Cryostat Sectioning of Undecalcified Mouse Appendage Joints for use in Immunohistochemical Staining Procedures

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Introduction

Immunohistochemical analysis was required to identity lymphoid cell subtypes in mouse appendage joint synovium tissue. Hamster and rat monoclonal antibodies directed toward lymphoid cell subtypes were found to be nonreactive in formalin-fixed decalcified appendages, and this necessitated the use of cryostat sections for immunohistochemical procedures. Initial attempts to section mouse joints using conventional cryostat techniques did not yield adequate sections, and it was clear that a different approach was necessary.

After collecting information from experienced technologists in the area of bone cutting (Lundin. pers. comm.), it became clear that a tungsten carbide knife and a firm embedding medium would be required. An approach based on a modification of a tape-transfer protocol which had been previously published (Sterchi and Eurell, 1990) was used. The original tape transfer publication had been applied to paraffin sectioning, and it was unclear if a similar approach would work in cryostat sections. The following protocol was developed after many steps of trial and error.

Specimen Collection (mouse)

Appendages were dissected and the skin portion removed using a scalpel blade. Specimens were placed in a minimal essential media at 4° C for transportation to the laboratory, where they were removed from the media, blotted off and placed in a disposable 24 mm x 24 mm plastic base mold. They were then covered with 5% aqueous polyvinyl alcohol (PVA) (Sigma P8136), and allowed to soak for 5 minutes.

The specimens were oriented to provide the correct section profile and then immersed in -50° C isopentane contained in a freezing bath (Histobath, Shandon/Lipshaw) for 3 to 5 minutes. The frozen specimens were removed from the base mold and placed in air-tight containers (Poly Con, Cole Parmer) and stored at -70° C until sectioning.

Sectioning

A tungsten carbide "C" profile microtome knife (Delaware Diamond Knives Inc.) was obtained and mounted into the knife holder of a cryostat (Leica 1800 Cryocut).

Chamber temperature was adjusted to approximately -25° C. Embedded appendage specimens were attached to the object clamp of the cryostat using 5% PVA and section thickness was set at 5 µm.

The surface of the block was faced with one end of the knife until the desired structures were present, in this case the joint and its synovium.

The knife was then positioned so that a sharper area was in front of the tissue. The block was adjusted to be parallel with the knife and some fine facing was performed to insure a full section.

Next, a small piece of double stick tape (Scotch Double Stick Tape -1/2 inch width, 3M) was placed over the surface of the exposed tissue and very lightly pressed over with a fingertip. The warmth from the fingertip firmly adhered the tape adhesive to the block surface.

The section was then cut by swiftly turning the wheel of the cryostat and the section was guided flat onto the knife blade by a sable brush.

The section on tape, while still in the cryostat chamber, was centered upon a microscope slide with the tissue side up, being careful not to trap air bubbles under the tape.

The slide was then removed from the cryostat and the tape around the section depressed to the slide with a flat-ended forceps. Care was taken not to depress the actual tissue.

The sections were dried for one hour at 37° C and then transferred to an

electronic dessicator for storage until staining.

Fixation

Excessive tape around the section was excised with a trimming blade and the slides were then placed In acetone (anhydrous) at 4° C for 5 minutes, then removed and fan-dried at room temperature for 15 minutes.

Alternatively, slides were fixed in neutral Tris-buffered, 1% paraformaldehyde for 3 minutes.

Both fixative solutions yielded immunoreactivity with the paraformaldhyde-fixed specimen exhibiting superior morphology for hemotoxylin and eosin staining (H&E).

Immunohistochemical Staining

Slides were rinsed or rehydrated in neutral phosphate buffered saline plus EDTA (1 mM EDTA in 10 mM phosphate buffer + 150 mM sodium chloride) and treated with sodium azide/hydrogen peroxide to inhibit endogenous peroxidase.

Non-specific protein binding was blocked using 5% non-immune goat serum diluted in PBS/Tween 20 detergent.

Hamster and rat biotinylated primary antibodies (Pharmingen) directed toward mouse lymphoid cell subtypes (CD3, CD4, CD8, CD45R, and CD11b) were applied for 60 minutes, followed by rinsing and an application of Streptavidin-horseradish peroxidase (Dako) for 30 minutes.

Following a rinse, peroxidase was reacted with 3-amino-9-ethylcarbazole (AEC) and hydrogen peroxide to produce an aqueous-insoluble red precipitate.

Sections were rinsed, counterstained with modified Schmitt's hematoxylin, blued in tap water, and coverslipped using Kaiser's glycerine mountant formulation.

Results/Comments

Sections were found to be acceptable for analyzing the appendages for the antigens mentioned above. The protocol was very time-consuming, but could be accomplished on almost any cryostat by anyone trained in cryotomy of other tissue types. The tungsten carbide knife was essential for performance and was very expensive to purchase and maintain sharpness. The technique worked well for the small mouse appendages, but would most likely not work well for larger specimens with thick bone. The primary advantage of this technique was that whole joint sections could be mounted in the proper orientation and successful immunohistochemical protocols for fixation-sensitive antigens as well as H&E's could be performed. ■

Lundin, D. Henry Ford Hospital, Detroit, MI. Personal communication to Timothy Plummer, October 1995.

Sterchi DL, Eurell JC, Journal of Histotechnology. 1990:13,11 p.207-208