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The Use of New Microwave Techniques to Facilitate the Immunostaining of Paraffin Sections on Glass Slides

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Microwave-assisted processing is gaining acceptance as a routine laboratory technique due to improved technology and better standardization of processing methods.[1] This evolution is primarily due to better control of the microwave environment during processing.[2] Recent development of the ColdSpot™ (Patent US6329645) has normalized the microwave environment and eliminated the necessity to employ a microwave calibration scheme as part of a protocol. A second component of the evolution has been the incorporation of continuous power control, from 50 to 750W, as an integral part of microwave technology (PELCO BioWave™, Ted Pella, Inc.).

The combination of these elements has made sample processing a simple process for electron microscopy. The efficacy of this combination as a viable tandem for immunocytochemistry was elegantly demonstrated by the work of Sanders and Gartner detailing the in vivo labeling of *Drosophila* embryos and *Allium* sp. root tips with nuclear vital stains.[3]

Previous reports using microwave methods to accelerate immunostaining applications for light or electron microscopy have reported variable results attributable to either differences between antibodies, length of microwave exposure or an incorrect temperature maximum.[4,5] To address the problem of the inconsistancies described by others a protocol for paraffin sections on glass slides was developed. The foundations were based on low power (250W-PELCO BioWave™), the ColdSpot™ and a slide rack with coverplates from ThermoShandon (Pittsburgh, PA). Control slides from DAKO® (Carpinteria, CA) were stained with IMMUNON™ pre-diluted primary antibodies (ThermoShandon, Pittsburgh, PA). An IMMUNON™ Universal Streptavidin/Biotin Immunoperoxidase Detection System with DAB (Kwik™ Kits, ThermoSandon) was used for the demonstration of each antigen in question.

The slide rack was used for all steps except the first two. When used, it was placed on top of the ColdSpot™ (Table 1). Steps 8, 10 and 12 (Table 1) were programmed as 2 minutes on, 2 minutes off and 2 minutes on. The addition of 0.1% Triton X-100 to all buffer rinse steps greatly improved the results of this method. Microwave heating was minimal due to the ColdSpot™ and a low microwave power setting (250W). Immunolabeling results were consistent run to run with little or no background staining. Figure 1 is indicative of the results obtained when using this technique. The problems and/or criteria ascribed to microwave-assisted labeling, as described by other authors, were not apparent using this new system.[4,5] Successful results did not depend on changing microwave parameters for any of the antibodies tested and all reagents were used at their supplied dilutions. When the protocol was not microwave-assisted, the results were inconsistent.

References

[1] Microwave Techniques and Protocols, Humana Press, Totowa, NJ, 2001.
[2] R.T. Giberson, Microsc. Microanal. 7 (Suppl. 2:Proceedings) (2001) 1192.

[3] M.A. Sanders and D.M. Gartner, in R.T. Giberson and R.S. Demaree, Eds., Microwave Techniques and Protocols, Humana Press, Totowa, NJ (2001) 155.
[4] J. Gu et al., Cell Vision. 2 (1995) 257.
[5] L. Chicoine and P. Webster, Microsc. Res. Tech. 42 (1998) 24.

TABLE 1. Protocol outline for microwave-assisted labeling of paraffin sections on glass slides.

Microwave Step	Time	Slide Holding Device in Microwave
1. Deparaffinization-xylene	4 min.	Coplin jar
2. 100% Ethanol	1 min.	Coplin jar
3. Isopropanol	1 min.	Slide rack
4. 70% Ethanol	1 min.	Slide rack
5. Proxidase block-3%H_2O_2	1 min.	Slide rack
6. Buffer rinse	1 min.	Slide rack
7. Blocking	1 min.	Slide rack
8. Primary antibody	6 min.	Slide rack
9. Buffer rinse	1 min.	Slide rack
10. Biotinylated secondary	6 min.	Slide rack
11. Buffer rinse	1 min.	Slide rack
12. Streptavidin peroxidase	6 min.	Slide rack
13. Buffer rinse	1 min.	Slide rack
14. DAB chromogen	4 min.	Slide rack
15. Water rinse	1 min.	Slide rack
16. Hematoxylin counterstain	3 min.	Slide rack

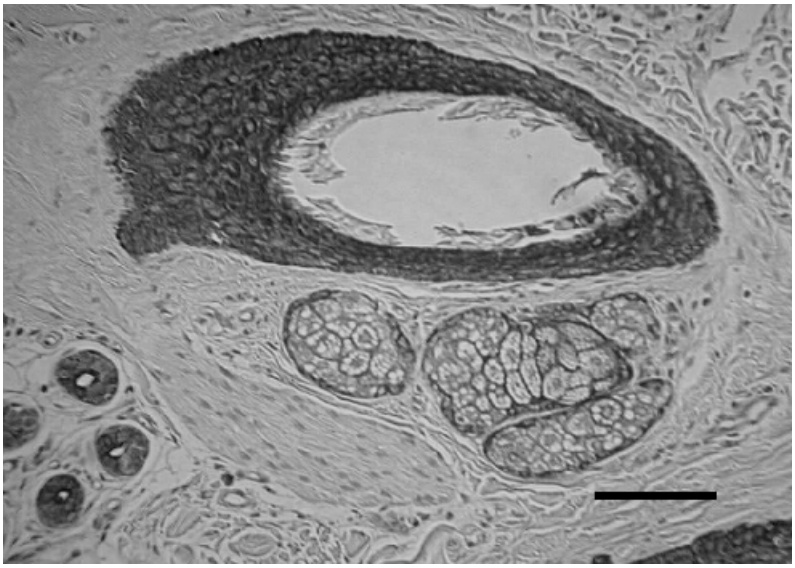

FIG. 1. DAKO® control slide S-100, Human Skin (Code T1072) stained by microwave-assisted methods with pre-diluted IMMUNON™ polyclonal Cytokeratin, wide spectrum and antigen detection with DAB using an IMMUNON™ Universal Streptavidin/Biotin Imunoperoxidase Detection System. Bar = 100µm.

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