Combined Pressure-Cooker and Microwave Antigen Retrieval

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The use of antigen retrieval techniques has been the foundation of the development of immunocytochemistry (ICC). After the pioneering work done on cryostat sections, the use of enzyme digestion made formalin-fixed archival and current material available for study by ICC. However, the relationship between the duration of fixation and the digestion time necessary for optimum results is a major drawback associated with enzyme digestion. The longer the tissue has been in fixative, the longer the required digestion time will be. Even after a standardised fixation time, some antigens require greatly extended digestion times. Whereas 10-15 minutes in trypsin may be a standard protocol after overnight fixation, immunoglobulin deposits in renal glomeruli may require 60-90 minutes.

The introduction of heat induced epitope retrieval (HIER) methods has done much to avoid these problems. Such protocols do not require modification to cope with extended fixation. Methods have been published using microwave ovens, pressure-cookers and autoclaves to provide the heat source, and all can achieve useful results. The critical feature common to all of these methods is the application of heat to sections immersed in solution. The nature of the solution used is of major importance - heavy metal salt solutions, citrate buffer at pH 6, glycine buffer, Tris buffer and EDTA solutions have all been used. The use of heavy metal salts has been discontinued on health grounds, but the others all have applications in ICC.

Most laboratories use solutions between pH 6 and pH 8, but some antigens give better staining if significantly higher, or lower, pH solutions are used. It is thought that these solutions act by chelating or precipitating cellular calcium which may be involved in cross-link formation during fixation. The heating of the sections ruptures cross-links and thus frees the calcium, which is chelated and precipitated.

In our laboratory we now routinely use a plastic pressure-cooker which fits inside, and is heated by, a microwave oven. This, of course, combines the effects of microwave antigen retrieval with those of pressure cooking. The results have been very impressive. The advantage of steaming is the lower risk of uneven heating (hot/cold spots) when using a domestic microwave. Our steamer can hold up to 1.5 litres of buffer (which is enough for two racks of 25 slides).

We preheat the buffer solution 15 minutes at full power for 1 litre (1 rack of slides) or 20 minutes for 1.5 liters (2 racks). This is started while the slides are being dehydrated, etc. At the end of the preheating period, the solution should be just boiling. We then drop the slides in, put on the lid and the pressure-regulating valve and start the microwave again, allowing about 3 minutes for it to reach full pressure (steamer has an indicator valve to show when this happens), then hold at pressure for 5 minutes.

After the time is up, we remove the steamer from the microwave and let it stand until it has lost its pressure (seen by watching the valve), open the lid and let the solution cool on the bench for twenty minutes before removing the slides. It is likely that the 20 minute cooling period is to allow the reconfiguration of protein molecules prior to immunostaining, and most workers stress the need to include this stage. The same protocol is also proving useful as a pretreatment for *in-situ* hybridisation.

This form of antigen retrieval is invaluable for dealing with referred material, when the fixative and fixation protocol is unknown, and is proving of immense value with a wide and increasing range of antibodies. Perhaps more advances in ICC lie in our use of domestic appliances! ■

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