General Procedure For Antigen Retrieval Using Microwave Heating

M.A.Hayat, Kean University

1. Fix the tissue without delay for 4 to 6 hours in 4% buffered formalin; the longer the issue remains in the fixative the lesser the chances of epitope retrieval (Hayat, 1999).

2. Wash in several changes of PBS; if available, an Autotecnicon should used in this step and in steps # 3 and 4, below.

3. Dehydrate in a series of ascending concentrations of ethanol.

4. Infiltrate and embed in paraffin.

5. Cut 4 µm thick sections with a microtome and float them onto a water bath which is kept at room temperature so as to preliminarily stretch the sections. An ordinary slide is used to transfer the sections onto another water bath kept at 58°C. Stretch the sections in this hot water, and lift them by the SuperFrost slides, thus mounting them in the process. The sections are allowed to dry in an upright position in a slide holder at a temperature of < 30°C. When the slide holder is full it is transferred to a conventional oven.

6. Dry the sections overnight at 58° C in the oven.

7. Remove the sections from the oven and deparaffinize them with 3 changes of 5 minutes each in xylene, followed by 3 changes of 100% ethanol, 2 changes of 75% ethanol, and 3 changes of distilled water. A wash in Tris- buffered saline (TBS) (pH 7.3) for 10 minutes is optional.

8. Place the slides in a microwave-proof (microwave transparent) jar containing 0.01 M sodium citrate buffer (pH 6.0); rectangular plastic jars are better than glass Coplin jars. Plastic Coplin jars are commercially available (Baxter Scientific, S7666). This jar is kept in another larger jar containing water to catch the boil-over from the smaller jar containing the slides. Place a jar containing the buffer or distilled water in the oven during the boiling of the slides so that when required to top off the slide jar, the liquid is at the same temperature as that of the slides. This jar also acts as neutral ballast in the oven, slowing down the speed at which the boiling temperature is achieved. The buffer is prepared as follows:

Solutions A and B are stock solutions.

Solutions A: 0.1 M citric acid is prepared by mixing 21.01 g citric acid with enough distilled water to make 1000 mL.

Solution B: 0.1 M sodium citrate is prepared by mixing 29.41 g sodium citrate with distilled water to make 1000 mL.

The working solution is prepared just before use by mixing 18 mL of solution A with 82 mL of solution B and adding enough distilled water to make 1000 mL and adjusting the pH to 6.0.

The jar containing the slides can be covered with loosely fitting lids or vented screw caps; do not tightly close the jar nor use aluminum foil to cover the jar. However, covering the jar is not obligatory if it has enough (2-3 cm) empty space above the buffer level.

9. Place the jars in the center of the oven on a rotary plate to ensure uniform heating of the slides.

10. Set the power to maximum; a power setting from 7 to 10 is

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11. Set the time to 10 to 15 minutes and check the buffer temperature with a temperature probe. The temperature of the buffer is different from that in the oven, so it is difficult to measure and control the temperature in the jar.

12. When the buffer begins to boil, allow it to cool for 5 minutes. Count the time of antigen unmasking from the boiling time. It is necessary to obtain vigorous boiling. The time for epitope unmasking depends upon the antigen, the antibody, the tissue type, the type of the fixative, and the duration of fixation. Thus, one has to standardize the microwave heating by trial and error. A known positive control is essential. As an average, the time for epitope unmasking varies from 2 to 10 minutes.

13. Check the level of the buffer in the jar, and add fresh buffer kept in the second jar, at the same time as the slides. The slides must remain fully immersed in the buffer.

14. After 5 minutes, again set the time to 5 minutes and restart the oven.

15. Repeat steps 12 and 13.

16. Remove the jar from the oven, and allow it to cool at room temperature for 20 minutes in a fume hood.

17. Rinse several times in 0.05 M PBS (pH 7.5).

18. Discard the used buffer.

19. When the DAB method is used, inhibit endogenous peroxidase by treating the sections with 50 µl of 30% H_20_2 in 50 mL of PBS for 30 minutes, followed by through washing in PBS.

20. Treat the sections with a mixture of 3% normal serum and 0.4% Trition X-100 for 30 to 60 minutes at room temperature to aid antibody penetration and block background staining.

21. Drain the excess serum without the slides, and incubate overnight at 4° C in a humid chamber with the primary antibody diluted appropriately in PBS. Incubation should be carried out with stirring to promote antigen-antibody contact.

22. Rinse in 3 changes of PBS.

23. Incubate for 30 minutes at room temperature in the linking agent (biotinylated anti-immunoglobulin; Vector lab, Burlingame, CA).

24. Rinse in 3 changes of PBS.

25. Incubate for 45 to 60 minutes in the avidin-biotin peroxidase or alkaline phosphatase or ABC elite or any other reagent or a third antibody if using the Double Indirect method.

26. Rinse in 3 changes of PBS.

27. Develop the color in 0.02-0.05% DAB activated with 0.003-0.01% H_20_2 in 0.1 M Tris buffer for 5 to 15 minutes. This step must be carried out in a flume hood. If using the alkaline phosphatase method, use the Alkaline Phosphatase Developing kit (Vector Red).

28. Wash in running tap water for 10 minutes.

29. Counterstain lightly for 20 seconds with hematoxylin, and rinse in water.

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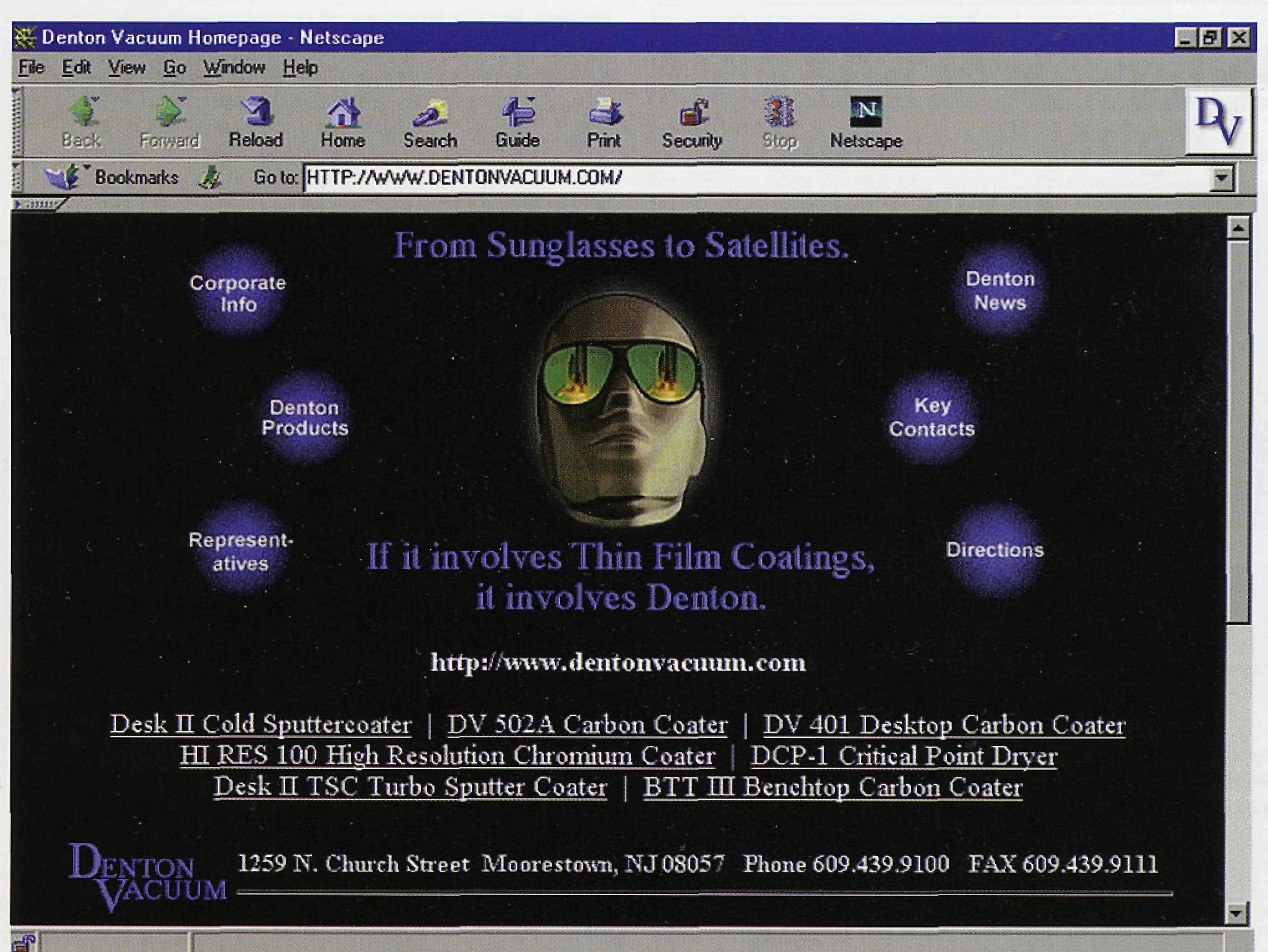

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30. Dehydrate in ethanol, clear in xylene, and coverslip with Permount.

Note: Instead of coating the slides with a glue, SuperFrost Plus slides (Fisher Scientific) should be used. These slides are charged positively and the sections are charged negatively; thus, preventing the sections from detaching from the slides while boiling. If these slides are unavailable, ordi nary glass slides can be coated with a glue such as poly-L-lysine (0.1%) or Neoprene (Aldrich Chemical, Milwaukee, WI).

However, the use of any type of glue on the slide may not prevent detachment of sections from the slide. The main reason for losing the sections during boiling is the air bubbles present between the section and the slide, and not due to lack of optimal gluing of the sections to the slide.

To avoid the risk of drying the sections during microwave heating, it is necessary to heat them in multiple four to five minute cycles, replenishing the jars between heating periods. Some evidence indicates that drying of sections on glass slides, prior to histological staining, in a microwave oven instead of in a conventional oven or on a hot plate has several advantages; paraffin sections adhere better to the glue-coated slides, drying time is reduced from 1 hour to 1 minute, and nonspecific background staining may be reduced.

It has been suggested that drying of paraffin sections, first at 38° C, and then at higher temperatures results in improved immunostaining of the proliferating cell nuclear antigen (PCNA) (Golick and Rice, 1992). Additional studies are required to evaluate the relationship between the temperature of slide drying and the extent of immunostaining. Another suggestion is that mild boiling of the epitope retrieval fluid has a gentle effect on tissue sections, so they are less likely to be dislodged from the slide. The microwave oven should be left at the high setting instead of changing it to medium setting because a change of setting does not affect the wavelength or actual power of the microwaves generated.

It is thought that the intensity of specific immunostaining can be enhanced and, simultaneously, background staining reduced by gentle orbital rotation (using a serological rotation) of slides during manual incubations (Butz *et al.*, 1994). Another advantage of this approach is shortening of the antibody incubation times, without sacrificing sensitivity.

Extreme antigen enhancement may cause false-positive staining. Such a staining has been observed in the case of p53 antigen (using monoclonal antibody D07) by employing Target Unmasking Fluid (TUF) containing 35% urea in the microwave oven at 96° C for 30 minutes (Baas *et al.*, 1996). This and other evidence indicates that there is a limit to the extent to which antigen enhancement can be applied to achieve optimal detection of a given antigen. An ideal antigen retrieval method should increase the clarity of immunostaining (*i.e.*, enhanced specific staining and absence of nonspecific background staining), and avoid tissue degradation (loss of morphologic detail) as well as aberrant immunoreactivity. The method should also be reproducible. ■

Baas, I.O., Van Den Berg, F.M., Mulder, J-W.R., Clement, M.J., Slebos, R.J.C., Hamilton, S.R.. and Offerhaus, G.J.A. 1996. Potential false-positive results with antigen enhancement for immunohistochemistry of the p53 gene product in colorectal neoplasms. J. Pathol. 178: 264.

Golick, M.L.. and Rice, M. 1992. Optimum staining of PCNA in paraffin sections is dependent on fixation, drying, and intenstification. J. Histoechnol. 15: 39.

Hayat, M.A. (1999). Principles and Techniques of Electron Microscopy: Biological Applications, 4th edition. Cambridge University Press, New York and Cambridge.

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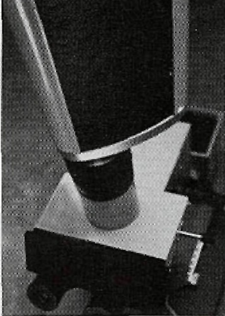

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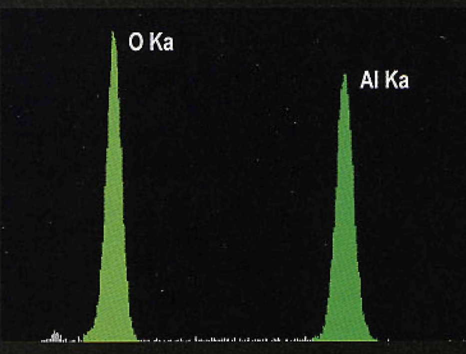

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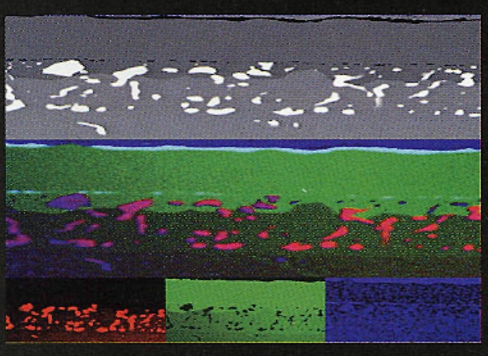

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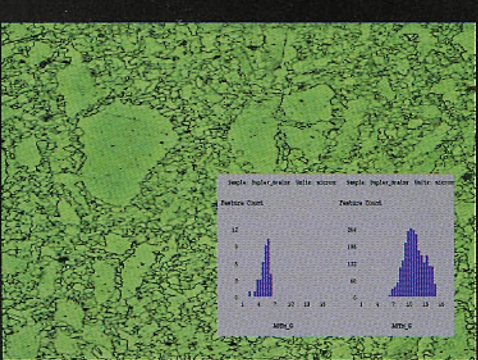

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