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Tissue microarray technique (TMA) for burkitt's lymphoma immunohistochemistry IHC

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Abstract
Immunohistochemistry (IHC) is an integral part of diagnostic pathology. Tissue microarray (TMA) technology is based on the idea of applying miniaturization and a high throughput approach to the analysis of intact tissues. TMA are composite paraffin blocks constructed by extracting cylindrical tissue core "biopsies" from different paraffin donor blocks and re-embedding these into a single recipient (microarray) block at defined array coordinates. However, its productivity has been restricted due to the confined thickness of traditional patient or donor block. Here, we use a high output TMA method that is applicable to a broader range of tissue samples. In this method, a 2 cm long and 2 cm wide recipient block with 36 square lattices (2 mm in width) was first prepared using several commercial tools. A 2 mm wide and 6 mm long tissue rod was then prepared using tissue steel needle from each paraffin embedded patient block of burkitt lymphoma tumors. These rods were manually arrayed one by one into the corresponding lattices of the 60°C pre-softened recipient block with the guide of holes. A 24 -rod TMA was made in this method.The prepared TMA had well defined array configurations, good tissue morphology. A total of 36 TMA sections could be easily obtained from a TMA block. This low-cost and time-saving method provides a sampling tool for high output TMA.

Keywords: *Immunohistochemistry, Tissue microarray, biopsies, burkitt lymphoma tumors*

Introduction
Burkitt's lymphoma is a highly aggressive B-cell non-Hodgkin lymphoma and is the fastest growing human tumour. The disease is associated with Epstein-Barr virus and was one of the first tumours shown to have a chromosomal translocation that activates an oncogene (c-MYC). Burkitt's lymphoma is the most common childhood cancer in areas where malaria is holoendemic. The incidence is very high in immune-suppressed patients in non-endemic areas, especially when associated with HIV infection. Tissue microarray (TMA) technology is based on the idea of applying miniaturization and a high throughput approach to the analysis of intact tissues. TMA consists of a histology slide on which representative tissue samples (cores) from a selection of different cases are assembled. TMAs are a valuable tool for investigations requiring a large number of histology sections, offering several advantages over the use of serial sections including :Better use of tissue sections, particularly important when these are a scarce resource, Improved experimental consistency as identical experimental conditions are used for each core, Decreased reagent use and the associated cost savings, Options for analysis at the DNA, RNA or protein level (using FISH, mRNA ISH and IHC, respectively). TMAs provide a high-throughput histology solution, allowing simultaneous analysis of protein expression in multiple formalin-fixed paraffin-embedded (FFPE) tissues. TMA blocks are generally made from cores of individual formalin-fixed, paraffin embedded tissue blocks inserted in the holes of a receptor block. There are also descriptions of TMA's made from fresh frozen tissue using blocks made from optimal cutting temperature (OCT) compound for example. However we will only address the process of making TMA blocks from paraffin blocks.

Kononen *et al* proposed and developed a high throughput TMA technique in 1998 to precisely re-embed up to 1,000 cores of donor tissue cylinders from a formalin-fixed paraffin embedded "donor" block into a "recipient" paraffin block with the help of punching tools [1]. As a high throughput research tool, TMA, also known as tissue chip, is considered as a recent innovation in the field of pathology. Sections from TMA can be using in situ immunohistochemistry (IHC), fluorescence in situ hybridization (FISH). TMA technique has dramatically changed traditional tissue sampling processes which are extremely boring, time consuming and costly.

In addition, TMA technology plays an important role in educating pathologists about internal quality control in IHC [2].

Although up to 1,000 different tissues can be analyzed in one TMA block, all TMAs are restricted by the thickness of paraffin embedded block because they are cylindrical cores obtained by punching the patient block. As a result, only about 100–300 sections are available from one TMA block [3].
In this study, we use method by various tools to obtain rods of tissues from patient's blocks and special mold to prepare the recipient block with 36 small wells. The rods of tissues were vertically embedded in the recipient block.

Methodology

Selection of appropriate paraffin blocks with Burkitt lymphoma (BLs) 24 cases were retrospectively collected from the histopathology department of many private laboratories in Khartoum state –Sudan. Prior written informed consent was signed by all patients and the study protocol was approved by the Ethics Committee Board

Preparation of recipient block, tissue "rod "and plantation of the donor tissue rods into the recipient block

First step was preparation of a receptor block manually: by putting the cassette on the metallic mold 24x2.0 mm; then pour the liquid paraffin onto the cassette; allow solidifying at room temperature; removing the screws of the metallic mold in a synchronized way; finally removing the block from the mold (Figure 1).

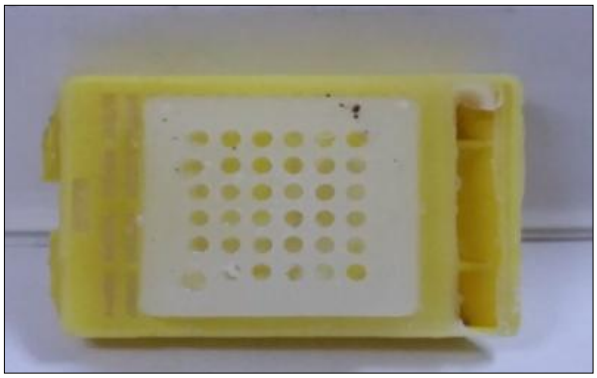

Fig 1: Recipient block

Once the recipient block is completed (TMA block) it is placed upside down onto a glass slide and into an oven at 40˚C overnight to facilitate bonding of the patients cores with the paraffin of the recipient block. In the next morning the block surface is leveled with the glass slide by gently pushing the cores into the block if needed. Then after cooling the blocks sectioned on the microtome like any other paraffin block.
The donor blocks (invariably stored paraffin blocks) are retrieved and sectioned to produce standard microscopic slides that are stained with hematoxylin and eosin. An experienced pathologist examined the slides and marked the area of interest, which is commonly an area of cancer, after which the samples were arrayed.
The checkerboard recipient block was a plastic board (2.0 cm in length and 2.0 cm in width) with 36 square lattices (2 mm in width). The board was etched to create 6 rows and 6 columns and marked as 1 to 5 in mirror phase in row except the first and last Lattice and from A to E in mirror phase in column except the first lattice. Melting paraffin was poured into the box to cover the whole area and cooled at room temperature until the plastic board could be separated from the transparent box. The recipient block together with the transparent box was incubated at 60 °C for 5 minutes prior to planting the rods of tissues to maintain the paraffin in a soft, but not melting status, and then placed on a flat board. Next, a hole was made at the starting spot A using a steel needle and the starting rod (Figure 2).

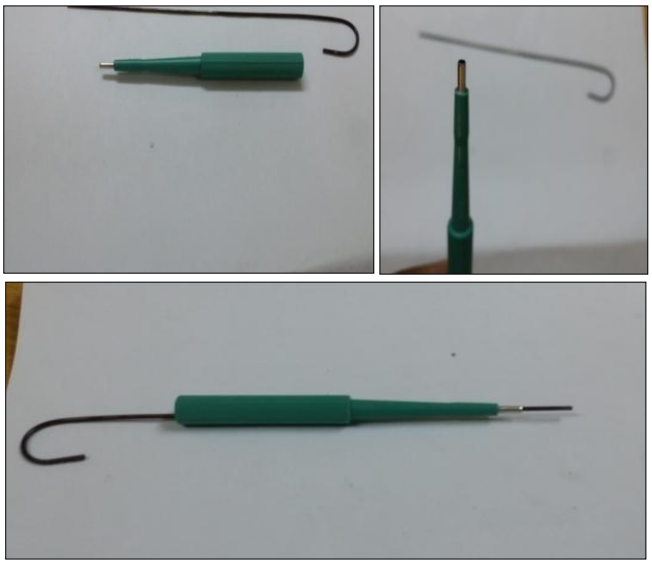

Fig 2: Steel needle

Tissue cores are punched from a predefined region of a donor paraffin wax embedded tissue block, Tissue cores are transferred to a recipient paraffin wax block, into a readymade hole, then manually planted into the recipient block one by one according to the corresponding location indicated by numbers.
The recipient block firmly holds the rods of tissues without slanting the rods since the paraffin pushed to the former hole due to the restriction of the transparent box when drilling the next hole. The course of planting stopped in any spot until all the desired tissue rods were planted. Then the recipient block with the transparent box was placed at 4 °C for 10 min until the paraffin easily separated from the transparent box. Aftermath, the TMA recipient block was taken out and sectioned on a routine microtome machine (Figure 3).

Fig 3: Donor or patient block

To prepare sections firstly we cut 4 µm thick sections by using a microtome, the sections transferred into the water bath then taken from the water bath immediately after they have

stretched out, to avoid melting of sensitive tissue spots. The section placed on a super frost glass slide.

The slides placed in a slide holder for drying in room temperature (RT) over night and stained by IHC (Figure 4).

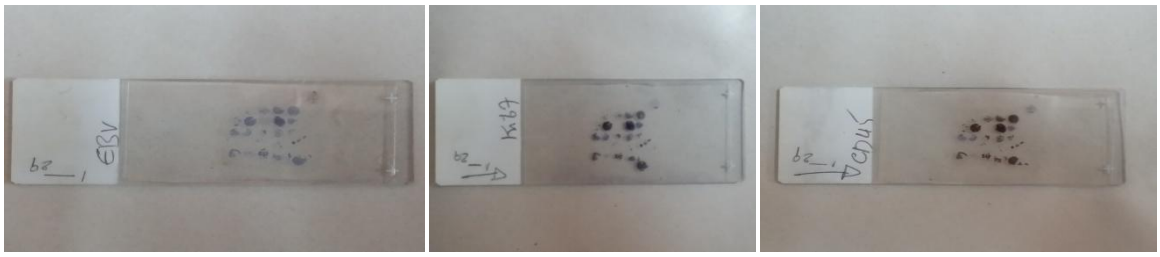

Fig 4: Slides from patients block

Quality control in IHC is one of the major problems in daily practice. Even though IHC has been in use now for decades, there is still a high variability of intra-laboratory and inter-laboratory results, mainly because of inter-laboratory differences in antigen retrieval, staining protocols, antibodies used, and in the interpretation of staining results. Therefore, approaches to guarantee a high level of quality are highly variable. The use of TMAs offers an alternative method of quality control for research and non-research purposes. A major issue in constructing TMAs for control purposes rests in the selection of tissues with expression of a large number of different antigens to use as positive control, at the same time tissue containing non related antigens used as negative control.

The main disadvantage of TMAs is that, because of the small diameter of the punches used, the sample may not be representative of the tumour as a whole, but this should be able to be circumvented by careful array design

Conclusion

High output TMA technology used in this study appears to be relatively easy, time-saving and cost-efficient. Therefore, this method is more feasible for general research groups to prepare high output TMA slides from appropriate tissue samples.

In summary, the widespread use of TMAs will become an integral part of daily practice in research and routine clinical laboratories. With this clear perspective, “pathology” as an old, largely morphology based medical specialty will find itself in a central position within these new developments. With the background of archives of well characterized tumour cases, pathologists will be in the position to use the potential of TMA technology to present their well-defined historical and current archives in an arrayed manner to the scientific community.

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