

Microwave Oven - Not Only for Cooking

Pages with reference to book, From 76 To 77

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One of the major technical advances in the field of histopathology during the last decade is the development of immuno-histochemical (IHC) techniques^{1,2}. Growing demand of antibodies that can be used for IHC on routine formalin fixed paraffin embedded tissues has stimulated efforts to develop antibodies that can recognize formalin-resistant epitopes. Alternatively, it is desirable to devise strategies to unmask cross-linked antigenic sites. One popular approach is to treat the sections with a protease such as trypsin or pronase before immunostaining. It is thought that the protease treatment breaks the cross-linking bonds of the fixative with the protein to reveal the antigenic sites. In our experience, this is helpful with only a handful of antibodies like cytokeratins. Our repeated full-hearted attempts to retrieve estrogen receptor antigenic sites with this method failed³. Clearly this means that the capability of retrieval of masked epitopes could not only significantly expand the range of antibodies useful in immunohistochemistry, but also reduce the incidence of false negative staining in over-fixed tissues, which is a frequent happening in our setting. With these goals and problems in mind, repeatedly failing with trypsin digestion technique and inspired with some other studies^{4,5}, the effects of microwave heating were tried for retrieval of estrogen receptor antigenic sites on fixed material. We found a dramatic enhancing effect of this treatment for unmasking estrogen receptor antigen and now routinely using it. In our setting as most of the breast tumours which are received for estrogen receptor determination are already formalin fixed. With conventional techniques, chances of false negativity are very high and in our opinion, this can only be circumvented by microwave digestion. For this purpose, we place de-paraffinized tissue sections in a plastic jar filled with 10 mM citrate buffer at pH 6.0. Then these sections are incubated in a microwave oven (buffer has to boil) six times for 5 minutes each, giving an interval in between. Domestic microwave ovens can be used for these applications with some reservations. For instance, heat distribution may not be uniform. The H2200 staining microwave processor (Energy Beam Sciences, P.O. Box 468, Agawam, MA 01001, USA) is specifically designed for histopathology and provides accurate time and temperature control⁶. For quality control purpose with reference to estrogen receptor determination, we choose tumour blocks which also contain normal breast lobules and are hesitant of calling any tumour negative if these breast lobules (built in control) are negative. Although the mechanism concerning microwave oven recovery of antigens is not clear, it is possible that the cross-linking of proteins caused by formaldehyde may be altered by microwave heating. We believe that a microwave oven in a laboratory means improved patient care.

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References

1. Pervez, S., Ahmed, R., Aijaz, F. et al. Application of immunocytochemistry in the differential diagnosis of undifferentiated tumours and lymphoproliferative disorders. *PJR* 1993;4:87-93.
2. Pervez, S. Immunohistochemistry in tumour diagnosis - Who actually needs it? *J. Pak. Med. Assoc.* 1994;44:199.
3. Pervez, S., Shaikh, H., Aijaz, F. et al. Immunohistochemical estrogen receptor determination in human breast carcinoma: Correlation with histologic differentiation and age of the patients. *J. Pak. Med.*

Assoc., 1994;44: 133-36.

4. Shi, SR., Key, M.E. and Kalra. K.L. Antigen retrieval fixed paraffin-embedded tissues: An enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J. Histochem. Cytochem.*, 1991 ;39:741-48.

5. Cattoretti, G., Pileri, S., Parravicini, C. et al. Antigen unmasking on formalin. fixed, paraffin embedded tissue section. *J. Pathol.*, 1993; 171:83-98.

6. Fox, C.H., Johnson, F.B., Whiting, J. et al. Formaldehy de fixation. *J. Histochem. Cytochem.*. 1985;33 :845-53.