Usefulness of Cytokeratin 5/6 and AMACR Applied as Double Sequential Immunostains for Diagnostic Assessment of Problematic Prostate Specimens

Kiril Trpkov, MD, FRCPC, Joanna Bartczak-McKay, BSc, MLT, and Asli Yilmaz, MD, FRCPC

Key Words: Prostate carcinoma; Immunohistochemistry; Cytokeratin 5/6; α-Methylacyl-CoA racemase; AMACR; P504S; Double immunohistochemistry

DOI: 10.1309/AJCPGFJP83IXZEUR

Abstract

We evaluated the usefulness of double immunohistochemical staining for cytokeratin (CK)5/6 and α-methylacyl coenzyme A racemase (AMACR) applied sequentially on 1 slide by assessing 223 foci in 110 consecutive prostate specimens. Double-chromogen reaction was used to visualize the antibodies: brown for CK5/6 and red for AMACR. Staining was scored as diffuse, focal, or negative. To establish the diagnosis, CK5/6 and AMACR were correlated with the morphologic features. All cancers lacked CK5/6 staining (100% specificity). AMACR showed diffuse or focal positivity in cancer, high-grade prostatic intraepithelial neoplasia, and atypia in 96.8% (120/124), 85% (22/26), and 80% (16/20) of cases, respectively. In atypical cases, diagnosis was because of non–immunohistochemical staining reasons in 80% of cases. In adenosis (n = 14), AMACR was diffusely positive in 4 cases (29%). Double immunohistochemical staining for CK5/6 and AMACR is a simple assay to perform and may be used as an alternative to antibody cocktails for routine evaluation of problematic prostate specimens.

Diagnostic assessment of problematic foci in prostate specimens aided by immunohistochemical analysis has increased pathologists’ certainty in establishing a definitive diagnosis. Pathologists typically use basal cell markers, which detect cytoplasmic high-molecular-weight cytokeratins, such as 34βE12 antibody, or a nuclear protein, p63, as negative cancer markers, usually combined with α-methylacyl coenzyme A racemase (AMACR) as a positive cancer marker. The usefulness of basal cell antibodies and AMACR has been previously examined and validated in prostate pathology practice by using the antibodies separately, typically in correlation with the H&E morphologic assessment of the focus of interest.1-15 To improve the diagnostic sensitivity and specificity of 34βE12 and p63 antibodies and to enable their use on 1 tissue section for immunohistochemical analysis, they were combined in double basal cell cocktails.16,17 Furthermore, by adding an AMACR antibody to 1 or 2 basal cell antibodies, double (p63 + AMACR)18-23 and triple (p63 + 34βE12 + AMACR)24-26 commercial and noncommercial antibody cocktails were used, which further improved the diagnostic immunohistochemical armamentarium for prostate evaluation.

Since 2003, in our daily practice we have used cytokeratins 5 and 6 (CK5/6) as our principal basal cell marker, which was assessed in correlation with the AMACR immunostain and H&E morphologic examination. Immunostains for CK5/6 and AMACR were performed separately on 2 histologic slides for evaluation of diagnostically problematic prostate foci. CK5 and CK6 are intermediate-sized cytokeratins, and, similar to 34βE12, they are expressed in the basal cells of the prostate and the seminal vesicle and typically exhibit continuous cytoplasmic staining in the basal cells of the benign glands. The usefulness of CK5/6 as a “negative” prostate marker has...
been documented previously, but CK5/6 is not as widely used as 34βE12 or p63. In our practice, CK5/6 is an excellent, consistent, and reliable basal cell marker for the assessment of routine prostate specimens from nontreated patients and from patients treated by radiotherapy, cryotherapy, and hormonal therapy. The lower sensitivity of CK5/6 for prostate cancer, reported in 2 studies, may be due to the use of nonformalin tissue fixatives and nonroutine specimens (eg, tissue microarray) and/or may be due to variability in the CK5/6 antibodies provided by various suppliers, which are different from the CK5/6 antibodies used in our practice and in this study. Recently, we introduced double combined immunohistochemical staining for CK5/6 and AMACR antibodies, which were applied sequentially on 1 tissue section, using an automated immunostainer. To our knowledge, double sequential immunohistochemical staining for CK5/6 and AMACR, visualized by double-chromogen reaction, has not been studied previously for the evaluation of problematic prostate specimens. The aim of this study was to assess the performance of a double-antibody immunostain for CK5/6 and AMACR in evaluating problematic foci in the routine surgical pathology practice involving prostatic specimens.

Materials and Methods

Case Sample

We performed double immunohistochemical staining for CK5/6 and AMACR antibodies for diagnostic evaluation of 223 problematic foci in 110 consecutive prostate specimens (99 needle biopsies, 6 transurethral resections of prostate [TURP], and 5 prostatectomies). The cases were evaluated consecutively in our institution during a 3-month period (May to July 2008); immunohistochemical analysis was performed only in the cases in which a workup was needed owing to diagnostic difficulty. The sample consisted of 124 prostate cancer foci (minimal prostate cancer, 71; nonminimal prostate cancer, 53), 26 high-grade prostatic intraepithelial neoplasia (HPIN) foci, 20 atypical, “suspicious” foci (hereafter referred to as atypical), and 53 benign foci. Minimal prostate cancer on needle biopsy was defined as cancer involving 5% or less of the core or 1 mm or shorter cancer length, and nonminimal cancer exceeded 5% of the core or 1 mm cancer length. The immunostains were used in strict correlation with the H&E morphologic features in establishing the definitive diagnosis. Two needle biopsy specimens were from treated patients (1 each postradiation and postcryotherapy), while remaining specimens were from nontreated patients. Only three biopsy specimens were excluded during the study period owing to tissue loss on immunohistochemical sections. Of note, our practice is a centralized academic urological pathology practice, and our monthly workload includes approximately 120 to 140 prostate needle biopsies, 30 prostatectomies, and 50 to 60 TURPs.

Tissue Processing

Prostate needle biopsy specimens were fixed in IBF fixative (Surgipath, Richmond, IL) for up to 24 hours, as previously described. IBF is an alcohol-based fixative and does not interfere with tissue immunoreactivity for the basal cell markers, such as 34βE12, p63 and CK5/6, or AMACR. For the initial H&E morphologic evaluation, biopsy cores were sectioned at 3 µm in 3 levels, and additional 3-µm sections were cut for immunohistochemical analysis only when required. In addition, 3 deeper H&E levels were sectioned routinely after the immunohistochemical sections to better assess the foci of interest. In our practice, we do not use spare slides between the initial H&E levels, although we are aware that this approach is practiced in some laboratories. Radical prostatectomy and TURP specimens were fixed in 10% neutral buffered formalin for at least 24 hours and were processed routinely according to a standard protocol, as previously described.

Double CK5/6 and AMACR Immunohistochemical Studies

Monoclonal antibodies were sequentially applied on a single histologic slide, and contrasting chromogens were used to visualize the antibodies: brown (diaminobenzidine [DAB]) for CK5/6 and red (fast red) for AMACR. Both antibodies were used individually for 5 years before the double method was introduced. After the initial workup and optimization, the double method was introduced in parallel with the individual immunostains for CK5/6 and AMACR during a 4-week period. Although no formal comparison between the individual and double methods was carried out, it was concluded that the dual method was preferable and more convenient because of the staining consistency and quality and because only 1 slide was assessed for each problematic focus instead of 2.

Paraffin-embedded sections were mounted on coated slides and were placed in an oven for 10 minutes at 60°C. The double-staining protocol was performed using the Ventana Benchmark XT automated immunostainer (Ventana Medical Systems, Tucson, AZ). Deparaffinization and on-board antigen retrieval were performed for 30 minutes at approximately 100°C with CC1 reagent, which is an EDTA-based proprietary Ventana solution (pH 8.0-8.5). CK5/6 mouse monoclonal antibody (clone D5/16 B4, dilution 1/20; Dako Canada, Mississauga, Canada) was applied and incubated for 32 minutes, followed by an ultraWash step (Ventana Medical Systems), to wash off excess antibody. Antibody denaturation for 4 minutes at 90°C was performed.
to ensure that the first primary antibody was completely inactivated before applying the second antibody. The AMACR rabbit monoclonal antibody (clone 13H4, dilution 1/400; Zeta, Sierra Madre, CA) was then applied as a second primary antibody and was incubated for 16 minutes.

The first primary antibody, CK5/6, was visualized using the polymer-based Ventana ultraView DAB detection kit (catalog No. 760-500), and the second primary antibody, AMACR, was visualized using the Ventana ultraView AP Fast Red detection kit (catalog No. 760-501). The slides were counterstained with hematoxylin, washed in Dawn soap and rinsed well in water, dehydrated in graded alcohols, cleared in a xylene sequence, and coverslipped. Negative control slides were routinely stained using a mouse monoclonal antibody (Ventana negative control); in addition, the internal negative control in the prostate tissue was assessed (ie, benign glands negative for AMACR, cancer glands negative for CK5/6). The slides were coverslipped in a timely manner because fast red washes off the slides if left too long in alcohol or xylene.

AMACR typically showed circumferential and subluminal or cytoplasmic finely granular red staining in the secretory epithelial cells in prostate cancer and HPIN glands. CK5/6 typically showed dark brown staining of the basal cells in the benign glands and HPIN glands. Staining for AMACR and CK5/6 antibodies was scored semiquantitatively: diffuse (all glands positive), focal (some glands or gland portions positive),
or negative. Intensity was scored as follows: negative, 0; weak, 1+; moderate, 2+; or strong, 3+.

**Results**

Results for CK5/6 and AMACR double immunostaining in relationship to the final diagnosis are shown in Table 1. The double CK5/6 and AMACR assay performed on 1 tissue section produced consistent immunostaining results and was a reliable tool for the evaluation of problematic prostate foci in all types of prostate specimens, including needle biopsy, prostatectomy, and TURP.

**Prostatic Cancer and HPIN**

Double immunohistochemical staining for CK5/6 and AMACR was not only a useful aid in evaluating minimal prostate cancer (n = 71) and nonminimal prostate cancer (n = 53) in general but also in specific cases representing prostate cancer variants (pseudo-hyperplastic, n = 5; intraductal cancer, n = 3; and foamy, n = 1), metastatic prostate cancer (n = 3), and prostate cancer after cryotherapy (n = 1). AMACR showed essentially identical staining results in nonminimal and minimal cancer (diffuse or focal positivity in 51 [96%] of 53 and 69 [97%] of 71, respectively). Overall, in prostate cancer and HPIN, AMACR was diffusely or focally positive in 120 (96.8%) of 124 and 22 (85%) of 26 foci, respectively. AMACR intensity was graded moderate (2+) to strong (3+) in 103 (83.1%) of 120 and 20 (77%) of 26 foci in prostate cancer and HPIN, respectively. CK5/6 exhibited excellent specificity for prostate cancer, which uniformly lacked CK5/6 staining (100% negative). Three prostate cancers in which an intraductal cancer component was present also contained foci of invasive prostate cancer that completely lacked CK5/6 staining.

**Table 1**

<table>
<thead>
<tr>
<th>Cytokeratin 5/6 (CK5/6)</th>
<th>α-Methylacyl Coenzyme A Racemase (AMACR)</th>
<th>Diagnoses</th>
<th>Diffuse</th>
<th>Focal</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cancer (n = 124)</strong></td>
<td>102 (82)</td>
<td>11 (15)</td>
<td>4 (3.2)</td>
<td>0 (0)</td>
<td>124 (100)</td>
</tr>
<tr>
<td>Minimal (n = 71)</td>
<td>58 (82)</td>
<td>11 (15)</td>
<td>2 (3)</td>
<td>0 (0)</td>
<td>71 (100)</td>
</tr>
<tr>
<td>Nonminimal (n = 53)</td>
<td>44 (83)</td>
<td>7 (13)</td>
<td>2 (4)</td>
<td>0 (0)</td>
<td>53 (100)</td>
</tr>
<tr>
<td>High-grade prostatic intraepithelial neoplasia (n = 26)</td>
<td>15 (58)</td>
<td>7 (27)</td>
<td>4 (15)</td>
<td>5 (19)</td>
<td>21 (81)</td>
</tr>
<tr>
<td>Atypical, “suspicious” (n = 20)</td>
<td>13 (65)</td>
<td>3 (15)</td>
<td>4 (20)</td>
<td>0 (0)</td>
<td>2 (15)</td>
</tr>
<tr>
<td>Benign (n = 53)</td>
<td>5 (9)</td>
<td>17 (32)</td>
<td>31 (58)</td>
<td>24 (45)</td>
<td>28 (53)</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage).

**Image 2**

**A** and **B**, Infiltrative microfocus of prostatic adenocarcinoma (**A**, H&E, ×400). Cancer glands are highlighted by strong cytoplasmic α-methylacyl coenzyme A racemase (AMACR) staining (red). They lack basal cells and are negative for cytokeratin (CK5/6). Adjacent benign glands show moderate to strong cytoplasmic staining for CK5/6 (brown) and are negative for AMACR (**B**, double immunohistochemical stain, AMACR-CK5/6, ×400).
Atypical, Suspicious

A final diagnosis of atypical, suspicious focus was established in 20 foci (10% of the foci evaluated by immunohistochemical analysis). Of these, 6 (30%) of 20 were considered atypical foci adjacent to HPIN, indicating a microfocus of atypical glands in proximity to HPIN that could not be reliably distinguished from HPIN outpouchings. Negative (n = 4) or focal positive (n = 3) AMACR and focal positive CK5/6 (n = 3) were considered contributory to the atypical diagnosis in 10 (50%) of 20 cases. However, the atypical diagnosis was considered to be primarily because of non–immunohistochemical staining reasons in 16 (80%) of 20 cases for the following reasons: microfocus containing fewer than 3 glands (n = 8), bland cytologic features (n = 2), glands at the edge (n = 5), and glands cut out on deeper H&E levels (n = 3) (some foci contained more than one of these features).

HPIN (n = 26) showed mostly fragmented basal cell staining for CK5/6, sometimes restricted to only rare positive basal cells. CK5/6 was graded focally positive in 21 (81%) of 26 foci. In 24 (92%) of 26 HPIN foci, CK5/6 was graded moderate (2+) to strong (3+).

HPIN (n = 26) showed mostly fragmented basal cell staining for CK5/6, sometimes restricted to only rare positive basal cells. CK5/6 was graded focally positive in 21 (81%) of 26 foci. In 24 (92%) of 26 HPIN foci, CK5/6 was graded moderate (2+) to strong (3+).
Benign

The category of benign foci (n = 53) usually demonstrated some elements of worrisome morphologic features, requiring immunohistochemical analysis to be performed. This group included benign crowded glands (n = 20), adenosis/atypical adenomatous hyperplasia (n = 14), atrophy (n = 8), partial atrophy (n = 6), mucinous metaplasia (n = 1), and urothelial metaplasia (n = 1). It is evident that the majority of these problematic foci represented benign mimickers of prostatic carcinoma, in which, not surprisingly, AMACR was diffusely or focally positive (eg, adenosis or partial atrophy), as previously reported. In adenosis (n = 14) and partial atrophy (n = 6), AMACR was diffusely positive in 4 (29%) and 1 (17%) foci, respectively. AMACR was negative in all foci of postatrophic hyperplasia, mucinous metaplasia, and urothelial metaplasia and 1 of 2 foci of basal cell hyperplasia (1 was focally positive). CK5/6 was completely negative in 1 focus of partial atrophy (Images 4C and 4D), while it was focally positive (or partially negative) in 28 (53%) of 53 foci, including 13 (93%) of 14 foci of adenosis, 2 (25%) of 8 atrophic foci, 5 (83%) of 6 partially atrophic foci, and 7 (35%) of 20 foci of benign crowded glands, and in 1 focus of postatrophic hyperplasia.

Discussion

In the present study, we demonstrated that double combined immunostaining for CK5/6 and AMACR represents a useful tool in establishing a definitive diagnosis when assessing problematic prostate specimens. Although the usefulness of prostate antibodies βE12 and p63, used with or without AMACR, has been previously demonstrated in prostate pathology practice,1-26,35-38 the usefulness of double immunostaining for CK5/6 and AMACR applied sequentially has not been examined. To our knowledge, only 1 study used a double sequential method combining AMACR and the basal marker p63 with a single detection chromogen, but this was not done for assessment of routine cases during sign-out.38

The double immunostain for CK5/6 and AMACR is a reliable and sensitive assay that was easy to perform and that produced consistent staining results. In the present study, double immunostaining was performed on consecutive, not preselected cases, and we thought that the examined cases were representative of the common diagnostic problems encountered in day-to-day prostate pathology practices. The double-staining method was particularly useful for the evaluation of minimal prostate cancer, which is a very common problem in assessing prostate needle core biopsy specimens. In this setting, a microfocus demonstrating appropriate morphologic features and a “malignant” immunoprofile (AMACR+, CK5/6–) confirms the diagnosis and increases pathologists’ confidence and accuracy. The double-staining method also enabled us to reliably distinguish prostate cancer from benign prostate cancer mimickers, such as adenosis, atrophy, partial atrophy, and basal cell hyperplasia, and prostate cancer from HPIN. In cases in which a final diagnosis of atypical or suspicious

![Image 3] A and B. Microfocus consisting of a single atypical and “suspicious” gland, which appears close to the edge of the core (A, H&E, ×400). The gland shows strong cytoplasmic α-methylacyl coenzyme A racemase (AMACR) staining and is negative for cytokeratin (CK)5/6 (B, double immunohistochemical stain, AMACR-CK5/6, ×400). In the absence of additional glands on deeper levels, a diagnosis of adenocarcinoma cannot be established with certainty, despite a “malignant” immunoprofile (positive AMACR, negative CK5/6).
was established, it was primarily because of non–immunohistochemical staining reasons in 80% of cases.

The overall sensitivity of 96.8% for AMACR in prostate cancer in this study, using the double assay, confirms the very good to excellent sensitivity of 80% to 100% documented previously in other studies examining the expression of AMACR in prostate cancer.4,5,7,8,12,13,16,21,24,26,29,37,39

In rare cases in which AMACR is negative in suspicious glands, the final diagnosis of cancer rests primarily on the architectural and cytologic findings on H&E morphologic examination, supported by the absence of basal cell staining on immunohistochemical analysis. The overall AMACR sensitivity of 85% for HPIN in this study is also within the range of the previously reported sensitivity of 75% to 95%.9,19,21,37,39,40

In our study, AMACR staining was typically graded moderate (2+) to strong (3+) in approximately 80% of the foci of prostate cancer and HPIN.

We confirm that CK5/6 is an excellent prostate basal cell marker, which can be used in a double immunostain or individually. As with the other basal cell markers, complete lack of CK5/6 staining, indicating absence of basal cells in a suspicious focus, lends support to the diagnosis of prostate cancer.

---

**Image 4**

**A** and **B**, Adenosis (or atypical adenomatous hyperplasia), a benign mimicker of prostate cancer (**A**, H&E, ×200). There is fragmented and focal basal cell staining for cytokeratin (CK)5/6, whereas α-methylacyl coenzyme A racemase (AMACR) shows subluminal staining that is weak to moderate (**B**, double immunohistochemical stain, AMACR-CK5/6, ×200).

**C** and **D**, Partial atrophy, another benign mimicker of prostate cancer (**C**, H&E, ×400), shows complete lack of basal cells and is negative for CK5/6. AMACR is essentially negative in this focus of partial atrophy, and there is only focal and faint cytoplasmic positivity (**D**, double immunohistochemical stain, AMACR-CK5/6, ×400).
One group showed that CK5/6 exhibited superior sensitivity and reliability for the routine assessment of prostate biopsy specimens in comparison with 34βE12 (CK903).\textsuperscript{27} CK5/6 also reliably differentiated atrophy and HPIN from prostatic adenocarcinoma in specimens from patients treated with and without adjuvant hormone therapy.\textsuperscript{28} We have previously shown that CK5/6, used as a single antibody, in combination with AMACR was a valuable marker for the evaluation of routine prostate specimens and in posttreatment specimens (radiotherapy, cryotherapy, and hormonal therapy).\textsuperscript{29,30} We have also observed that the staining for CK5/6 is preserved in the basal cells of nonmalignant glands that are cauterized, crushed, or distorted (eg, close to the tissue edge), which also testifies to the robustness and reliability of the antibody. Of note, focal or diffuse basal cell positivity may be seen with CK5/6 (as with 34βE12 and p63) in some distinct types of prostatic carcinoma, such as ductal carcinoma and intraductal carcinoma (or carcinoma with intraductal spread).\textsuperscript{25}

CK5/6 can be considered an excellent alternative to the other basal cell markers, 34βE12 and p63, which have demonstrated some shortcomings when used individually, resulting in staining variations.\textsuperscript{16,21,22,41-44} For example, 34βE12 staining was shown to be dependent on the type and duration of the tissue fixation and the antigen retrieval technique,\textsuperscript{41-43} while p63 demonstrated loss of immunostaining intensity in stored slides\textsuperscript{22,44} and showed spurious cytoplasmic staining of the luminal cells when used in low dilutions.\textsuperscript{21} Hence, one can understand better the drive to improve the performance of these basal cell markers by combining them in a cocktail, although this was shown to be only marginally advantageous for diagnostic usefulness.\textsuperscript{16,17} We have not noticed any significant performance variations of the CK5/6 antibody using different antibody suppliers (eg, Cell Marque, Hot Springs, AR; DAKO) in a busy day-to-day practice in more than 5 years. Although we have not conducted a formal study of CK5/6 under different circumstances or compared it head to head with the other basal cell markers in a formal study, in our experience and as demonstrated in this study, CK5/6 is an excellent and dependable basal cell marker.

Double immunostaining for CK5/6 and AMACR has several advantages, which make it an attractive alternative to the use of separate immunostains. First, the technique was easy to introduce and was simple to perform using an automated immunostainer and produced immediately consistent staining results for CK5/6 and AMACR, which were previously in use as individual antibodies. Second, this method is performed on 1 tissue section (same as with antibody cocktails), which eliminates the use of separate slides for immunohistochemical analysis and reduces the possible loss of problematic foci during block cutting. Using 1 instead of 2 slides also increases the available space for slide placement in automated immunostainers. Third, assessing the stains on 1 section allows for more convenient microscopic evaluation of the foci of interest and eliminates switching between individual CK5/6 and AMACR immunostains to match the foci under examination. Fourth, the use of contrasting chromogens, such as brown (DAB) for a basal cell marker CK5/6 and red (fast red) for AMACR, allows for easier assessment of the prostate foci in question. The use of a double-chromogen brown and red reaction is advantageous over the monochromatic detection method (brown and brown) for both primary antibodies. For example, a brown cytoplasmic or nuclear basal cell stain may be obscured by the often strong cytoplasmic AMACR brown stain. If only 1 chromogen is used for both antibodies, it may be difficult to see 1 or 2 basal cells in a worrisome glandular microfocus to establish a definitive diagnosis of cancer with confidence. Fifth, double sequential immunostaining can be used as an alternative to commercial or in-house–developed antibody cocktails for the assessment of prostate specimens that combine the basal cell markers 34βE12 and/or p63 with AMACR. Commercial cocktails are typically provided pre diluted, which may limit the options for optimizing the cocktail staining if there are staining heterogeneities or if there is prominent non-specific background staining. When using antibody cocktails, the secondary and tertiary immunostains are often not as high quality as the primary immunostain, being less sensitive and/or less specific. We have not encountered sensitivity as a problem when using the antibodies sequentially, and there is no background staining of the stroma. However, we have noticed that the secondary antibody (AMACR) tends to be picked up slightly more in benign glands in some cases. Regardless whether double sequential or combined antibody cocktails are used, it is necessary for antibodies, detection systems, and the other reagents to be properly titrated, optimized, and evaluated in each laboratory to achieve a level of quality control with consistent staining results before routine diagnostic use.

A minor limitation with the double sequential immunostain- ing is that the procedure is slightly longer than immunostaining with 1 antibody, roughly proportional to incubation with the second primary antibody. The overall length of the double sequential staining method in our laboratory is approximately 3 hours and 30 minutes. Another minor pitfall in the assessment of the prostate specimens by AMACR is that some benign glands may show focal, typically luminal and noncircumferential AMACR staining of weaker intensity (1+), which is much weaker than the staining of the cancer glands. However, this was also present when the antibodies were used individually in our practice, and it is also reported uniformly for the AMACR antibody in other studies, regardless of the technique used.\textsuperscript{4,24} This is usually easy to evaluate on morphologic examination, and it is not a problem because of the uniform positive CK5/6 staining of the basal cells in the benign glands.

This study also confirms that by using the double CK5/6-AMACR immunoassay, some benign mimickers of prostate
cancer, such as adenosis (atypical adenomatous hyperplasia), can be diffusely positive for AMACR in up to 29% of cases
described6,7 and that partial atrophy can be positive for AMACR
or negative for basal cell markers and can even exhibit a malignant
immunoprofile (AMACR positive, basal cell marker
differentiated cases of a benign examination). As reported previously,9,35,36 The awareness of these
scenarios emphasizes the fact that the immunostains should be
used to aid, support, and confirm the diagnosis, which is pri-
marily established by histomorphologic studies. By using this
approach, a small number of cases with a malignant immuno-
profile will still be diagnosed as atypical or suspicious, which,
in this study, was primarily for non–immunohistochemical
staining reasons. Of note, in our practice, the rate of atypical
cases has been consistent (approximately 4% for atypical and
atypical foci adjacent to HPIN cases together), despite the use
of CK5/6 and AMACR antibodies.45
We demonstrated that the double combined immuno-
staining assay, using sequentially applied CK5/6 and AMACR
antibodies, is a valuable aid for the routine assessment of prob-
lematic prostate specimens. This sensitive and specific assay
was easy to perform using an automated immunostainer, and
it allowed for simultaneous evaluation of both markers on 1
slide containing the focus of interest by using a brown and red
double-chromogen reaction. Double CK5/6-AMACR immu-
nohistochemical staining also eliminated the use of separate
slides for each individual antibody, which reduced the risk of
tissue loss, particularly in limited needle biopsy specimens.
The double assay can also be used as an alternative to commer-
cial or in-house–developed antibody cocktails, combining
basal cell antibodies or basal cell antibodies with AMACR.
We also reaffirmed that CK5/6 can be used as an alternative
to other prostate basal cell antibodies because it is an excellent
basal cell marker.

From the Department of Pathology and Laboratory Medicine,
Calgary Laboratory Services and University of Calgary, Calgary,
Canada.

Address reprint requests to Dr Trpkov: Anatomical
Pathology, Rockyview General Hospital, Department of Pathology
and Laboratory Medicine, Calgary Laboratory Services and
University of Calgary, 7007 - 14 Street SW, Calgary, Alberta,
Canada, T2V 1P9.

Acknowledgments: We acknowledge the contributions of
Tracy Lenek and Susan Hut for technical assistance with the
introduction of CK5/6 and AMACR in the Immunolab of Calgary
Laboratory Services. We also thank and acknowledge the staff of
the Immunolab for excellent technical assistance in performing the
immunohistochemical stains.

References

1. Hendrick L, Epstein JI. Use of keratin 903 as an adjunct

2. Wojno KJ, Epstein JI. The utility of basal-cell specific anti-
cytokeratin antibody 34BE12 in the diagnosis of prostate


immunohistochemical detection in 403 prostatic specimens
2002;26:1588-1596.

racemase, a useful marker for the diagnosis of small foci of
2002;26:1169-1173.

methylacyl-CoA racemase (P504S) in atypical adenomatous

usefulness of monoclonal antibody P504S in the workup of
2003;120:737-745.

racemase: a variable sensitive immunohistochemical marker for

of alpha-methylacyl-CoA racemase (P504S) in foamy gland

methylacyl-CoA racemase contribute to resolving an atypical
diagnosis on prostate needle biopsy beyond that provided by

11. Faninola MA, Epstein JI. Utility of immunohistochemistry
for alpha-methylacyl-CoA racemase in distinguishing
atrophic prostate cancer from benign atrophy. Hum Pathol.

evaluation of AMACR (P504S) and basal cell markers in the
assessment of routine prostate needle biopsy specimens. Hum Pathol.

boosts diagnostic resolution in “suspicious” foci in prostate

14. Weinstein MH, Signoretti S, Loda M. Diagnostic utility of
immunohistochemical staining for p63, a sensitive marker for

cell-specific markers 34BE12 and p63, in the diagnosis of prostate

p63) improves the detection of prostate basal cells. Am J Surg Pathol.

17. Shah RB, Kunju LP, Shen R, et al. Usefulness of basal cell
cocktail (34BE12 + p63) in the diagnosis of atypical prostatic

of a p63/alpha-methylacyl-CoA racemase (p504s) cocktail in

p63/alpha-methylacyl coenzyme A racemase immunohistochemical
cocktail stain in prostate needle biopsy specimens and tissue


