Application of microwave heat to paraffin sections: rapid adhesion of sections to slides

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Tissue sections of paraffin-embedded tissue cut and mounted on glass slides for subsequent staining must be heated to melt the paraffin wax in order to adhere the tissue section to the slide. The protocol employed in most cellular pathology departments is heating for 10–30 min in a 70°C oven. This is deemed sufficient to melt the paraffin wax and give adequate adhesion for haematoxylin and eosin (H&E) staining, special stains and immunocytochemistry (ICC). However, a problem can arise when dealing with urgent specimens, where this may delay subsequent ICC and special staining techniques.

This brief study investigates the use of microwave heating to melt the paraffin wax and adhere sections to the slide. A microwave should be an integral part of the complement of equipment used in any histopathology laboratory, as it is a common tool in antigen retrieval for immunocytochemistry[1] and for heated special staining methods involving silver impregnation.[2]

All sections were cut on a rotary microtome at 3 μm (those for Congo red staining were cut at 6 μm) and placed on glass microscope slides. Water was allowed to drain from the slides and they were placed either in a 70°C oven for 20 min or heated in a microwave oven on full power for either 30 or 60 sec until dry .

Sections for H&E staining were cut from blocks of prostate, ovarian cyst, keratinised skin, skin, large bowel, urinary bladder, uterus (myometrium/endometrium), lipoma, products of conception (POC), pilar cyst and appendix. A batch of 30 appendix sections was cut and placed in close proximity in a staining rack prior to microwave adhesion to test the effects of slide proximity on adhesion.

Microwave-heated slides were assessed for staining quality (eosin intensity and crispness of haematoxylin staining) relative to their oven-melted duplicates and for quality of section adhesion. Staining quality showed no observable differences between either heating method.

Section adhesion following microwave heating was good for all sections at 30 sec (with the exception of highly keratinised and overly bloody tissue). At 60 sec, all sections, including those that showed poor adhesion at 30 sec, showed good adhesion comparable with that achieved in a 70°C oven. The batch of 30 appendix slides placed in close proximity in a rack all stained well and showed no deterioration in adhesion.

Duplicate sections of special stain control material were cut for standard oven heating and microwave heating. Sections were stained with Congo red (Highman), Perl's Prussian blue, periodic acid Schiff (PAS), haematoxylin and van Gieson (HVG), Ziehl Neelson and reticulin (Gordon and Sweet).

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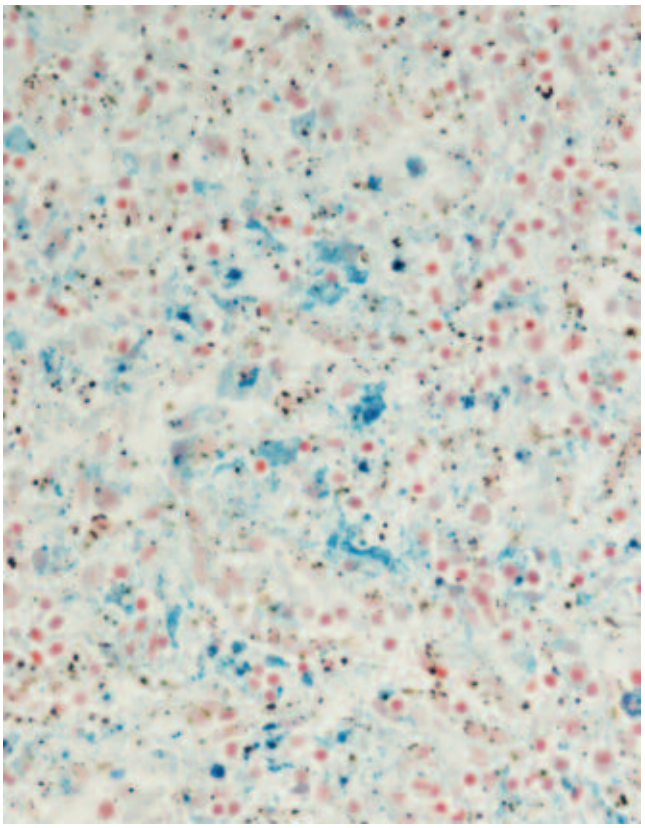

Fig. 1. Perl's Prussian blue staining of liver following 60 sec microwave heating (full power) to adhere section (original magnification x400). *See this image in colour at www.bjbs-online.org*

Microwave-heated sections were assessed for staining quality (intensity and specificity) relative to the oven-melted duplicates and for quality of section adhesion. Staining quality showed no observable differences between the two forms of heating. While adhesion was poor after PAS and HVG staining on sections heated in a microwave oven for 30 sec, all sections heated for 60 sec showed good adhesion.

Duplicate sections of ICC control material were cut for desmin, factor VIII (von Willebrand factor [VWF]), S100, prostate-specific antigen (PSA), smooth muscle actin (SMA), carcinoembryonic antigen (CEA), HER-2/neu, CD23, CD56, CD79a, MIB-1, CD10, CD21, calponin, cytokeratin (CK; AE1/AE3), Cam 5.2, CK7 and oestrogen receptor (ER).

Microwave-heated sections were assessed for antibody specificity and sensitivity relative to the oven-melted duplicates and for quality of section adhesion. Slides that required heat-mediated antigen retrieval (i.e., HER-2/neu, CD23, CD56, CD79, MIB-1, CD10 and ER) showed no loss of specificity and sensitivity in relation to their oven-heated duplicates. Slides subjected to proteolytic antigen retrieval (i.e., desmin, VWF, PSA, CEA and CD21) showed no loss of specificity and sensitivity in relation to their oven-heated duplicates. Sections not subjected to antigen retrieval (i.e., SMA) showed no loss of specificity or sensitivity in relation to the oven-heated counterparts. Section adhesion was good for all ICC slides following microwave heating for 60 sec.

The results showed no loss of staining quality with H&E or common special stains. Immunocytochemistry, a technique

that utilises microwave heating for antigen retrieval of some markers, showed no detrimental effects with any of the antigens assessed, regardless of the retrieval method. This was to be expected as the buffer that is important for antigen retrieval was not present on the slides during the heating step to achieve adhesion.

There was no evidence in any of the sections of the 'hot spots' that are reported to occur in a microwave oven, as adhesion and staining quality were consistent across individual sections.

Adhesion proved adequate for most specimen types after microwave heating for 30 sec; however, heating for 60 sec produced satisfactory results with all tissue types, including those with a tendency to detach during staining (e.g., heavily keratinised skin, excessively bloody specimens).

There seems to be no limit to the number of slides that can be heated in a batch to achieve adhesion. This is important if a backlog of slides for drying, which would negate the time advantage inherent in microwaving, is to be avoided. Although this technique lends itself to all applications, the greatest advantage will be seen with urgent sections, as almost 20 min can be saved, thus allowing sections to be passed to the histopathologist for reporting at the earliest opportunity.

The use of microwave heating to adhere paraffin sections to microscope slides prior to staining is a viable alternative to traditional use of a 70°C oven. Adhesion is comparable and there is no degradation of, or interference with, the results of subsequent staining techniques.

References

1 Leong AS. Microwaves in diagnostic immunohistochemistry. *Eur J Morphol* 1996; **34** (5): 381–3.

2 Minbay FZ, Kahveci Z, Cavusoglu I. Rapid Bielschowsky silver impregnation method using microwave heating. *Biotech Histochem* 2001; **76** (5–6); 233–7.

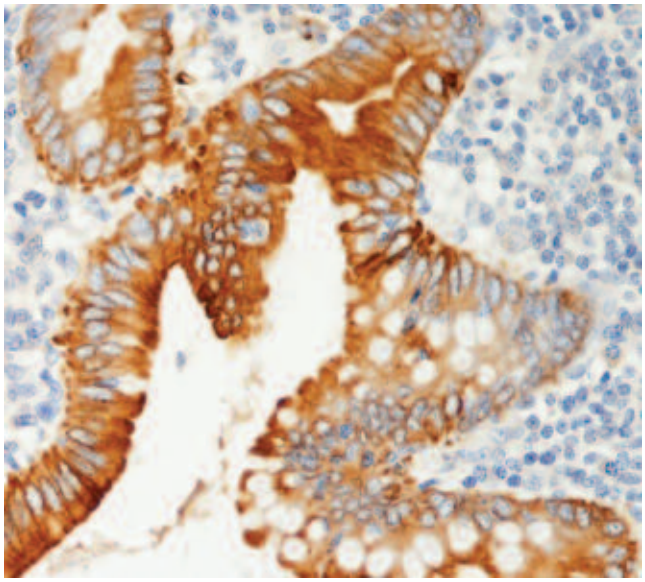

Fig. 2. Cytokeratin (AE1/AE3) immunostaining (dilution: 1:50, enzyme pretreatment: 8 min) of appendix following 60-sec microwave heating (full power) to adhere section (original magnification x400).
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Should long-haul flights carry antibiotics on board to treat acute bacterial meningitis and meningococcal septicaemia?

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Acute bacterial meningitis and meningococcal septicaemia represent an import medical emergency in medical microbiology. Several studies suggest that early intervention with intravenous (iv) antibiotics, particularly the β-lactam agents (e.g., benzylpenicillin) in patients with signs and symptoms of the disease, has a positive outcome on morbidity and mortality in infected individuals.[1–3] Consequently, it is now standard UK practice for GPs to carry iv antibiotics in their bag for immediate administration to individuals suspected of presenting with signs and symptoms of these infections, prior to immediate admission to an intensive care unit (ICU) in secondary care or to tertiary paediatric care.

In 2003, a case report[2] was published that described a fatal case of meningococcal meningitis and meningococcaemia in a 20-year-old male student travelling from Tel Aviv, Israel, to Newark, New Jersey, USA, who developed signs and symptoms of infection some 90 minutes prior to landing at Newark, where the patient died two hours after arrival. The airline did not carry antibiotics in its medical kit. The authors of the report[2] recommended that the appropriate authorities should require airlines to add a broad-spectrum antibiotic preparation to the emergency medical kit, as the authors speculated that earlier intervention with an appropriate onboard antibiotic may have saved the life of the young man.

The authors of the current paper have contacted several airlines flying long-haul routes out of the UK to estimate the current level of awareness of this disease among crews of commercial airlines, and what actions would help to raise awareness. It was noted that some airlines carried antibiotics for the treatment of acute bacterial meningitis and meningococcal septicaemia, and some crew members were trained in the recognition of the signs and symptoms of bacterial meningitis and meningococcal septicaemia. One airline welcomed receiving educational resources on meningitis as an information/educational resource for their crews and passengers.

Acute bacterial meningitis and meningococcaemia are serious infections in medical microbiology and should be treated as medical emergencies. This is facilitated on the ground in the UK by the availability of iv benzylpenicillin in

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