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Rapid Communication

Antigen Retrieval in Formalin-fixed, Paraffin-embedded Tissues: An Enhancement Method for Immunohistochemical Staining Based on Microwave Oven Heating of Tissue Sections

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We describe a new approach for retrieval of antigens from formalin-fixed, paraffin-embedded tissues and their subsequent staining by immunohistochemical techniques. This method of antigen retrieval is based on microwave heating of tissue sections attached to microscope slides to temperatures up to 100°C in the presence of metal solutions. Among 52 monoclonal and polyclonal antibodies tested by this method, 39 antibodies demonstrated a significant increase in immunostaining, nine antibodies showed no change, and four antibodies showed reduced immunostaining. In particular, excellent immunostaining results were obtained with a monoclonal antibody to vimentin as well as several different keratin antibodies on routine formalin-fixed tissue sections after pre-treatment of the slides with this method. These results showed that after antigen retrieval: (a) enzyme predigestion of tissues could be omitted; (b) incubation times of primary antibodies could be significantly reduced, or dilutions of primary antibodies could be increased; (c) adequate staining could be achieved in long-term formalin-fixed tissues that failed to stain by conventional methods; and (d) certain antibodies which were typically unreactive with formalin-fixed tissues gave excellent staining. (*J Histochem Cytochem 39:741-748, 1991*)

KEY WORDS: Immunohistochemistry; Antigen retrieval; Formalin-fixed tissue; Paraffin sections; Microwave.

Introduction

Growing interest in immunohistochemical staining procedures has led to the development of a wide range of highly specific immunostains which are of value to the surgical pathologist in diagnostic and investigative studies (1,2). Although formalin remains the most popular fixative used in pathology, it is clear that this fixative is not always the best choice for preserving antigenicity of tissues to be used in immunohistochemical procedures. Despite many studies on the intermolecular cross-links formed between formalin and proteins (3,4), the molecular mechanism underlying tissue fixation is not well understood (5).

The demand for a broader selection of antibodies that can be used for immunohistochemical staining on routine formalin-fixed, paraffin-embedded tissues has stimulated efforts to develop antibodies that can recognize formalin-resistant epitopes. Although this strategy has been effective in developing many useful antibodies, it has not been entirely satisfactory in resolving all problems. A persistent concern in immunopathology is choosing the correct fixative and duration of fixation that will provide maximal preservation of tissue morphology with minimal loss of antigenicity.

One approach to resolve this dilemma was the introduction of protease digestion of formalin-fixed sections to unmask antigenic sites hidden by cross-linked proteins (6,7). However, Leong et al. (8) showed that, aside from cytokeratins and desmin, digestion with trypsin did not substantially improve immunostaining of the other antigens studied. At present it is not clear whether or not the formalin-induced cross-linking of proteins is a reversible chemical reaction. However, a recent study concerning formalin sensitivity of a GFAP epitope supported the hypothesis that the sensitivity of some epitopes was not due to the direct effect of the aldehyde but rather was due to the binding of other molecular structures to the epitope (9).

Clearly, the capability of retrieval of masked epitopes could significantly expand the range of antibodies useful in immunohistochemistry as well as reduce the incidence of false-negative staining in over-fixed tissues. In addition, antigen retrieval could provide greater diagnostic accuracy by improving immunohistochemical procedures. With these goals in mind, we studied the effects of microwave oven heating of tissue sections in the presence of metal solutions. We found a dramatic enhancing effect of this treatment on the recovery of many antigens, which is particularly intriguing

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in view of the presumed deleterious effects of high temperatures on protein antigens.

Materials and Methods

Tissues and Paraffin Blocks. Fresh tissues were obtained from the Cooperative Human Tissue Network (CHTN; Columbus, OH), and were fixed immediately in either 10% neutral buffered formalin or 90% ethanol. Fixed tissues were dehydrated in ethanol, cleared in xylene, and embedded in paraffin blocks. Some tissues which had been fixed in formalin from 24 hr to up to 2 years were obtained from the Department of Pathology, National Medical Center of the City of Hope (Duarte, CA) and Hartford Hospital (Hartford, CT). Five-μm sections were cut and mounted on poly-L-lysine (Sigma; St Louis, MO)-coated slides. The use of a tissue adhesive was important, as without an adhesive tissues tended to detach from the slides during the antigen retrieval process.

Microwave Oven. A Toshiba model ER-855BT operating at a frequency of 2.45 GHz with nine power level settings was used at the highest power setting (720 w). Three Coplin jars were filled with either de-ionized water, 1% zinc sulfate, or saturated lead thiocyanate, and placed in the center of the microwave oven. Because the number of Coplin jars placed in the microwave oven might influence the temperature, three jars were always used, and they were always placed in the same positions. When heated at the indicated settings, the boiling point (100 ± 5°C) for these aqueous solutions was reached in 140–145 sec.

Protocol for Antigen Retrieval. Although different antigens may behave differently under similar conditions of treatment, the following protocol was found to be acceptable for most antigens tested in this study. The steps for antigen retrieval were as follows.

1. Tissue sections were deparaffinized and rehydrated to water.
2. Endogenous peroxidase was blocked with 3% H_2O_2 for 5 min.
3. Slides were washed with distilled water for 5 min.
4. Slides were then placed in plastic Coplin jars containing either distilled water, a metal solution of saturated lead thiocyanate, or 1% zinc sulfate.
5. Jars were covered with loose-fitting screw caps and heated in the microwave oven for either 5 or 10 min. Sometimes a 10-min heating time was divided into two 5-min cycles with an interval of 1 min between cycles to check on the fluid level in the jars.
6. After heating, the Coplin jars were removed from the oven and allowed to cool for 15 min.
7. Slides were then rinsed in distilled water twice and in PBS for 5 min.
8. Treated slides were immunostained as described below.

For comparison, a conventional oven was also used to heat the slides. The solutions were heated to the specified temperature. The slides were placed in the pre-heated solutions for 5–10 min and then treated as previously described for the microwave procedure.

Immunohistochemistry. All polyclonal and monoclonal antibodies listed in Table 1 were obtained from BioGenex Laboratories (San Ramon, CA). Unless otherwise specified, the detection system for the immunohistochemical staining was the Super Sensitive system, also from BioGenex. In some studies a MultiLink detection system (BioGenex) was also evaluated. Both horseradish peroxidase with AEC chromogen and alkaline phosphatase with Fast Red chromogen were used.

Immunohistochemical staining was performed according to the manufacturer's instructions. Briefly, all incubations were performed at room temperature as follows: (a) primary antibodies were incubated from 30 min to 24 hr according to the manufacturer's instructions; (b) link antibody was incubated for 20 min; (c) streptavidin-conjugated enzyme was incubated for 20 min; (d) peroxidase substrate was incubated for 5 min, or alkaline phosphatase substrate was incubated for 20 min. After immunostaining, slides were counterstained with hematoxylin and coverslipped with an aqueous mounting medium.

Non-immune rabbit serum or nonspecific mouse ascites was used as negative control for rabbit and mouse primary antibodies, respectively. Contribution of nonspecific staining of primary antibody was evaluated by substitution of the primary antibody with the negative controls or with PBS.

Enzyme Digestion. In some cases, deparaffinized tissues were pre-treated by protease digestion before application of the primary antibody. Tissue sections were incubated with 0.1% trypsin (Sigma) in PBS for 30 min at 37°C. After enzyme digestion, slides were rinsed in PBS and immunostained as previously described.

Table 1. *Immunostaining of formalin-fixed, paraffin-embedded tissue after antigen retrieval*[a]

Improved staining	No change	Decreased staining
Pan-cytokeratin (F12-19)	Tubulin (P)	Ferritin (P)
Cytokeratin (AE1)	Desmin (P)	Ferritin (M3.170)
Cytokeratin (AE3)	Desmin (33)	C3 (P)
Cytokeratin (AE8)	Myoglobin (P)	Gastrin (P)
Cytokeratin 7 (CK7)	Myoglobin (MG-1)	
Cytokeratin 8,18,19 (5D3)	β-endorphin (P)	
IgD (IADB6)	α-1-anti-trypsin (P)	
GFAP (P)	Transferrin	
GFAP (GA-5)	(HT1/13.6.3)	
NF (2F11)	Calcitonin (P)	
CEA (P)		
CEA (SP-651)		
VIP (P)		
Serotonin (P)		
Estrogen receptor-related protein (D5)		
C-erb-B2 (CB11)		
CMV (P)		
Albumin (P)		
Macrophage (LN5)		
Blood group A (81 FR2.2)		
Blood group B (81/11)		
Cathepsin B (P)		
Vimentin (V9)		
NSE (P)		
NSE (MIG-N3)		
Chromogranin (LK3H10)		
ACTH (R)		
α-hCG (02-310-94)		
PSA (8)		
Thyroglobulin (P)		
Factor VIII (P)		
Myeloid, CD15 (Tü9)		
T-cell (MT1)		
T-cell (MT2)		
B-cell (MB1)		
B-cell (MB2)		
Kappa chain (KP-53)		
Lambda chain (HP6054)		
AFP (A-013-01)		
Total 39	9	4

[a] P, polyclonal antibodies; others are monoclonal.

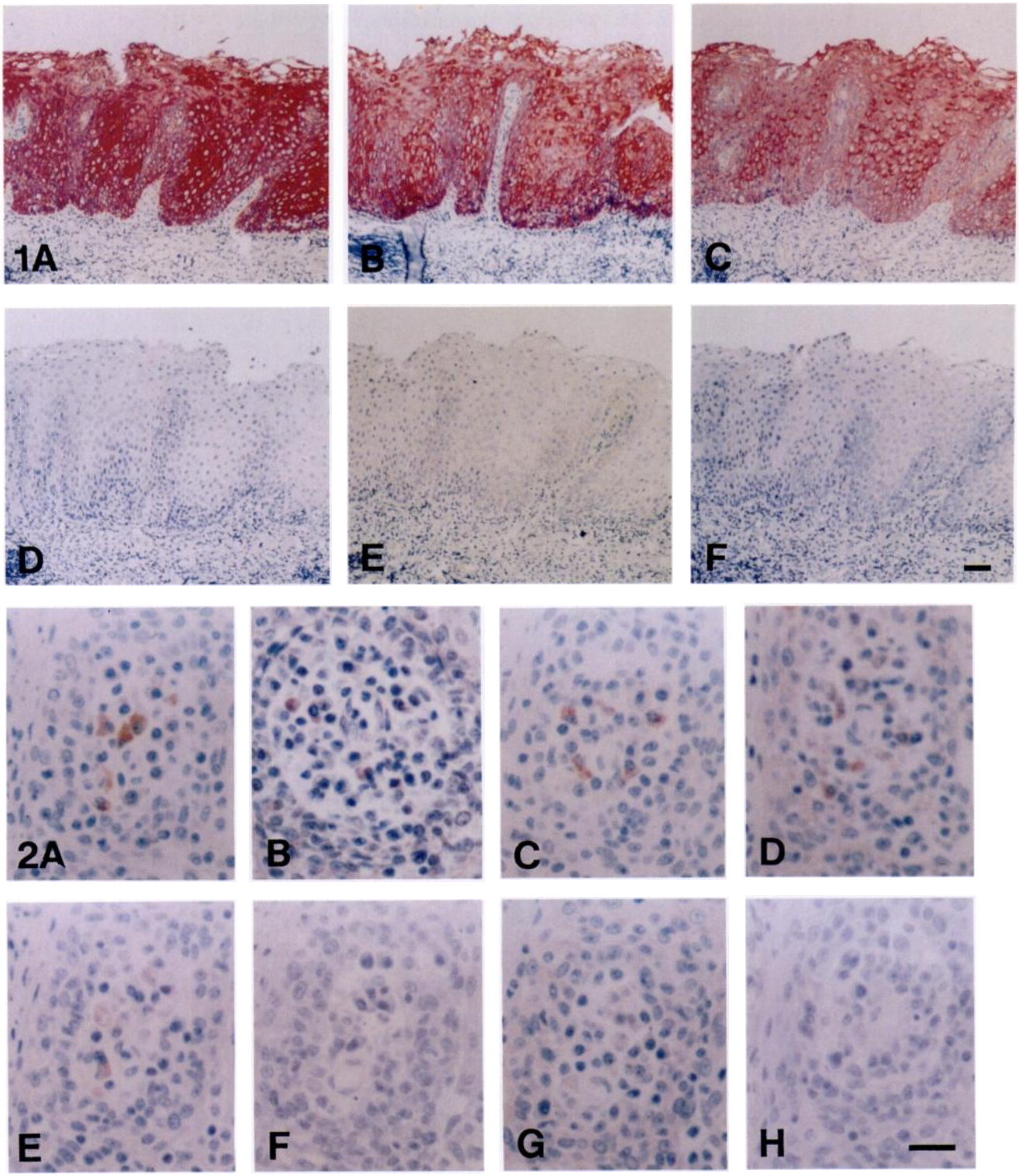

Figure 1. Sensitivity of antigen retrieval method. Monoclonal antibody to pan-cytokeratin was tested on formalin-fixed, paraffin-embedded sections of human tonsil. (**A**) Microwave (MW) + lead thiocyanate; (**B**) MW + zinc sulfate; (**C**) MW + water; (**D**) no treatment: (**E**) negative control (non-immune ascites) following MW + lead thiocyanate; (**F**) trypsin pre-digestion before immunostaining. Note strong staining of epithelium in **A** and **B**, moderate staining in **C**, and negative staining in **D–F**. Bar = 50 μm.

Figure 2. Comparison of microwave (MW) and conventional heating (CH) protocols for antigen retrieval. Monoclonal antibody to IgD was tested on formalin-fixed paraffin sections of human tonsil. (**A**) MW + lead thiocyanate; (**B**) MW + zinc sulfate; (**C**) MW + water; (**D**) CH + lead thiocyanate; (**E**) CH + water; (**F**) no treatment; (**G**) negative control (non-immune ascites) with MW + lead thiocyanate; (**H**) trypsin pre-digestion of tissues before immunostaining. Note strong positive staining of cells in **A**, moderate staining in **B–D**, weak staining in **E**, and no staining in **F–H**. Bar = 20 μm.

Table 2. *Comparison of conventional heat with microwave heat for antigen retrieval in formalin-fixed tissues*

Antibody[a]	Number of tissues tested	Conventional oven				Microwave oven (Boiling)	
		80°C		Boiling			
		H_2O	Lead	H_2O	Lead	H_2O	Lead
CK 8, 18, 19	2	-[b]	+	+ +	+ + +	+ + +	+ + + + +
IgD	2	+	+ +	+ +	+ + +	+ + + +	+ + + + +
Pan-CK	39	+	+ +	+ + +	+ + + +	+ + + +	+ + + + +
Vimentin	39	+	+ +	+ + +	+ + + +	+ + + +	+ + + + +

[a] CK, cytokeratin.
[b] Immunoreactivity was scored on a scale of - to + + + + +. The reactivity score was an average value over all the tissues tested.

Results

Immunohistochemical Staining Using Microwave Heat

Immunostaining results using 52 different primary antibodies on tissues treated for antigen retrieval are summarized in Table 1. Most of the antibodies tested showed increased intensity of immunostaining after microwave oven heating in the presence of either distilled water or metal solutions. In general, the intensity of immunostaining was stronger with the metal solutions, particularly using the lead solution (Figure 1). In some cases, such as with monoclonal antibody to IgD, the use of zinc sulfate solution caused strong background staining of tonsil epithelium and some false-positive staining of lymphocyte nuclei. This type of false-positive staining was not observed with the lead solution (Figure 2).

Best results were obtained when slides were heated in the microwave oven using the intermittent heating method of two 5-min cycles with an interval of 1 min between the heating cycles. Another advantage of this method was that additional solution could be added to the jars if necessary.

Immunohistochemical Staining Using Conventional Heat

Heating slides in distilled water or metal solutions by conventional heat in an oven also resulted in some increased immunostaining; however, there were noticeable differences compared with microwave heating (Table 2).

For tissues fixed in formalin for 24 hr or longer, heating the slides by microwave oven was clearly superior to heating the slides with conventional heat. Similarly, the use of lead solution led to better results than zinc solution, which in turn was better than water (Figure 2).

Sensitivity of the Antigen Retrieval Method

To demonstrate the increased sensitivity achievable with this method, selected antibodies were tested at titers that failed to produce positive stains when tested by a conventional immunostaining procedure. Furthermore, immunoreactivity could not be demonstrated with these antibodies, even with the use of trypsin pre-digestion of tissues. When these antibodies were then tested on the same tissues after antigen retrieval, strong immunostaining was observed (Figures 1, 2, and 3; Table 3).

Specificity of the Antigen Retrieval Method

The specificity of the antigen retrieval method was tested by immunostaining tissues known either to contain or to lack certain antigens. For these studies, tissues were immunostained with monoclonal antibodies to cytokeratin 7 or estrogen receptor-related protein p29. Both of these antibodies detected formalin-sensitive but ethanol-resistant epitopes. Tissues were first categorized as being antigen positive or antigen negative by immunostaining frozen sections of each tissue fixed in ethanol. The remainder of the tissues were then fixed in formalin and embedded in paraffin. When paraffin-embedded tissues known to contain antigen were tested with antibody to cytokeratin 7, no staining occurred in any formalin-fixed tissue regardless of the length of fixation time. Similarly, with antibody to p29 no staining occurred in antigen-positive tissues that had been fixed in formalin for 48 hr or longer. Although neither antibody detected antigen in formalin-fixed tissues before antigen retrieval, after retrieval both gave strong staining of their respective antigens (Figure 4). Furthermore, when formalin-fixed, paraffin-embedded tissues that were negative for these antigens were immunostained for cytokeratin 7 or p29, no staining occurred either with or without antigen retrieval.

Antigen Retrieval in Long-term Formalin-fixed Tissues

Thirty-nine different tissues which had been fixed in formalin for periods of time ranging from 2–4 weeks and one tissue which had been stored in formalin for 2 years were tested for immunoreactivity to vimentin and pan-cytokeratin (Figure 3). As shown in Table 4, without treatment only a minority of the tissues were stained, and the staining that did occur was usually weak. However, after antigen retrieval with lead solution, immunoreactivity for these two antibodies was significantly enhanced, suggesting that retrieval of antigen in long-term formalin-fixed tissues was possible.

Effect of Formalin Fixation on Formalin-sensitive Antigens

A single sample of malignant melanoma was divided into several

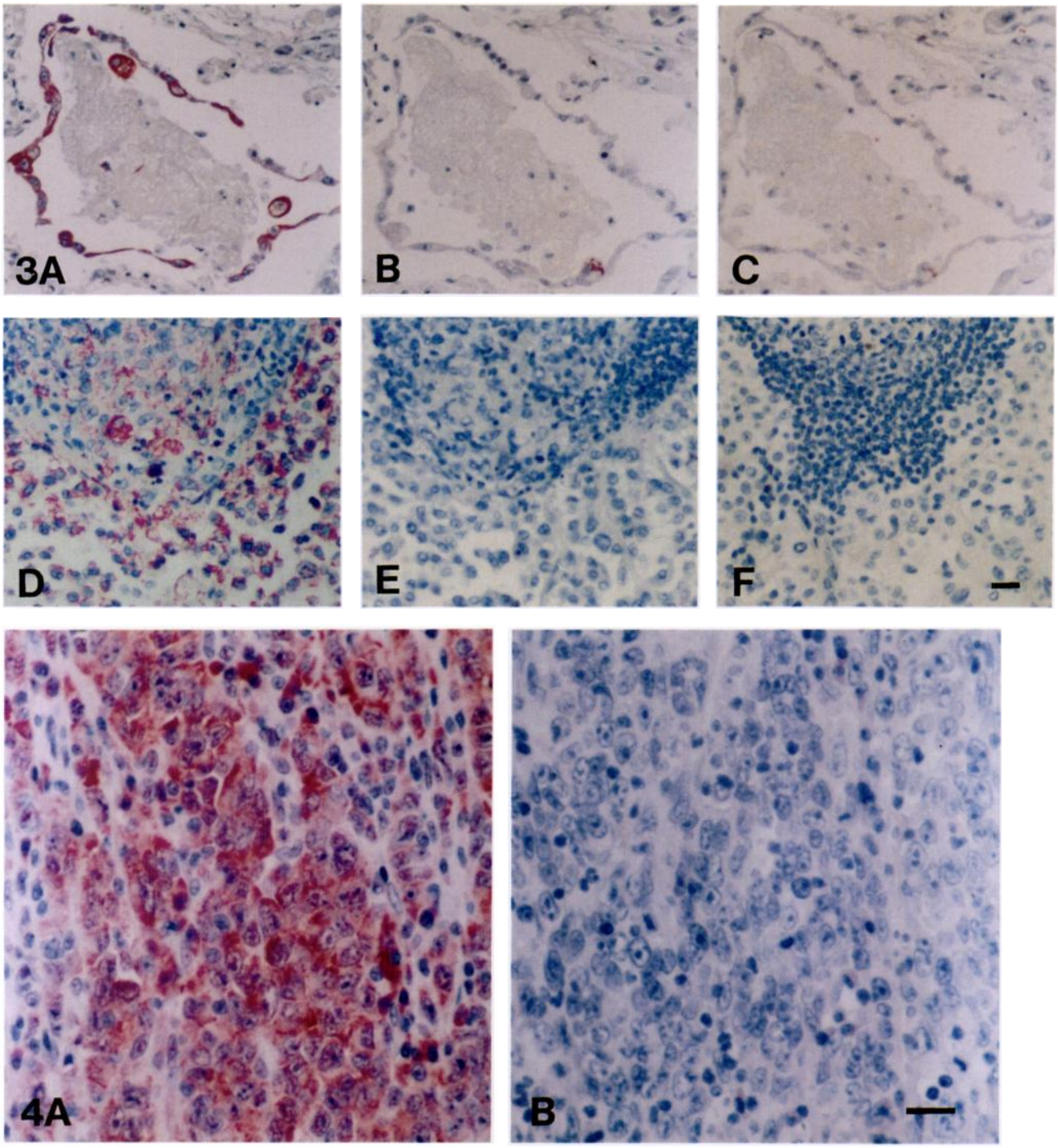

Figure 3. Antigen retrieval of formalin-sensitive epitopes. (**A–C**) Two-year formalin-fixed lung tissue was immunostained for cytokeratin 8, 18, 19, and (**D–F**) metastatic melanoma in lymph node fixed in formalin overnight at 37°C was immunostained for vimentin. (**A,D**) Microwave (MW) + lead thiocyanate; (**B,E**) no treatment; (**C,F**) negative control (non-immune ascites) after MW + lead thiocyanate. Note strong positive staining in **A** and **D** and negative staining in panels **B**, **C**, **E**, and **F**. Bar = 25 μm.

Figure 4. Formalin-fixed tissue of poorly differentiated adenocarcinoma. (**A**) Tumor stains positively for cytokeratin 7 after antigen retrieval with microwave + lead thiocyanate, but exhibits no staining for cytokeratin 7 without treatment (**B**). Bar = 20 μm.

Table 3. *Comparison of immunostaining results using diluted primary antibodies*

Antibody dilution	Tissue	Non-treatment	Trypsin digestion	Microwave oven[a] DW	Zinc	Lead	NC[b]
Pan-CK[c]	Tonsil	-	-	+ +	+ + +	+ + +	-
CK AE1	Tonsil	-	-	+	+ +	+ + +	-
CK AE3	Tonsil	-	-	+ +	+ + +	+ + +	-
CK AE8	Tonsil	-	-	+	+ +	+ + +	-
CK 7	Adeno-carcinoma[d]	-	+ / -	+ +	+ +	+ + +	-
IgD	Tonsil	-	-	+ +	+ +	+ + +	-

[a] Microwave oven with: DW, distilled water; Zn, zinc sulfate solution; lead, lead thiocyanate.
[b] NC: negative control or PBS was used to replace primary antibody on the slide treated by microwave oven with metal solution or distilled water.
[c] CK, cytokeratin.
[d] Adenocarcinoma, breast adenocarcinoma.

pieces and each piece was fixed in formalin for 22 hr at a temperature of 4°C, 25°C, or 37°C. Because the process of formalin fixation is temperature dependent, higher temperatures produce more rapid fixation (5). After paraffin embedding these tissues were subjected to immunostaining for vimentin. Because the epitope recognized by this vimentin antibody (clone V9) is partially formalin sensitive (10), this system was used to investigate whether antigen retrieval could be used to reverse the deleterious effects of fixation in formalin. As shown in Table 5 and Figure 3, the observed decrease in vimentin immunoreactivity was directly related to an increase in temperature of the formalin fixative. However, after antigen retrieval in the presence of lead solution, vimentin immunostaining was completely restored to a level even surpassing that observed in tumor fixed at 4°C without subsequent treatment (Table 5). These results suggest that, at least for some epitopes, the deleterious effects of formalin fixation are reversible.

Controls

For most antibodies diluted to their optimal titer, the signal-to-noise ratio was usually much better with tissues treated for antigen retrieval compared with untreated tissues, as the background was usually lower after antigen retrieval. However, in some tissues that were already prone to high background (staining in the absence of primary antibody), treatment by antigen retrieval further enhanced background staining. This type of background was associated with the direct binding of the secondary biotinylated antibody to the tissue, and could usually be eliminated by appropriate dilution of the secondary antibody.

Table 4. *Immunoreactivity of long-term formalin-fixed tissues*[a]

Antibody	No treatment Antibody	NC[b]	Microwave + lead Antibody	NC
Vimentin	6/40[c]	0/40	40/40	0/40
Pan-CK[d]	5/40	0/40	26/40[e]	0/40

[a] Staining method was by the MultiLink alkaline phosphatase system.
[b] NC, non-immune ascites negative control.
[c] Represents the number of tissues staining positive (+ to + + +) over the total number of tissues tested.
[d] CK, cytokeratin.
[e] Of the 40 tissues tested, only 26 contained epithelial cells that would be positive for keratin staining.

Table 5. *Effect of fixation temperature on vimentin immunoreactivity of malignant melanoma*

Fixation temperature[a]	No treatment Vimentin	NC[b]	Microwave + lead Vimentin	NC[b]
4°C	+ +[c]	-	+ + +	-
25°C	+	-	+ + +	-
37°C	-	-	+ + +	-

[a] Fixation in 10% neutral buffered formalin for 22 hr.
[b] NC, non-immune ascites negative control.
[c] Immunoreactivity scored on a scale of - to + + + .

Alcohol Fixation

When antigen retrieval was performed on sections of tissues fixed by alcohol there was no enhancement of immunoreactivity, whereas all sections fixed in 10% formalin, irrespective of the length of fixation time, showed increased immunoreactivity.

Discussion

The microwave oven has been used for tissue fixation (8,11,12) and for rapid histochemical and immunohistochemical staining (13–20). One recent report has also observed enhanced immunohistochemical staining after microwave drying of slides (21). However, in these cases only short periods of irradiation and low temperatures were used. To the best of our knowledge there have been no published reports concerning antigen retrieval by the use of the microwave oven and metal solutions. Similarly, no studies have indicated that the immunohistochemical staining intensity could be increased by heating slides to high temperatures. From this point of view, the excellent immunostaining results demonstrated in this study could not have been predicted on the basis of current knowledge.

Although the mechanism concerning microwave oven recovery of antigens is not clear, since this treatment did not affect alcohol-fixed paraffin sections it is possible that the cross-linking of proteins caused by formaldehyde may be altered by microwave heating.

The use of heavy metal salts in combination with formalin for tissue fixation has recently been introduced (22,23). Some studies have demonstrated the superiority of zinc–formalin as a fixative for antigen preservation (23). Furthermore, when routine formalin-fixed tissues were re-fixed in zinc–formalin, immunoreactivity was improved (24). The metal solutions used in the present study were formulated based on the hypothesis that heavy metal salts act as protein precipitants, forming insoluble complexes with polypeptides (22), and that protein-precipitating fixatives frequently display better preservation of antigens than do cross-linking aldehyde fixatives (23).

In the present study we found that the use of metal solutions in combination with microwave oven heating, and to a lesser ex-

tent conventional heating, could substantially improve the immunoreactivity above that achievable with no treatment or with treatment consisting of distilled water and heat. This was particularly evident in tissues that had been fixed in formalin for greater than 24 hr. Our preliminary study has demonstrated that lead solution is better than zinc solution during microwave oven treatment, since the lead solution demonstrated stronger immunoreactivity with less background.

When compared with protease digestion, the influence of microwave pre-treatment was clearly superior, particularly with antigens, such as vimentin, which are typically not enhanced by proteolysis. Battifora and Kopinski (7) have reported that the optimal length of time for proteolytic digestion increased in direct proportion to the length of time that tissues were fixed in formalin. In the present study we found that tissues fixed in formalin for periods ranging from 2 weeks up to 2 years could still be immunostained for pan-cytokeratin and vimentin after antigen retrieval, even though enzyme pre-digestion failed to restore immunoreactivity. These results suggest that other tissues that were previously considered unsuitable for immunohistochemical analysis can potentially be salvaged and successfully evaluated with a broad range of antibodies.

Although the methods described in this report are relatively simple and straightforward, a few precautions should be noted. First, our data suggest that background staining may be increased by this method in certain tissues already prone to high backgrounds. Therefore, it is important when evaluating this method to carefully compare positive staining to an appropriate negative control. Second, the use of certain metal salts, such as lead, poses a potential health risk which should be minimized by appropriate measures. In our laboratory we utilize the microwave oven within a chemical fume hood to reduce environmental exposure to lead aerosols.

Immunohistochemical staining methods represent a relatively new diagnostic tool which can contribute to our understanding of human pathology. The continued refinement of these tools has significantly expanded the capabilities of the surgical pathologist in diagnostic procedures. Despite these important advances, there is no standard method of fixation that can be applied to tissues submitted for immunohistochemical analysis. Indeed, in most cases the requirements for tissue fixation are dictated by other considerations, not the least of which is personal preference. Although standardization of immunohistochemical methods has been frequently advocated (25), it is unlikely that such standardization will gain widespread acceptance, at least in the near future. Furthermore, even if standardization of fixation were achieved, the need to apply immunohistochemistry to archival material would still persist. Consequently, new techniques that can compensate for certain deficiencies relating to tissue processing are needed. If techniques such as antigen retrieval could be used to visualize antigens that were otherwise undetectable, the range of useful immunohistochemical methods would be greatly expanded. Furthermore, a simplified method for antigen retrieval could reduce the incidence of false-negative immunostaining results. In clinical applications this may translate into increased diagnostic accuracy and improved patient care.

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Literature Cited

1. Taylor CR, Kledzik G. Immunohistologic techniques in surgical pathology—a spectrum of "new" special stains. Hum Pathol 1981;12:590
2. DeLellis RA. Diagnostic immunohistochemistry. New York, Paris, Barcelona, Milan, Mexico City, Rio de Janeiro: Masson Publishing USA, 1981
3. Kelly DP, Dewar MK, Johns RB, Shao WL, Yates JF. Cross-linking of amino acids by formaldehyde. Preparation and 13C NMR spectra of model compounds. In Friedman M, ed. Protein crosslinking. Symposium on protein crosslinking, San Francisco, 1976. New York: Plenum Press, 1977:641
4. Harlan JW, Feairheller SH. Chemistry of the crosslinking of collagen during tanning. In Friedman M, ed. Protein crosslinking. Symposium on protein crosslinking, San Francisco, 1976. New York: Plenum Press, 1977:425
5. Fox CH, Johnson FB, Whiting J, Roller PP. Formaldehyde fixation. J Histochem Cytochem 1985;33:845
6. Huang SN, Minassian H, More JD. Application of immunofluorescent staining on paraffin sections improved by trypsin digestion. Lab Invest 1976;35:383
7. Battifora H, Kopinski M. The influence of protease digestion and duration of fixation on the immunostaining of keratins. J Histochem Cytochem 1986;34:1095
8. Leong ASY, Milios J, Duncis CG. Antigen preservation in microwave-irradiated tissues: a comparison with formaldehyde fixation. J Pathol 1988;156:275
9. Bell PB Jr, Rundquist I, Svensson I, Collins VP. Formaldehyde sensitivity of a GFAP epitope, removed by extraction of the cytoskeleton with high salt. J Histochem Cytochem 1987;35:1375
10. Azumi N, Battifora H. The distribution of vimentin and keratin in epithelial and nonepithelial cells. Am J Clin Pathol 1987;88:286
11. Mayers CP. Histological fixation by microwave heating. J Clin Pathol 1970;23:273
12. Hopwood D, Coghill G, Ramsay J, Milne G, Kerr M. Microwave fixation: its potential for routine techniques, histochemistry, immunocytochemistry and electron microscopy. Histochem J 1984;16:1171
13. Login GR, Dvorak AM. Microwave energy fixation for electron microscopy. Am J Pathol 1985;120:230
14. Brinn NT. Rapid metallic histological staining using the microwave oven. J Histotechnol 1983;6:125
15. Estrada JC, Brinn NT, Bossen EH. A rapid method of staining ultrathin sections for surgical pathology TEM with the use of the microwave oven. Am J Clin Pathol 1985;83:639
16. Hafiz S, Spencer RC, Lee M, Gooch H, Duerden BI. Use of microwaves for acid and alcohol fast staining. J Clin Pathol 1985;38:1073
17. Brinn N, Terrell W. Selected enzyme histochemical techniques facilitated by the microwave oven. J Histotechnol 1986;9:231
18. Valle S. Special stains in the microwave oven. J Histotechnol 1986;9:237
19. Chiu KY. Use of microwaves for rapid immunoperoxidase staining of paraffin sections. Med Lab Sci 1987;44:3
20. Van de Kant HJG, Boon ME, de Rooij DG. Microwave-aided technique to detect bromodeoxyuridine in S-phase cells using immunogold-silver staining and plastic-embedded sections. Histochem J 1988;20:335
21. Sharma HM, Kauffman EM, McGaughy VR. Improved immunoperoxidase staining using microwave slide drying. Lab Med 1990;21:658

22. Jones MD, Banks PM, Caron BL. Transition metal salts as adjuncts to formalin for tissue fixation. Lab Invest 1981;44:32A

23. Herman GE, Chlipapa E, Bochenski G, Sabin L, Elfont E. Zinc formalin fixative for automated tissue processing. J Histotechnol 1988;11:85

24. Abbondanzo SL, Allred DC, Lampkin S, Banks PM. Enhancement of immunoreactivity in paraffin embedded tissues by refixation in zinc sulfate-formalin. Proc Annu Meeting US and Canadian Acad Pathol, Boston: March 4–9, 1990

25. Elias JM, Gown AM, Nakamura RM, Wibur DC, Herman GE, Jaffe ES, Battifora H, Brigati DJ. Special report: quality control in immunohistochemistry. Am J Clin Pathol 1989;92:836