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► **To cite this version:**

Anurag Joshi, Murali Varma, Bharat Jasani, Sue Wozniac. Potential unreliability of normal tissue as positive control in diagnostic immunohistochemistry of poorly differentiated carcinoma. *Histopathology*, 2010, 57 (5), pp.760. 10.1111/j.1365-2559.2010.03692.x . hal-00593445

HAL Id: hal-00593445

<https://hal.science/hal-00593445>

Submitted on 16 May 2011

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Potential unreliability of normal tissue as positive control in diagnostic immunohistochemistry of poorly differentiated carcinoma

Journal:	<i>Histopathology</i>
Manuscript ID:	HISTOP-03-10-0150.R1
Manuscript Type:	Short Report
Date Submitted by the Author:	08-Apr-2010
Complete List of Authors:	Joshi, Anurag; University Hospital of Wales, Histopathology Varma, Murali; University Hospital of Wales, Histopathology Jasani, Bharat; Cardiff University, Histopathology Wozniac, Sue; University Hospital of Wales, Histopathology
Keywords:	immunohistochemistry, internal control, tumour differentiation, prostate cancer



Review

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Running title:

Lesson of the month

Keywords:

immunohistochemistry, internal control, tumour differentiation, prostate cancer

Lesson of the Month

Potential unreliability of normal tissue as positive control in diagnostic immunohistochemistry of poorly differentiated carcinoma

Judicious use of positive and negative tissue controls is an important part of the technical quality assurance in immunohistochemistry. Positive controls may be 'external', where known positive tissue is immunostained in parallel to the test slide; or 'internal', with known positive tissue/ cells present within the tested tissue section. Internal controls are generally considered superior to external controls as the control cells have been subjected to identical conditions of fixation and immunostaining as the test cells. We describe 2 cases to demonstrate that even an apparently good quality internal control result is not adequate proof of assay sensitivity.

High-molecular weight cytokeratin (HMWCK) antibody clone 34 β E12 is commonly used to help distinguish high-grade urothelial carcinoma from prostatic adenocarcinoma as it is consistently expressed by urothelial carcinoma but negative in prostate cancer^{1,2}. The two cases described below were both referred to us for consultation with a differential diagnosis of urothelial vs. prostatic carcinoma. Tumour cells were judged to be immunonegative for prostatic markers as well as 34 β E12 immunostaining (supported by adequately strong positive reactions observed in the internal control tissue elements). Despite this, unequivocal positivity for 34 β E12 was observed using a more sensitive assay in our laboratory- indicating a diagnosis of urothelial carcinoma.

CASE HISTORIES

CASE 1

Prostate needle biopsies performed on a 66 year old man with a 'malignant feeling' prostate and serum PSA <0.1ng/L, revealed a poorly differentiated carcinoma (Fig 1-A) that was negative for immunohistochemical staining with PSA (Prostate Specific Antigen) and HMWCK (clone 34 β E12, DAKO, 1:50, Labvision autostainer, pre-treatment with Trypsin for 12 minutes, 37°C, pH7.8). The basal cells of adjacent benign prostate glands were strongly 34 β E12 positive (Fig 1- B). The appearances were considered consistent with a high grade prostate carcinoma, however, in the absence of PSA staining, urothelial carcinoma could not be excluded and the case was referred to us.

At our institution, we confirmed the negativity for PSA and PSAP (prostate specific acid phosphatase). However, staining for HMWCK in our laboratory (clone 34 β E12; DAKO; 1:40; ChemMate/ HRP detection kit; microwave pre-treatment 800W 20min, EDTA pH7.0) revealed diffuse and intense staining of tumour cells (Fig 1- C). A diagnosis of high grade urothelial carcinoma was made and confirmed on a subsequent radical cysto-prostatectomy (Fig 1- D).

CASE 2

Transurethral resection of the prostate (TURP) performed on a 73-year-old man for urinary retention (PSA 1.5ng/L) showed a poorly differentiated carcinoma (Fig 2- A) that was negative for PSA, PSAP and HMWCK (clone 34 β E12, DAKO, Ventana autostainer, Protease 1 pretreatment) (Fig 2- B). Background benign urothelium was strongly 34 β E12 positive (Fig 2- B, inset). Because of this immunohistochemical

profile, the origin of this tumour was considered uncertain and the case was referred for our further opinion.

Immunohistochemistry performed in our laboratory confirmed that the tumour cells were negative for PSA and PSAP, however, HMWCK (clone 34 β E12; DAKO; 1:40; ChemMate/ HRP detection kit; microwave pre-treatment 800W 20min, EDTA pH7.0) stained the tumour cells diffusely and intensely (Fig 2- C). The immunohistochemical staining profile was thus interpreted as in keeping with high-grade urothelial carcinoma.

Two months after the TURP, the patient expired because of unrelated medical causes. There had been no clinical, serological or radiological evidence of prostate cancer.

COMMENT

Positive controls are used to exclude false negative results resulting from suboptimal immunostaining assay performance. In the two cases presented, 34 β E12 immunostaining had been considered satisfactory due to strong immunoreactivity in the internal positive control tissue. However, the original immunohistochemical method used in these two cases has been shown to be suboptimal with respect to the sensitivity of 34 β E12 immunostaining as an urothelial marker (a heat induced antigen retrieval method is preferred to enzyme predigestion in this scenario)³.

The cases highlight an important issue regarding reliance upon internal positive controls in routine immunohistochemistry. Internal controls are generally regarded as superior to external controls by virtue of these 'control' cell populations having been subjected to identical fixation as the tumour cells being investigated. In these cases, although the original

34 β E12 immunostaining showed strong positivity in the positive internal controls (basal cells of benign prostatic acini in case 1 and benign urothelium in case 2) the urothelial carcinoma cells were falsely negative, as subsequently shown by a more sensitive method. The sensitivity of 34 β E12 as an urothelial marker has been shown to be higher with heat retrieval pre-treatment as used in our laboratory as compared to enzyme pre-digestion that had been employed in the referring laboratories³.

Internal positive controls are most reliable when they are similar in their antigen content and distribution to the “tumour cells”. Under these circumstances, the quality control of assay sensitivity afforded by them is considered ideal; as is the case when 34 β E12 is used as a basal cell marker to support the diagnosis of prostatic adenocarcinoma (where the basal cells of benign prostatic glands serve as the internal control).

It should be noted that the same issues would also apply to external positive controls. The external positive control tissue must be appropriate to the tissue being tested. Thus, when 34 β E12 is used as a prostatic basal cell marker, benign prostate would be a suitable internal/external positive control. However, if the same antibody is used as an urothelial marker, the ideal external control would be high-grade urothelial carcinoma.

Figure 1

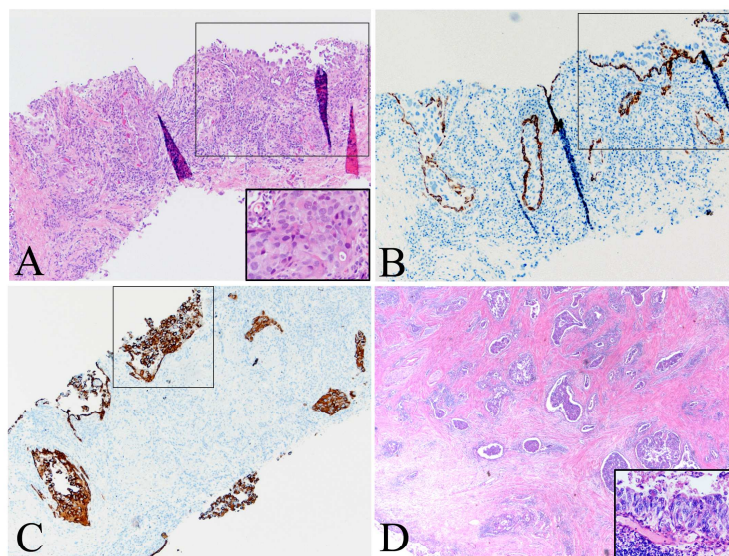


Fig 1

- A) Prostate biopsy with poorly differentiated carcinoma (H&E)
 B) Tumour cells are negative on 34βE12 immunostaining after enzyme predigestion, note positive staining in prostatic basal cells (internal control)
 C) Diffusely positive tumour cells on 34βE12 immunostaining after heat mediated antigen retrieval.
 D) Follow-up cystectomy specimen showing invasive urothelial carcinoma and urothelial carcinoma in situ (inset) (H&E)

209x297mm (300 x 300 DPI)

Figure 2

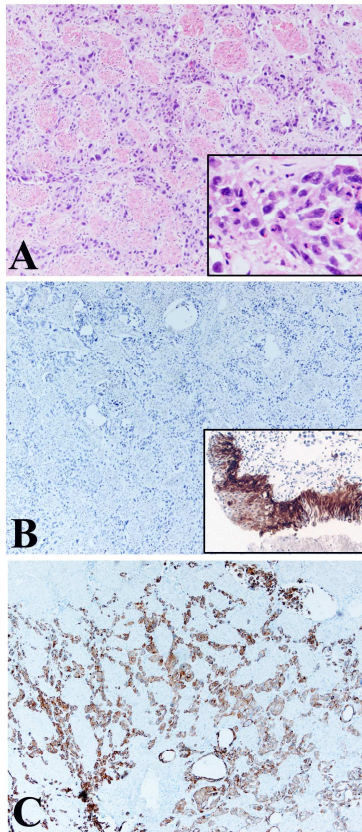


Fig 2

- A) TURP specimen with poorly differentiated carcinoma (H&E)
B) Tumour cells are negative on 34βE12 immunostaining after enzyme predigestion, note the strongly positive staining in normal urothelium present elsewhere in the section (inset).
C) Tumour cells diffusely positive on 34βE12 immunostaining after heat mediated antigen retrieval.

209x297mm (300 x 300 DPI)

FIGURE LEGENDS

Fig 1

- A) Prostate biopsy with poorly differentiated carcinoma (H&E)
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Fig 2

- A) TURP specimen with poorly differentiated carcinoma (H&E)
- B) Tumour cells are negative on 34 β E12 immunostaining after enzyme predigestion, note the strongly positive staining in normal urothelium present elsewhere in the section (inset).
- C) Tumour cells diffusely positive on 34 β E12 immunostaining after heat mediated antigen retrieval.

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