

Use of image analysis for interpretation of PIN-4 immunohistochemical staining in prostate needle biopsies

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Introduction

Several immunohistochemistry markers are routinely used by pathologists in the interpretation of prostate biopsies, including P504S (racemase), p63, and the HMW cytokeratins (CK5 and CK14)¹. P504S is a protein preferentially expressed in the cytoplasm of prostatic adenocarcinoma as well as high-grade prostatic intraepithelial neoplasia (HGPIN). p63 and the HMW cytokeratins are expressed in the nucleus and cytoplasm respectively of prostatic basal cells surrounding benign prostatic glands, but not in the secretory cells of these glands². The combination of these markers in the PIN-4 antibody cocktail (Biocare) is useful to the pathologist in the distinction between adenocarcinoma, HGPIN, and benign glands, particularly in cases with limited tissue³. However, correct interpretation of multiple IHC markers staining different subcellular compartments of different cell types can be challenging. An image analysis (IA) algorithm was therefore developed to assist the pathologist in the interpretation of prostatic tissue stained with the PIN-4 cocktail.

Design

Fifty formalin-fixed, paraffin-embedded prostate biopsy cases, each consisting of corresponding H&E and PIN-4 stained slides were selected. DAB chromagen was used to visualize the p63 and HMW cytokeratin antibodies, and AEC chromagen was used to visualize the P504S antibody. Slides were scanned at 20x magnification on the BioImagene iScan™ Slide Scanner. Manual interpretation (manual digital read) was performed on a computer monitor that allowed the pathologist to view whole slide images at magnifications from 1x to 40x. After a one-week wash-out period, the same cases were reviewed using the PIN-4 IA algorithm and BioImagene Virtuoso™ software for selected regions of interest. For manual scoring, cases were categorized as benign, HGPIN, atypical/ASAP or adenocarcinoma. For IA, the algorithm categorized cases as benign, HGPIN or adenocarcinoma.

Figure 1a-i. Examples of different prostate biopsies stained with H&E (a) with corresponding area in PIN-4 IHC study before and after IA (b and c). (20x unless otherwise noted)

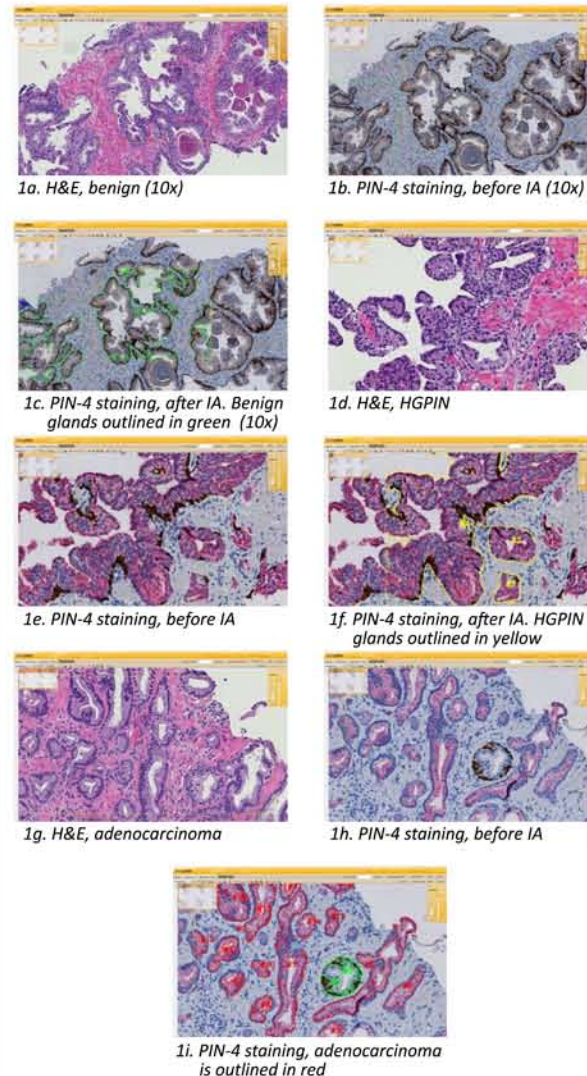


Table 1. Manual digital read vs. IA for interpretation of benign versus abnormal PIN-4 IHC staining. Benign classification includes benign and HGPIN cases. Abnormal classification includes atypical/ASAP and adenocarcinoma cases. Concordance = 98%.

Manual Digital Read	Image Analysis		
		Benign	Abnormal
	Benign	29	1
Abnormal	0	20	

Table 2. Manual digital read vs. IA for interpretation of benign versus benign/HGPIN PIN-4 IHC staining. Benign classification includes benign cases while benign/HGPIN classification includes benign cases with HGPIN. Concordance = 90%.

Manual Digital Read	Image Analysis		
		Benign	Benign + HGPIN
	Benign	16	1
Benign + HGPIN	2	10	

Results

- There is close agreement between manual digital reading and image analysis for interpretation of PIN-4 IHC staining
- IA correctly categorizes glands into benign, HGPIN and malignant categories in most cases
- There is a 98% concordance for benign (benign and HGPIN) vs. abnormal diagnoses (adenocarcinoma and atypical) (refer to Table 1)
- There is a 90% concordance for benign vs. benign/HGPIN (benign and HGPIN) diagnoses (refer to Table 2)
- The IA algorithm does not currently have an atypical category for small numbers of glands lacking basal cell staining. This explains the handful of cases where the manual interpretation was benign or atypical, but the IA interpretation was adenocarcinoma (see Figures 2 and 3).

Literature Cited

1. Weng Leong Ng V, et al. Is Triple Immunostaining With 34BE12, p63, and Racemase in Prostate Cancer Advantageous? *Am J Clin Pathol* 2007;127:248-253
2. Ayala AG, et al. Prostatic Intraepithelial Neoplasia: Recent Advances *Arch Pathol Lab Med* 131(8):1257-1266
3. Molinie V, et al. Diagnostic utility of a p63/alpha-methyl-CoA-racemase (p504s) cocktail in atypical foci in the prostate *Mod Pathol* 2004 17(10):1180-90

Conclusions

- IA is a useful adjunctive tool to aid the pathologist in the interpretation of PIN-4 IHC studies
- The PIN-4 algorithm can identify glands in three categories (refer to Figure1), and there is a high degree of concordance between manual interpretation and automated image analysis.
- To our knowledge, this is the first example of an algorithm to incorporate three color image analysis of an IHC cocktail.
- Planned future versions of the PIN-4 algorithm will include an atypical/ASAP category, perform whole slide pre-analysis, and provide quantification of adenocarcinoma in the sample (% gland and surface area involvement).

Figure 2. Example of an atypical focus (ASAP) in a prostate biopsy stained with H&E (a) with corresponding area in PIN-4 IHC study before and after IA (b and c). (20x) Figure 3. Example of a focus of HGPIN that was interpreted as positive (adenocarcinoma) by IA. This was the discrepant case in the comparison of manual digital reading versus IA (Table 1). H&E and corresponding areas on PIN-4 stained prostate biopsy before and after IA. (20x)

