A Novel Method for Streamlined Immunohistochemistry

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Introduction

Immunohistochemistry (IHC) has long been the standard method for the detection and localization of biomarkers within tissue samples. While manual IHC provides reproducible tissue staining it is time consuming and laborious. Here we present a novel IHC format using a vacuum driven Immuno detection system (SNAP i.d.® 2.0 protein detection system) that enables western blotting and immunostaining with the same platform. Blots can be developed in only 30 minutes, while tissue slides can be processed usually in about 2 hours using any typical manual protocol with no detectable staining artifacts or loss in sensitivity. The vacuum driven IHC system can handle 1 to 24 slides using formalin fixed paraffin embedded tissue (FFPE) or fresh frozen samples.

Advantages of the vacuum driven IHC system over the traditional manual format include: reduction in slide handling time, no need of Pap pen, fast and efficient washing steps that help in the decrease of non-specific staining and effective recovery of the antibody for future re-use. Furthermore, the consistency of the vacuum driven IHC process allows for a high degree of reproducibility and standardization. The system facilitates the optimization of antibodies or other reagents for future studies. It can also be helpful during the scaling up process for automated staining systems. Antibody incubation times can be performed at room temperature for short periods of time or even overnight at 4˚ C with no loss in antibody volume.

In this poster, we describe how the system works, provide the data to demonstrate the system versatility, show the consistency in the results and compare the sensitivity with the traditional IHC manual method.

Key features of the vacuum driven SNAP i.d.® 2.0 protein detection system for IHC:

- Flexibility of multiple slide configurations enables the processing of 1 to 24 slides at a time
- Compatible with standard IHC slides and protocols
- Easy-to-use format
  - Incorporates blocking, washing, and antibody incubation steps
  - Streamlines handling multiple slides without the cost of automation
  - Eliminates the use of a Pap-pen
  - Allows for antibody collection and reuse

Material and Methods

Samples & Equipment:

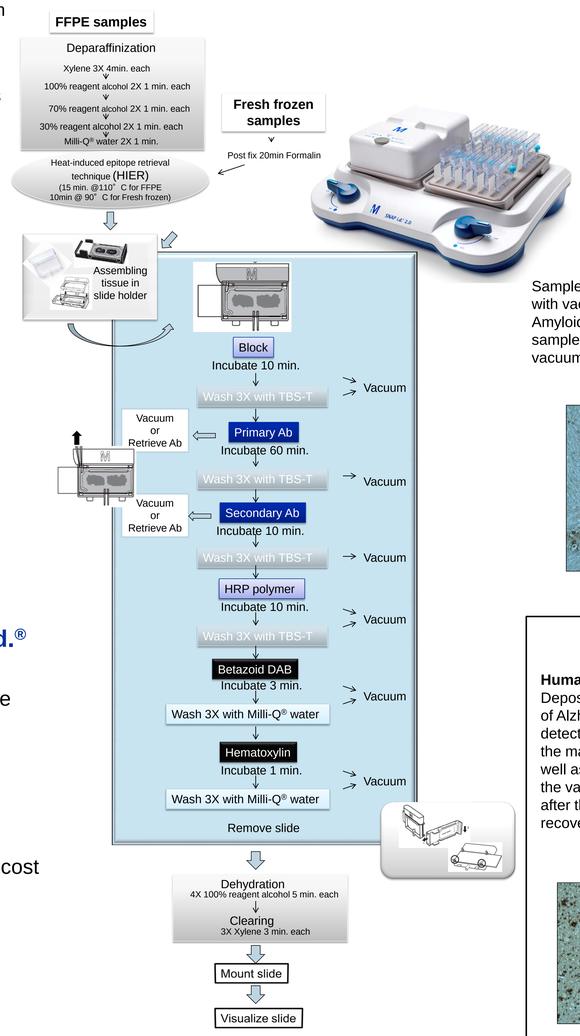
- FFPE and fresh frozen tissue slides of human kidney and brain assembled on ProbeOn™ slides (Fisher Scientific Cat. # 15-188-51)
- Antigen retrieval reveal Decloaker Chamber™ NxGen instrument (Biocare Medical Cat. # DC2012)
- SNAP i.d.® 2.0 immunodetection system
  - SNAP i.d.® 2.0 base (Cat. # SNAP2BASE)
  - IHC frame (Merck Millipore Cat. # SNAP2FRIHC)
  - Slide holder (Merck Millipore Cat. # SNAP2SH)
- Aperio® Scanscope (Leica Biosystems)
- Evos® microscope (Life Technologies)
- Super Pap pen (American Master Tech Cat # WISPP)

Reagents:

- Xylene (Merck Millipore Cat. # XX0060-4)
- Ethanol (Merck Millipore Cat. # EX0276-1)
- Milli-Q® water (Merck Millipore Integral 15 system)
- Modified Mayer Hematoxylin (Sigma Cat. # MHS16)
- Tris buffer saline with 0.05% Tween®-20 [TBS-T] (Boston BioProducts Cat. # IBB-181)
- Betazoid DAB chromogen kit (Biocare Medical Cat. # BDB2004)
- Background punisher (Biocare Medical Cat. # BP974L)
- CoverSafe™ mounting medium (American Master Tech Cat. # MMC0226)
- ProLong® Gold Antifade reagent with Dapi (Life Technologies Cat.# 36935)

Antibodies (Ab):

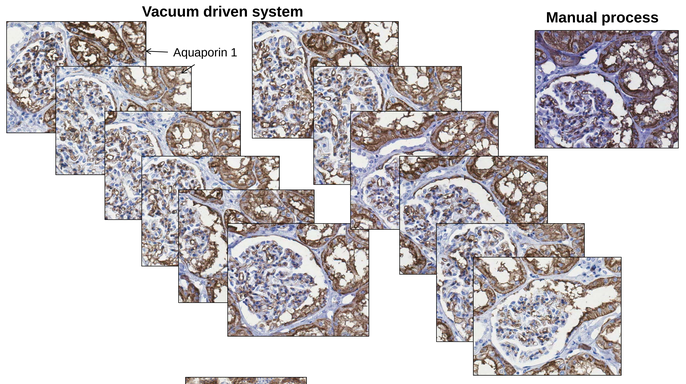
- Rabbit anti Aquaporin 1, anti Amyloid β, anti Glial Fibrillary Acidic protein and anti Sox 11 (Merck Millipore Cat. #'s AB2219, MABN10, MAB3402 and ABN105)
- Mach 3™ Rabbit probe and Rabbit HRP polymer (Biocare Medical Cat # RH531H)
- Mach 3™ Mouse probe and HRP polymer (Biocare Medical Cat. # MH530H)
- Goat anti mouse DyLight 488 and goat anti Rabbit DyLight 549 (Biocare Medical Cat #'s FDM488AK,CK and FDR549 AK, CK)



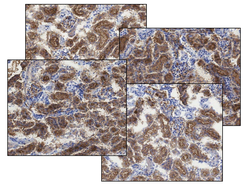
Results

Consistency in the staining process

Twelve slides of **FFPE human kidney tissue** were simultaneously processed using vacuum driven IHC and compared with the traditional manual process. High sensitivity and lower background was observed in all the samples.



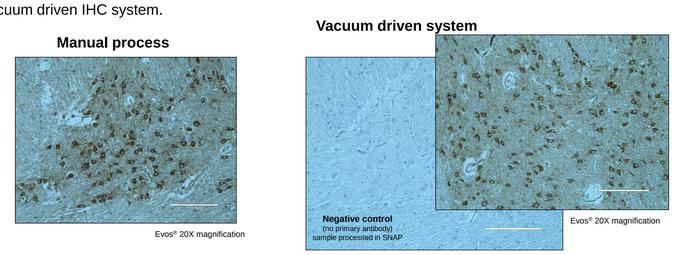
Detection of Aquaporin 1 in 20 µm **Fresh frozen** rat kidney tissue processed in the vacuum driven IHC system



Aquaporin 1, (stained in brown) is an integral membrane protein capable of forming water-specific channels to increase permeability. It was detected in the proximal tubes of human and rat kidney samples using anti Aquaporin 1 antibody at 1:2000 dilution and stained with HRP-DAB. (Amperio® 40X magnification)

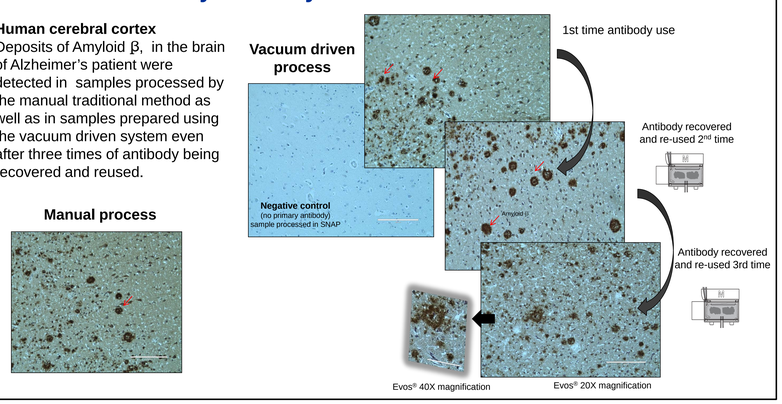
Comparison of manual process vs. vacuum driven IHC

Samples of human brain thalamus were tested with anti Amyloid β using the traditional manual process and comparing with vacuum driven IHC system.
Amyloid β peptide, one of the major constituents of the plaques occurring in Alzheimer's disease, was detected in the samples processed by both methods. A negative control slide (no primary antibody) was also processed using the vacuum driven IHC system.

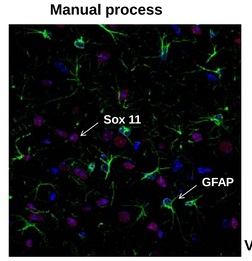


Antibody recovery and reuse in vacuum driven IHC

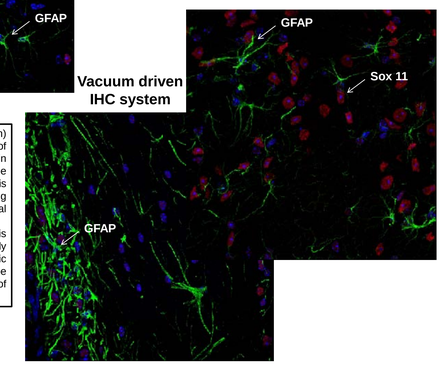
Human cerebral cortex
Deposits of Amyloid β, in the brain of Alzheimer's patient were detected in samples processed by the manual traditional method as well as in samples prepared using the vacuum driven system even after three times of antibody being recovered and reused.



Fluorescent staining using vacuum driven IHC *vs.* the traditional manual process



Analysis of FFPE mouse brain using a "cocktail" of two different primary antibodies [anti-mouse Glial Fibrillary Acidic Protein ("GFAP" diluted 1:1000) and anti-rabbit Sox 11 ("Sox 11" diluted 1:500)]. Fluorescent detection was performed after the addition of goat anti-mouse DyLight 488 and goat anti-rabbit DyLight 549 and counterstained with Dapi (blue stained nuclei).



GFAP protein (stained in green) is the main constituent of intermediate filaments in astrocytes. It is considered to be the cell specific marker for this type of differentiated cell during development of the central nervous system.
Sox 11 protein (stained in red), is a neural transcription factor, highly expressed during embryonic development. In adults, it may be involved in the regeneration of neurons after nerve injury.

Conclusion

The vacuum driven system helps to streamline the immunohistochemistry process by reducing the slide handling time and by eliminating the tedious process of using a pap pen. While, this system is low to medium throughput, it can be helpful in the antibody optimization process, and/or during the reagent scale up process required for automated systems.

The results presented here indicate that different manual protocols can be reproduced easily in the vacuum driven IHC system and that it can be used for a variety of tissues prepared by either formalin-fixed paraffin-embedded techniques, or fresh frozen samples.

Summary

- SNAP i.d® 2.0 IHC system is versatile and it is compatible with typical manual protocols
- It can process FFPE or fresh frozen tissue sections
- Advantages of the vacuum driven system includes :
  - Handles 1 to 24 slides
  - It is useful for antibody optimization or antibody/reagent scale up
  - Eliminates the use of a Pap-pen
  - Decreases background with fast, and efficient vacuum driven washes
  - Reduces the tracking, set-up and handling time
  - Allows for antibody collection and re-use

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