Microwave-assisted staining procedures in routine histopathology

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Summary
The use and practicability of microwave-assisted staining procedures in routine histopathology over more than three years has been evaluated. A domestic microwave oven was used to speed up the following staining procedures: Haematoxylin-Eosin (for frozen sections), Romanowsky-Giemsa, Periodic acid-Schiff (PAS), Ziehl-Neelson, Papanicolaou, Feulgen and Grocott - stain on buffered formalin fixed sections or cytologic smears. These staining procedures can be made highly reproducible providing; (1) Staining vessels are placed in the same position inside the oven; (2) Accurate timing in seconds is observed. Microwave-assisted staining procedures are equal to or even superior to those of the standard methods. Staining times can be reduced to 2%-10% of the conventional staining procedures. The basic staining protocols are presented.

Introduction
This paper describes the experience obtained in microwave-assisted staining procedures in a routine histopathological laboratory dealing with 50-80 biopsies and the equivalent cytological and surgical specimens daily. Microwave-assisted fixation can easily be performed in cases of small biopsy specimens, as shown previously, and may reduce the cost of reagents (Kayser et al., 1988). Formalin concentration can be reduced from a 5%-7% solution to a 0.5% solution or even completely replaced by TRIS buffer (Kayser et al., 1988; Boon & Kok 1989).

In histopathological laboratories, automated staining machines are available reducing the cost of manpower for bulk slides. However, special diagnostic problems need special staining procedures. These procedures, performed by hand, are a necessity in each laboratory, fairly expensive in reagents and man hours. The quality of these methods and the time taken to do them are important. Any technique reducing the staining time without loss of quality is valuable in reducing costs and improving the service to the clinicians. Microwaves may aid in this by increasing the rate of diffusion of the dye into the cells and the binding of the dye to the substrate (Boon & Kok, 1986; Kok et al., 1988). The first success of microwave technology in metallic staining was reported by Brinn (1983). Moorlag et al. (1987) have reduced the staining time in the PAS reaction to 10 min by microwave irradiation (Moorlag et al., 1986). Boon & Kok (1986) describe a series of staining procedures improved by microwaves (Boon & Kok, 1989). Horobin & Boon (1988) documented the use of microwaves on Romanowsky-Giemsa stains applied to plastic embedded bone marrow specimens (Horobin, 1986; Boon et al., 1987; Horobin & Boon, 1988). The greatest staining benefit of microwave irradiation might be anticipated in staining procedures using metals due to the polar and charged dye molecules and the time-consuming staining procedures (Boon et al., 1987). Brinn reported a reduction of the 180 min staining time in Grocott's silver stain to 20 min by use of microwave irradiation (Brinn, 1983; Brinn, 1986). Similar results were given by Boon and Kok (1989) for the Grimelius silver technique and Perls' iron, etc. Based upon the need in a routine histopathological laboratory, the staining procedures discussed below were selected and introduced into routine performance.

Materials and methods

General features
A domestic microwave oven was selected with a maximum power of 650 W (Philips). The machine has a power reduction switch allowing discrete power selections ranging from 10%—90% and a programmable time switch (range in seconds). The machine was placed beside the work station for frozen sections. A conventional set of baths for dewaxing, hydration and dehydration was placed to hand. The staining procedures were performed in Petri dishes filled...
with the appropriate staining solution to allow the staining of two specimens at the same time. The position of the dishes inside the microwave oven was marked and the dishes were placed in the same position every time to avoid unrepeatable results due to the inhomogeneous electromagnetic field. The power control of the microwave oven was usually set at 80% of maximum energy. Commercially available staining solutions (Merck Darmstadt, Sigma, Munich, FRG) were used for all protocols given below.

**Haematoxylon-Eosin for frozen sections**

This staining procedure was developed for speeding up frozen section diagnosis. After snap freezing with liquid nitrogen cryostat sections (Jung 2700) were made.

Haematoxylon was added for 3 s at 80% maximum power; rinsed in tap water for 5 s then 1–2 drops of HCl–ethanol were added and rinsed again in tap water for 5 s. Eosin was added for 3 s at 80% maximum power; rinsed in tap water for 3 s then rinsed in ethanol for 5 s and mounted; total time taken was 25 s.

The procedure can be improved by use of Kryofix (Merck Darmstadt) prior to the staining procedure. In the case of frozen sections of lymph nodes, especially possible lymphomas, staining with and without Kryofix fixation is helpful.

**Periodic Acid-Schiff reaction (PAS)**

The staining procedure was developed for use with frozen sections. It may also be used on paraffin sections for rapid results.

Paraffin sections were placed in water and oxidised in 0.75% periodic acid for 5 s at 80% maximum power. The sections were rinsed in water for 5 s and then Schiff's reagent added for 5 s at 80% maximum power. Sections were rinsed in SO₂-water for 5 s and then rinsed in water for 5 s. The sections were then counterstained with Gill's Haematoxylin for 3 s at 80% maximum power and mounted.

The PAS staining procedure is somewhat sensitive to the microwave irradiation time due to the thermolabile Schiff's reagent. It is easy to 'overcook' and it is essential to place the specimens exactly at the premarked position inside the microwave oven. Test series should be carried out to determine the best irradiation time.

**Papanicolaou stain**

Microwave irradiation in Papanicolaou stain is used in cases of possible malignancy.

Sections were fixed in the ether-ethanol and hydrated in distilled water. Haematoxylon was added for 3 s at 80% maximum power and then rinsed in water for 5 s, then differentiated in HCl-ethanol for 3 s. Sections were rinsed in water for 5 s. Orange-G-solution was added for 3 s, at 80% maximum power and then rinsed in ethanol for 5 s. EA65 was added for 3 s at 80% maximum power and mounted. Total time taken was 40 s.

The procedure is easy to perform and is nearly independent of the fixation. The ether-ethanol fixation can be replaced by microwave assisted Kryofix fixation.

**Grocott's silver stain**

This stain is somewhat difficult due to its photoreaction in general. It is commonly used to identify Pneumocystis carinii. The staining protocol given below can also be applied to cytological specimens.

Sections were placed in water. 10% chromic acid solution was added for 10 s at 80% maximum power and then rinsed in water for 5 s and the slides cleared in sodium bisulphite for 60 s. Methenamine silver working solution was added for 8 s at 80% maximum power and then left to stand in the hot working solution for an additional 10 s. Rinsed in water for 5 s and toned in 1% gold chloride for 5 s. Rinsed in water for 5 s and then drops of 5% sodium thiosulphate added for 5 s. Rinsed in water for 5 s and counterstained with Haematoxylin and Eosin (see above) and mounted. Total time taken was 140 s.

Grocott's staining procedure depends upon the fixation of the specimens and the age of the stock solutions. The time in the hot methenamine silver working solution can be used as a regulator. Old stock solutions tend to crystallise on the slides. The microwave assisted staining is as easy or as difficult as the standard technique.

**Ziehl-Neelsen stain**

The staining procedure needs heat in its standard technique and thus differs from the other microwave assisted procedures. It can be applied to formalin fixed specimens as well as to cytological smears.

Sections were placed in water and Carbol-fuchsin added for 30 s at 80% maximum power. Rinsed in running water for 10 s and differentiated in HCl-ethanol for 10 s. Rinsed in water for 10 s and then counterstained with Methylene-Blue for 3 s at 80% maximum power and mounted. Total time taken was 70 s.

The staining procedure should be tested with smears with positive controls prior to general use. The time in the carbol-fuchsin may be prolonged to 40 s if the stain of the myobacteria is not brilliant. 'Overcooking' is not really critical.

**Romanowsky-Giems stain**

Some authors consider the Romanowsky-Giems stain as the basic histopathological staining procedure.

Sections were placed in water and Giemsa working solution was added for 10 s at 80% maximum power. Rinsed in water for 5 s and then rinsed in HCl-ethanol for 5 s and mounted. Total time taken was 25 s. The time of microwave irradiation is not very critical considering the nuclear chromatin pattern and is somewhat dependent upon the quality of fixation and the fixation medium used. In order to

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Fig. 1. Microwave-assisted HE stain, intraoperative section showing a moderately differentiated adenocarcinoma. ×150.
Fig. 2. Microwave-assisted PAS stain showing a slightly hypersecretive bronchus mucosa. ×150.
Fig. 3. Microwave-assisted Papanicolaou stain of a cytologic smear showing ciliated cells, macrophages, lymphocytes and granulocytes. ×150.