontoothed).

Tissue Selection and Sampling

Tissue pieces (cubes, rectangular prisms, or less defined shapes) of 3 to 5 mm in greatest dimension are dissected from paraffin blocks or processed unembedded tissue with a 1-sided razor blade. Preferably, the sampling is taken from 1 side of the block, with the help of a matching hematoxylin and eosin stained section. The obtained pieces can also be of irregular shapes, but 1 side should be flat and straight to allow stable placement onto the bottom of the embedding mold. The cut surface, to be placed facing the bottom of the embedding mold, can be marked with a permanent ink marker (different colors and different patterns) to allow for identification of individual tissue pieces and monitoring the correct embedding order and placement onto the bottom of the mold. It is beneficial to embed the tissue at a 90-degree angle (perpendicular to original block surface) in order to obtain blocks with a greater tissue depth, whenever a smaller tissue profile is acceptable. Use of smaller tissue profiles also allows for placing a larger number of tissues in the block. The tissue pieces can be collected on a slide tray for immediate embedding (Fig. 1A), or they may be taped on sheets of paper waiting for embedding at a later date. The sampling phase takes 1 to 4 hours, depending on how detailed attention is given to the sampling. Samples can also be obtained from processed, unembedded tissue, though with less accurate sampling to yield representative tumor tissue (estimate for accuracy of blind sampling, 70% to 80%).

Placement of Tissue Pieces Into Embedding Mold and Embedding

The dissected pieces are initially placed in paraffin bath in the desired order and let to melt completely. Nontoothed forceps are recommended to avoid tissue damage and improper attachment. Then the pieces are lifted one by one and placed in horizontal rows in a desired order into a relatively deep (5 mm or deeper) embedding mold filled with melted paraffin. The flat side, marked by ink, should be placed facing the bottom of the embedding mold. The mold should be kept on hot surface (63 to 64°C to prevent premature paraffin solidification. Approximately 5 to 10 pieces can be fit in each row, depending on the piece

size. Normal tissue (placenta, liver) can be used for scaffolding or stabilization, when needed. Alternatively, 1 side of the block can be used as supportive scaffolding. Sufficient space (approximately 1/4 of the width or length of the mold, depending on its positioning in the microtome) should be left empty in each row or column to allow space for more effective separation of tissue ribbons during microtome cutting.

After all tissues have been placed into the mold, the mold is carefully lifted on a cold plate to allow attachment of the pieces to the bottom, and then the appropriately numbered cassette is placed onto the mold (with label side at the first row) and filled with paraffin. Then the block is left to cool on the refrigerated surface.

The resulting block can be used to obtain up to 200 to 500 tissue sections, depending on the depth of the block. The ink markings representing different tissue pieces are seen on the tissue surfaces, helping to verify the tissue order and proper placement at the block surface (Fig. 1B). The slides will then contain rectangular or irregularly shaped issue profiles measuring 1 to 5 mm in greatest diameter (Figs. 1C, D). The embedding phase takes 15 to 30 minutes, depending on the number of tissues included in the block.

Any solid tissue is suitable, but fragmented specimens, such as endometrial curettings or very necrotic tissue would be difficult to manage. However, such tissues could be converted to stable pieces by embedding them in agarose before tissue processing (or reprocessing) after being recovered from conventional paraffin blocks.[6]

DISCUSSION

Different multitumor block and tissue microarray techniques have been described in order to facilitate efficient immunohistochemical analysis of a large number of samples for screening of antibodies or for analysis of immunophenotypes of particular types of non-neoplastic tissues or tumors.[1–5]

The method described here allows for an easy generation of blocks without any special equipment beyond what is available in a normal histology laboratory. Also, the above-described method can be modified to generate deep, long-lasting blocks that can be cut multiple times, potentially yielding several hundreds of sections. The depth of the tissue usually well exceeds that obtained from microarrays made from punches from modern, usually relatively thin blocks. Other advantages of the multitumor blocks described herein are flexible sampling sizes and shapes.

Generation of this block is moderately labor intensive. However, because collection, selection, and preparation of tissues is the most labor-intensive step in generation of any multitissue/microarray block, the effort for generation of the above described multitissue blocks remains very reasonable.

Starting material for the described multitumor block can be pieces dissected from paraffin blocks with a razor blade. Equally possible is to use pieces dissected from processed but unembedded tissue. In the latter case, the samples tend to be more irregular in shape, larger

in size, resulting in fewer pieces per block. Also, blind sampling is less effective, yielding 70% to 80% accuracy for specific tissue content in our experience.

Compared with the widely popular “punch” tumor microarrays,[5] generation of the block is faster with the herein described manual method, but the content of cases is lower (25 to 50 cases), as opposed to maximum 500 cases in microarrays. However, practice of use of multiple tissue cores from 1 case and spacing used between specimens or groups of them lessens the numerical efficiency of the microarray blocks.

This manual method enables cost- and time-effective immunohistochemical analysis of multiple tissues in research setting. Multitissue blocks are also excellent tools for screening and evaluation of new antibodies. We have also found H&E-stained and immunostained slides derived from multitumor blocks useful for teaching purposes, demonstrating variety within a group of tumors within any entity and allowing instant comparison between different cases.

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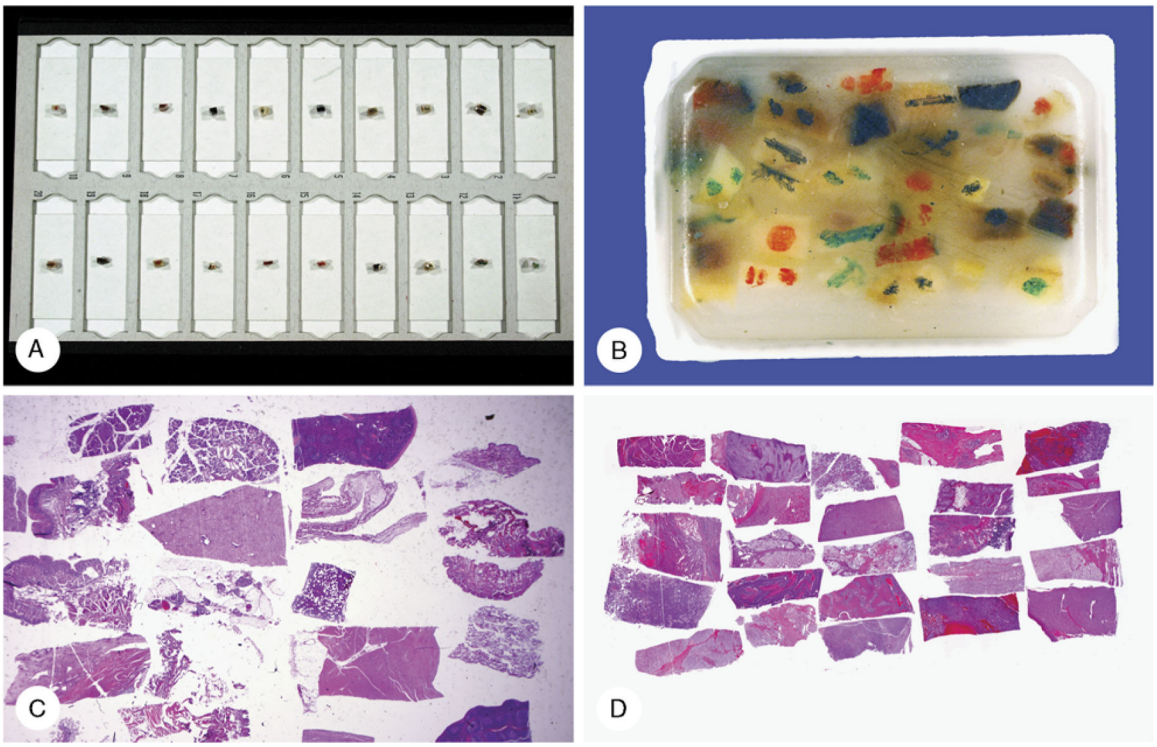

FIGURE 1.

A, Dissected tissue pieces ready for embedding. B, Undersurface of the block containing tissue pieces marked with permanent ink for identification and proper placement. C, D, Examples of H&E sections generated from 2 blocks. C, Normal tissues. D, Different types of renal cell carcinomas (n = 25).