Method To Improve Handling Characteristics Of Free-floating Brain Sections After Immunohistochemical Staining For Amyloid

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ABSTRACT

Detection of beta amyloid in sections from brains of humans with Alzheimer's disease, mouse models of the disease and aged non-human primates is accomplished with antibodies against different peptide sequences (e.g. Aß1-42, Aß1-40, 6E10, 4G8). Pretreatment of the sections with formic acid is necessary to 'open up' the A molecule to expose the different epitopes. This pretreatment, prior to standard immunochistochemical processing with DAB as the chromagen, on free floating sections often results in sections that have differential shrinkage across brain structures and consequently difficult to mount. For all the brain sections processed at NSA, the otherwise successful 'wrinkle free' mounting solutions (Neurosci Lett. 1979;103-5; acetate buffer and alcohol based) do not result in 'easy to mount' sections and with frequent fragmenting of the sections, especially the larger format sections (human and monkey sections). One large section could take 10' to achieve a flat, wrinkle-free section.

Based on results from other preparations, sections were removed from the 'antigen preserve' collection solution (50%ethylene glycol in phosphate buffered saline), rinsed in Tris-buffered saline (TBS) pH 7.07 and incubated in formaldehyde + TBS (1 part 37% formaldehyde: 9 parts TBS) for 1, 2, and 24 hours. The sections were then rinsed in TBS and mounted from the acetate buffer mounting solution above. The sections incubated for 2hr in the formaldehyde-TBS solution mounted the easiest with the mounting time reduced to 3 minutes and with less fragmentation of the sections. Furthermore, the staining quality seemed crisper and better defined. We have adopted the practice of pretreating sections in the formaldehyde-TBS for other staining protocols with very positive results.

METHODS

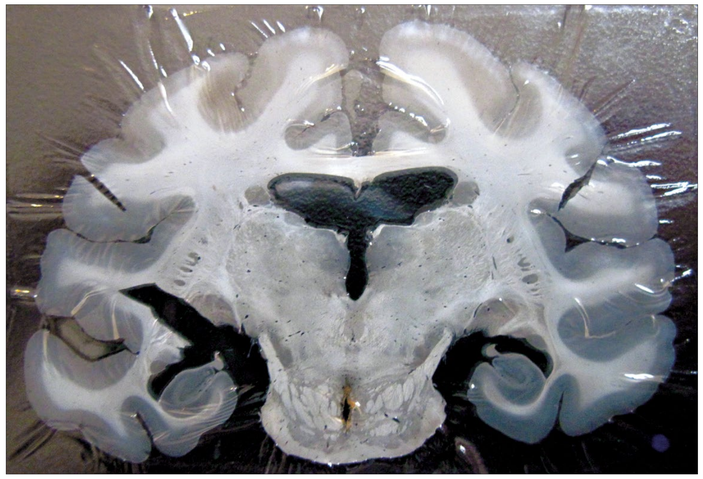
1. Removal of sections into rinses (3x5') with Tris buffer-saline* (TBS)
2. 3.7% Formaldehyde-TBS (1:9 dilution of 37% formaldehyde in TBS)
3. Rinses TBS (3x5')
4. Peroxide-TBS- 30'
5. Rinses TBS (3x5')
6. Formic Acid (undiluted from bottle ~95%)- 15'
7. Rinses TBS (3x5')
8. Proceed to serum blocking step (if used) and remainder of procedure

**TBS normally is used in NSA's antibody protocol instead of PBS*

COMPARISON

Below, images compare the untreated vs. pretreated near adjacent sections from a cynomolgous monkey brain embedded in MultiBrain® gelatin matrix.

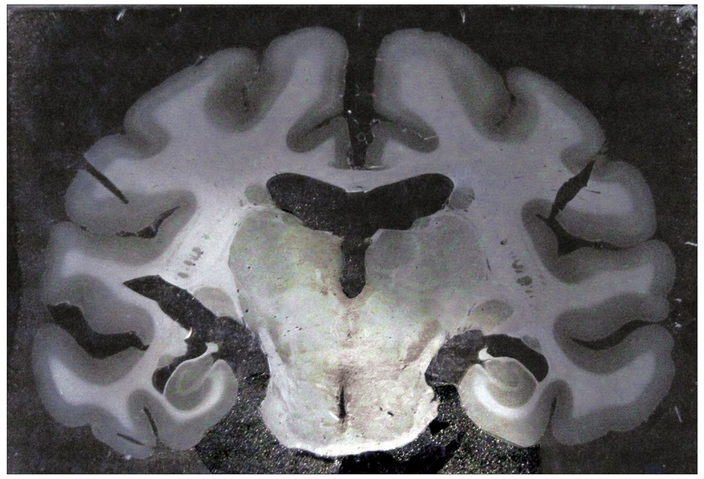
No Pretreatment with Formaldehyde

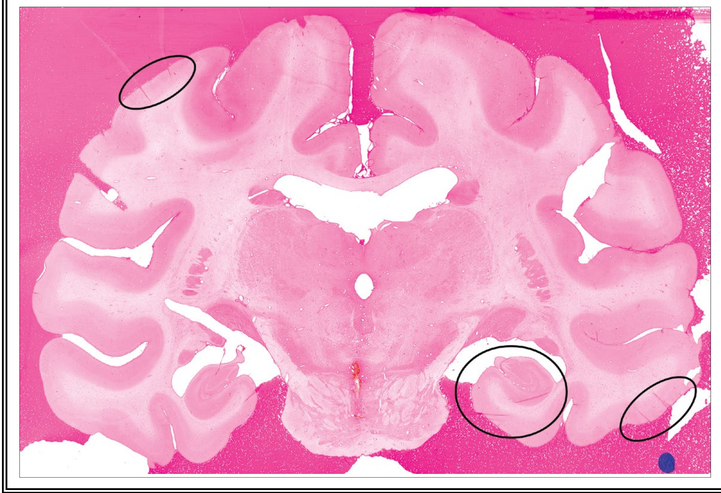


In the left image, wrinkles are the result of differential shrinkage or the expansion between tissue and the gelatin matrix.

In the right image, no wrinkles persist in a section treated with formaldehyde prior to formic acid treatment.

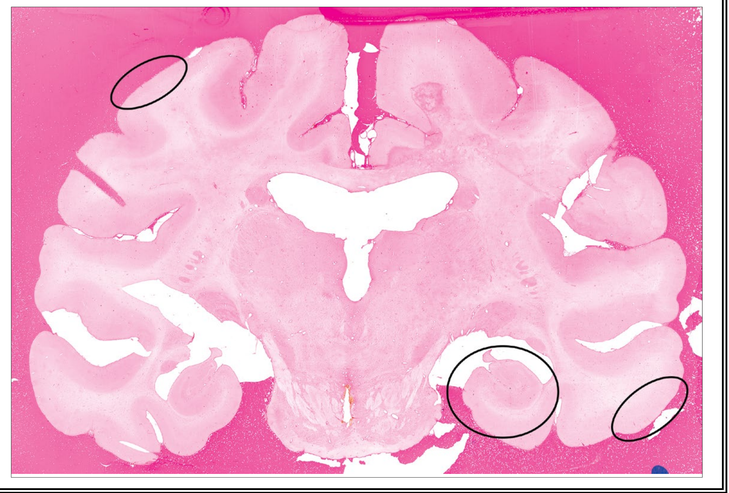
Pretreated with Formaldehyde





Mounted sections stained with eosin reveal relationship of tissue to the gelatin matrix.

Wrinkles shown by circles (left) indicate differential shrinkage/ expansion of tissue with respect to the gelatin matrix wheras the treated tissue (right) displays no shrinkage/expansion.



CONCLUSION

Formaldehyde treatment of free-floating tissue sections, that are pretreated with formic acid in preparation for staining with Aß-related antibodies, results in sections that are easier and faster to mount and yield better quality.