

# An Alcian Blue/PAS combined with TTF-1 and Napsin A Staining Procedure

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## Introduction

Non-small cell lung cancer (NSCLC) can be classified into several histological subtypes, most commonly adenocarcinoma (LADC) or squamous cell carcinoma (SqCC). With the introduction of targeted therapies that can result in dramatically different outcomes based on subtype, the importance of accurate classification has been amplified. Limited availability of tissue from needle core biopsies has challenged routine diagnosis, particularly for poorly differentiated samples. Studies have shown that histochemical stains Alcian blue/PAS are useful in lung cancer diagnosis (1,2). Immunohistochemical TTF-1 and Napsin A double stains have also been used in lung biopsies and were effective in identifying LADC. In this study, we explore the possibility of combining all four stains on a single section of formalin-fixed paraffin embedded (FFPE) tissue. We are presenting the debut of a quadruple stain using Alcian Blue, PAS, TTF-1 and Napsin A in lung tissue.

## Materials and Methods

FFPE lung tissues were cut at 4 microns and processed in the usual way. Tissues were stained with a modified Alcian Blue/PAS special stain. Tissues were then rinsed in deionized water and antigen retrieval was performed using a Decloaking Chamber (pressure cooker, Biocare Medical) at 125°C. Sequentially, immunohistochemistry was performed using a TTF-1 and Napsin A antibody cocktail, followed by a double stain polymer detection kit. For visualization DAB and Warp Red chromogens (Biocare Medical) were applied at 5 and 7 minutes respectively.

### Protocol

1. Deparaffinize and hydrate sections to deionized water.
2. Peroxide Block: incubate for 5 minutes.
3. Pretreatment: retrieve sections with Diva Decloaker (Biocare Medical, DV2004) under pressure at 125 °C (Decloaking Chamber, Biocare Medical). Allow solution to cool for 10 minutes; then wash in distilled water.

## Alcian Blue/PAS Procedure

1. Rinse slides in running tap water and then deionized water.
2. Apply Alcian Blue for 15-20 minutes at RT.
3. Immerse slides in Periodic Acid Solution for 5-10 minutes at RT.
4. Rinse slide in several changes of distilled water.
5. Immerse slides in Schiff's reagent for 15-20 minutes at RT.
6. Immerse slides in EDTA (Biocare Medical, CB917) for 5 minutes to stop the reaction.
7. Wash slides in running tap water for 5 minutes
8. Rinse slides in TBS Buffer then keep slides in TBS Buffer.

## IHC Procedure

1. Primary Antibody: incubate for 30 minutes at RT.
2. Double Stain Detection: incubate for 30 minutes at RT using Biocare's MACH 2 Double Stain #2.
3. Chromogen: incubate for 5 minutes at RT using Biocare's Betazoid DAB and for 5-7 minutes at RT with Fast Red.
4. Dehydrate, clear and mount sections in toluene or xylene-based mounting media

## Results

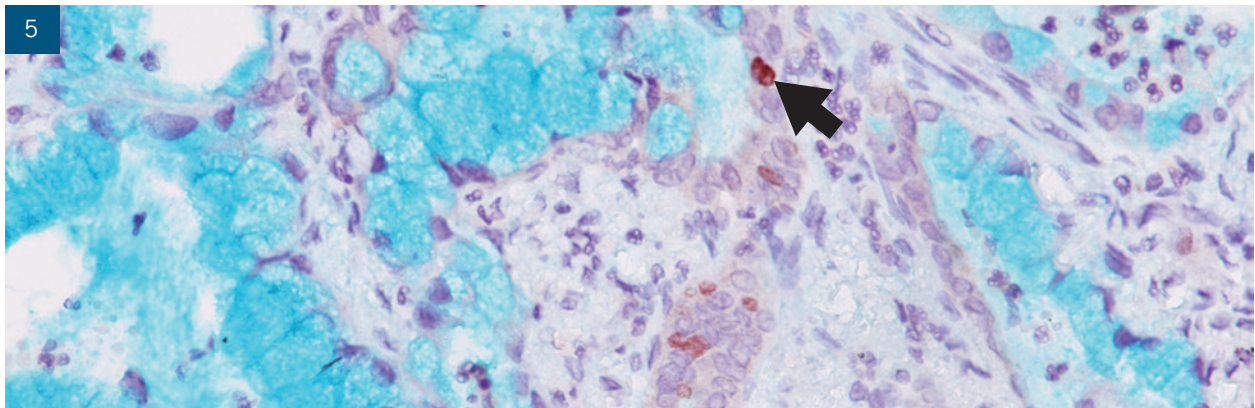
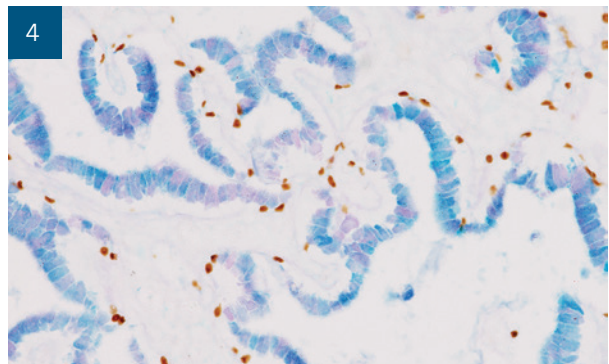
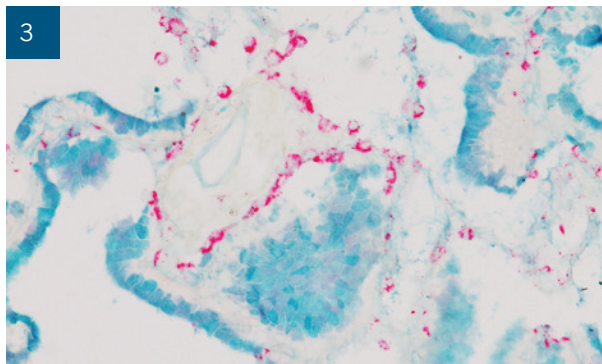
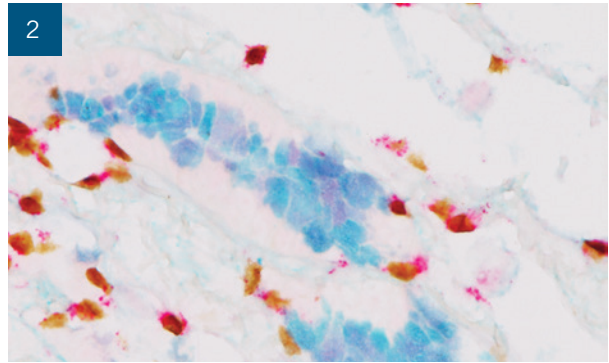
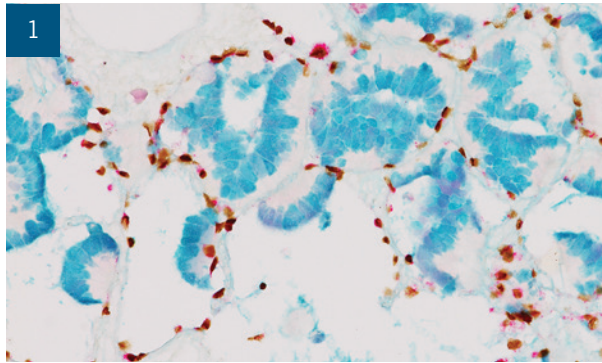
### Staining interpretation

#### Alcian Blue/PAS

Acidic mucins .....Blue  
Neutral mucins .....Magenta/Pink  
Mixtures of above .....Blue/purple

Simultaneous quadruple staining in a single tissue section using Alcian Blue/PAS and TTF-1+ Napsin A was achieved in less than 3 hours. The Alcian Blue/PAS stained shades of blue, to magenta, to purple/blue, TTF-1 stained nuclei brown (DAB) and Napsin A stained cytoplasm (granular) red (Warp Fast Red) (Figures 1-2). Individual stains for Napsin A and TTF-1 with Alcian Blue/PAS staining (Figures 3-4) were compared to the quadruple stain and the single antibodies demonstrated equal staining. Alcian Blue with TTF-1 was compared to the Alcian Blue/PAS with TTF-1 stain and demonstrated absence of PAS staining (Figure 5).

## Figures



Figures 1-5: Lung cancer

Figure 1: Alcian Blue/PAS Napsin A + TTF-1 Cocktail (10x). Figure 2: Alcian Blue/PAS Napsin A + TTF-1 Cocktail (20x). Figure 3: Alcian Blue/PAS + Napsin A. Figure 4: Alcian Blue/PAS + TTF-1. Figure 5: Alcian Blue only + TTF-1 (arrow).

## Conclusion

Alcian Blue/PAS combined with the TTF-1 + Napsin A antibody cocktail in a single section was achieved and may be useful in the evaluation of lung biopsies and tumors of unknown origin. Staining four different sites in a single section saves tissue and provides easier, quicker interpretation for the pathologist. The ability to combine other histochemical stains with immunohistochemical staining may prove to have additional advantages.

## References

1. Kennedy A, Burgin PD. A comparison of different methods of detecting mucin in adenocarcinomas of the lung. *Br J Dis Chest*. 1975 Apr;69(2):137-43
2. Loo PS *et.al*. Subtyping of undifferentiated non-small cell carcinomas in bronchial biopsy specimens. *J Thorac Oncol*. 2010 Apr;5(4):442-7.