

Novel Instrument for Heat Induced Epitope Retrieval

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ABSTRACT

Heat Induced Epitope Retrieval (HIER) has become a mainstay of the immunohistochemistry laboratory. However, despite reliance on HIER current methods using microwave ovens, steamers, pressure cookers and waterbaths are poorly controlled, leading to inconsistent results. We introduce a new instrument, the PreTreatment Module PreTreatment Module™ (PTM™)*, specifically designed to tightly control the parameters of HIER. The PTM is a computer-controlled instrument in which the user can choose the exact incubation time and temperature desired and precisely repeat those conditions for all HIER runs. When compared to other methods the PTM gives more consistent results. We show that true standardization for HIER is now a reality.

INTRODUCTION

Heat induced epitope retrieval (HIER, aka, *Antigen Retrieval*) has been in use for many years and is a mainstay of the pathology laboratory (1). The essential factor is that a buffer solution of the appropriate pH be heated to a suitable temperature (2). While the original method employed a microwave oven, many other methods have been employed including water bath, steamer and pressure cooker (3). A common problem with all these methods is lack of control of the heating method. We introduce here a novel instrument, the PreTreatment Module, that uses computer control to set the time and temperature in a reproducible manner. For ease of use, the PTM is designed to work with the racks from these autostainers: DakoCytomation Autostainer, BioCare Nemesis, Richard-Allan HMS 710i and all Lab Vision Autostainers.

METHODS

Serial tissue sections were HIER-treated in either the PreTreatment Module (PTM), microwave oven (MW), pressure cooker (PC), or vegetable steamer (ST). When MW or S was used, the slides were deparaffinized using xylene/alcohol and HIER was performed using the appropriate pH HIER buffer. When PTM or PC was used, the slides were deparaffinized and HIER-treated using a one-step deparaffinization/HIER buffer (the appropriate pH HIER buffer for the antibody). Primary antibody used for comparison (calretinin or AE1/AE3, each from Lab Vision) was applied at the same dilution regardless of treatment. Detection of primary antibody was performed with biotinylated poly-valent antibody followed streptavidin/HRP conjugate for 10 minutes each. AEC or DAB chromagen was applied for appropriate time to obtain best possible reaction. All reagents were obtained from Lab Vision Corporation.

Results

Figures 1-4 demonstrate the comparison between PTM, PC, ST and MW. The staining for calretinin achieved by using the PTM is at least equal in intensity to that achieved by use of the PC, and is stronger than the intensity obtained by S or MW. HIER in the PTM achieved stronger signal that that obtained using the MW (Fig. 5,6 respectively). Two other antibodies (CD3, Ki67, Lab Vision) were tested using the PTM treatment but were not compared to other methods of HIER. Each showed very strong signal with no background (Fig 7, 8).

Pretreatment Module™ HIER vs Other Methods

Calretinin, mouse monoclonal, clone CRT01

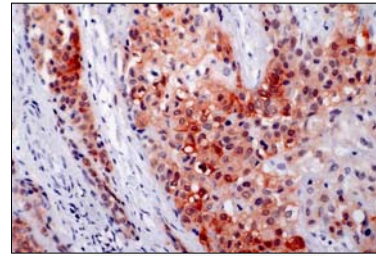


Fig.1 PTM HIER

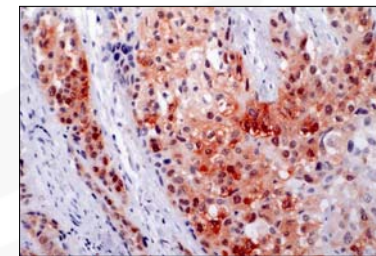


Fig. 2 PC HIER

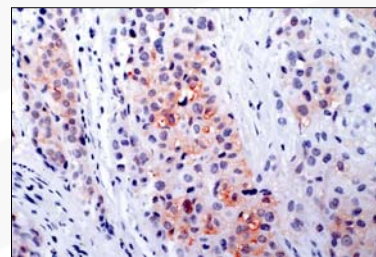


Fig. 3 Steamer HIER

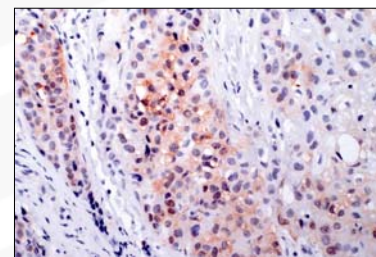


Fig. 4 MW HIER

Cytokeratin, Clone AE1/AE3

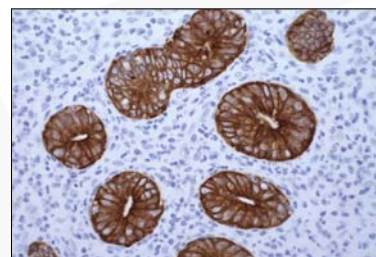


Fig. 5 PTM HIER

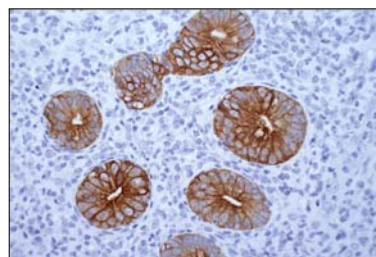


Fig. 6 MW HIER

Other examples

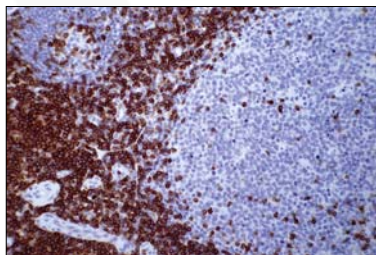


Fig. 7 CD3, clone SP7, PTM HIER

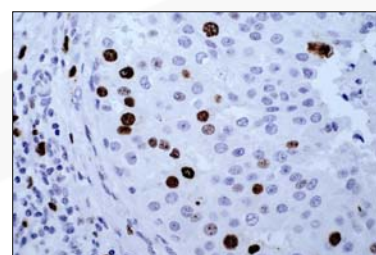


Fig. 8 Ki67, clone SP6, PTM HIER



PreTreatment Module

Instrument Specifications				
Instrument	Manufacturer, Model	Slide capacity	Controllable parameters	Max Temp
Pretreatment Module	Lab Vison PTM-1	48	Time, temperature	102C
Microwave	Panasonic MS-S5438FR, 1300W	60	Time, power	100C
Pressure Cooker	Panasonic NSR-106NW, 800W	60	Time	120C
Steamer	Oster 5711, 900W	60	Time	98C

Use parameters						
Instrument	Deparaffinization	HIER	Steps to perform	HIER time	Total time*	Ease of use
Pretreatment Module	One-step	see Depar	1	40	70	1
Pressure Cooker	One-step	See Depar	2	10	50	2
Steamer	Xylene, alcohol series	Aqueous buffer	14	30	65	3
Microwave	Xylene, alcohol series	Aqueous buffer	13	20	60	4

* Total time is for one method. Methods may vary significantly between laboratories.

Discussion

The PTM™ proved to be at least equal to the most common HIER methods used in the laboratory at the present time. The ability to use the Autostainer racks significantly reduces slide handling time. The use of a one-step deparaffinization/HIER solution significantly reduces procedure steps. Computer-control of time and temperature variables allows reproducibility not achievable with other methods. The combination of these features makes the PTM™ a very powerful yet user-friendly instrument for HIER.

Conclusion

Heat induced epitope retrieval using the PreTreatment Module™ is equivalent or better than HIER using other common methods.

References

- Shi S-R, Key ME, Kalra KL, **Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections.** J Histochem Cytochem 39:741-748 (1991).
- Major Factors influencing the effectiveness of Antigen Retrieval Immunohistochemistry,** Shi S-R, et al, pp 41, in *Antigen Retrieval Techniques, Immunohistochemistry and Molecular Morphology*, Ed Shan Rong Shi, et al. Eaton Publishing, 2000.
- Appendix: Antigen Retrieval Techniques,** Shi S-R, et al, pp 311, in *Antigen Retrieval Techniques, Immunohistochemistry and Molecular Morphology*, Ed Shan Rong Shi, et al. Eaton Publishing, 2000.