Improved Method for Trypsin Digestion of Paraplast Sections Before Immunofluorescence Staining

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Formalin-fixed, Paraplast-embedded tissue sections mounted on subbed slides were digested with crude trypsin in modified Sorensen phosphate buffer containing sodium fluoride before immunofluorescence staining. This method prevented detachment of sections from slides during processing, thereby permitting immunofluorescence and histological examinations of adjacent serial sections.

Pretreatment of Formalin-fixed, paraffin-embedded tissue sections with trypsin enhances the immunofluorescence observed after antibody staining of viral antigens (1, 2, 3). This technique has great appeal because it extends the usefulness of fluorescent-antibody staining to Formalin-fixed tissues when no fresh or frozen tissue is available, it allows routine histological processing, which preserves morphological detail often obscured in cryostat-cut sections of unfixed frozen tissue, and it permits correlation of the distribution of antigen with histopathology in serial sections.

We were unable initially to use this technique to full advantage because sections frequently and unpredictably detached from the glass slides during the required processing after trypsin treatment. Several modifications were attempted, including the use of synthetic glue (1); this minimized, but did not eliminate, the problem. We developed an experimental protocol which resulted in firm attachment of the sections, permitting more thorough washing and thus reducing nonspecific fluorescence.

Tissue sections were mounted on subbed slides which were prepared by the following method. Gelatin (5 g, laboratory grade, 100 bloom, Fisher Scientific Co., Pittsburgh, Pa.) was dissolved in 800 ml of hot distilled water, and 0.5 g of chromic potassium sulfate was added. The mixture was filtered. Slides were dipped in the solution several times and then drained and dried in a vertical position.

Sections were embedded in Paraplast (Lancer, St. Louis, Mo.), cut at 6 μ m, mounted on subbed slides, and incubated overnight at 56°C. The sections were deparaffinized in two changes of xylene (2 to 3 min each), rehydrated in 100% alcohol (2 to 3 min) and 95 and 80% alcohol (15 s each), and washed in modified Sorensen phosphate

buffer (4). This buffer, also used as the diluent for the trypsin, consisted of 0.1 g of sodium fluoride, 0.4 g of sodium chloride, 13 ml of Sorensen phosphate buffer A (9.08 g of potassium dihydrogen phosphate and distilled water to make 1,000 ml), and 87 ml of Sorensen phosphate buffer B (9.47 g of sodium hydrogen phosphate and distilled water to make 1,000 ml).

Sections were digested at room temperature for 1 h with a solution of 0.1 g of crude trypsin (either Difco Laboratories, Detroit, Mich., or GIBCO Laboratories, Grand Island, N.Y.) in 100 ml of buffer and then washed in three changes of buffer (10 to 20 min each). Tissues were stained for 15 min at 37°C in a humid chamber with specific antisera diluted in buffer as described previously (1, 2, 3), followed by three washes in buffer and one brief rinse in distilled water. Stained sections were mounted in buffered glycerin. Adjacent tissue sections were stained with hematoxylin and eosin.

This procedure allowed us to examine adjacent sections of mouse brain tissue by light and fluorescence microscopy because the tissue sections did not detach from the slides. The immunofluorescence was as bright as and better defined than that seen with stained frozen sections prepared from the same tissue.

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