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A simple and effective heat induced antigen retrieval method

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A B S T R A C T

In this paper, we describe an additional step to the standard method of heat induced antigen retrieval to improve the detection of antibody staining of formalin fixed paraffin embedded tissue sections. Direct heating of tissues in buffer is an efficient epitope retrieval method but often results in the damage or loss of tissues. In this modified method, before keeping in buffer for heating, we overlapped the tissue on the slide with a plain slide by clipping one end using a normal paperclip, keeping a minimum gap between the slides. Tissues heated in this way in buffer had following advantages over normal heat treatment for epitope retrieval.

- Tissues were intact even at high temperatures which improved the quality of staining by preventing fold, damage or detachment of tissues from the slides.
- The method is very safe and economical compared to the methods using microwave or pressure cooker.
- This simple method also appears to be very effective and less time consuming compared to the existing methods.

A R T I C L E I N F O

Method name: Modified HIER method

Keywords: Antigen retrieval, Immunostaining, Tissue damage, New method

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Methodology background

Antigen retrieval is an important step in immunohistochemistry staining. The simple technique of boiling formalin-fixed paraffin embedded (FFPE) tissue sections in water has played a major role in extending the reach and use of immunohistochemistry [1]. However, direct boiling of tissues in buffer

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often results in the damage of tissues or falling off tissues from the slide. Several reasons such as insufficient fixation, improper sectioning and drying, poor adherence and even uncleaned slides have been reported to contribute to the issue of falling off or detachment of tissues from slide [2–5]. Even after taking care of above factors, the sections often tend to detach from adherent coated slides while boiling. In this paper, we present an additional step to the standard heat induced epitome retrieval (HIER) of tissues by which the damage or detachment of tissue from the slide while boiling can be prevented.

Tissue collection and preparation

Tissues collected from male Sprague-Dawley rats aged 8–12 weeks used for this study. After fixation in 10% neutral buffered formalin, paraffin embedded tissues were cut at 4–5 μm and transferred to HistoGrip coated glass slides. Tissue sections were dried on a slide warmer at 60 °C prior to immunostaining.

Antigen retrieval method

Deparaffinised and rehydrated slides were kept in a solution of sodium citrate (pH 6.0) or Tris/EDTA (pH 9.0), once the temperature has reached 95 °C in a water bath.

Additional step (Fig. 1) in antigen retrieval method is described below.

Before keeping the slides in buffer for heating in a temperature controlled water bath or in a beaker on hotplate, the tissue bearing slide was overlapped with another plain slide by clipping one end using a normal paperclip (U clip of 2″). Clipping is done only at one end in such a way that the other end gets slightly widened allowing the buffer to go to the tissue and the tissue does not get jammed between the slides. Also one end is slightly shifted laterally (clockwise) in order to make it easier to remove the overlapping slide after the heat treatment. Each tissue slide was paired with a plain slide separately in this way before keeping in buffer for boiling. Tissues were boiled for 15 min at temperature ranging from 95 to 100 °C. Slides were then taken out of water bath and allowed to cool in a vessel of tap water for 10 min. The clips were removed safely when the tissues were in cold water.

Immunostaining

Immunoperoxidase staining was performed as per the standard Avidin-Biotin Complex (ABC) method. Precisely, primary antibody was incubated for 1 h and secondary antibody for 30 min to 1 h at

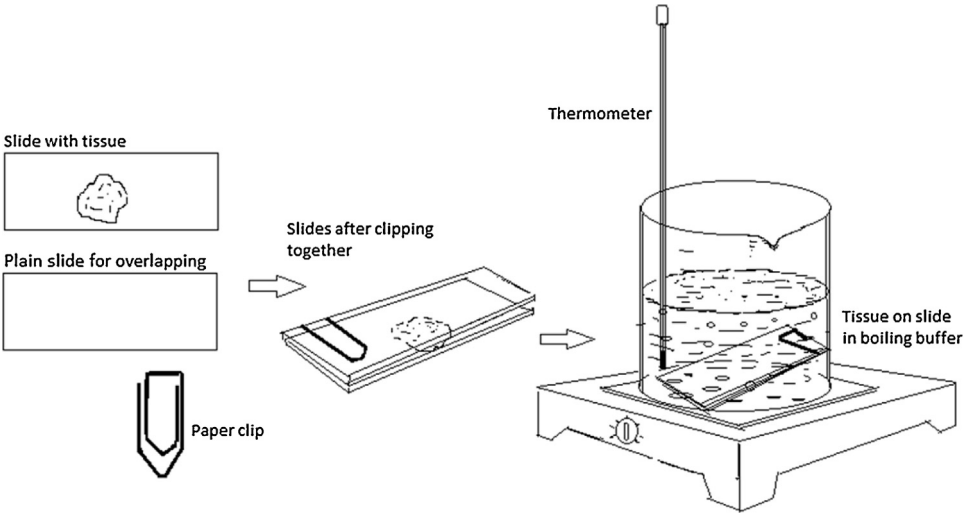

Fig. 1. Additional step in antigen retrieval.