Markers of genitourinary tract in NordiQC assessments

Jan Klos, MD
Stavanger University Hospital
Stavanger, Norway
2004 -2017

- PSA, PAP, PROSTEIN, NKX3.1, AMACR/P504S
- P63, p40, HMWCK
- PAX8 (PAX2)
- GATA3
- PLAP, OCT3/4, SALL4
2004-2017

- 14 markers
- 1-4 assessments/marker
- 28 Assessments
- 136 Tissue cores
- 132 Evaluations of RTU products
- 253 Evaluations of concentrated antibodies
- 3095 Evaluations of individual laboratories
- 9 - 284 Participating laboratories/Assessment
PSA and PAP, Prostein/P501S

Proteins which are highly specific for prostate glandular epithelium.

**PSA:** 4 assessments; pass rate 74-90%; 726 participants

**PAP:** 1 assessment; pass rate 88%; 24 participants

**Prostein:** 1 assessment; pass rate 77%; 11 participants
Run 40. PSA. Photo 4a and 4b
Run 12. PAP. Photo 2a and 2b

Run 27. Prostein/p501S.
Photo 1b and 2b
**Optimal results**

**5-10 concentrated/14 RTU**

**PSA**: mAb 35H9 and ER-PR8*, rmAb EP109 and pAb 0562

*Lower frequency of optimal results using recommended vendor`s protocol settings for RTU indicates the need of protocol modification for optimal results.

**2 concentrated**

**PAP**: mAb PASE/4LJ and pAb A0627

**1 Concentrated / 1 RTU**

**Prostein/P501S**: mAb 10-E3
NKX3.1

Nuclear transcription factor with critical function in prostate development and tumor suppression.

Gives nuclear staining of prostate epithelium, spermatogonia, bronchial mucous glands and focally urothelium of the ureters.

Application: confirming the origin from prostate: (60-90% of prostate adenocarcinoma including 20% of small cell prostate ca), but positive also in 15-30% lobular and 2-5% of ductal breast carcinoma.

The epitope seems to be fixation sensitive according to internal data from NordiQC

1 assessment; 65% pass rate; 49 participants
5 concentrated / 5 RTU

**Optimal results:** rmAb clone EP356 (10/15), pAb CP422 (5/17), mAb UMAB196 (1/1) and RTU based on EP356*.

*Protocol settings recommended by vendor have to be modified for optimal results*
AMACR/p504S

Enzyme involved in the beta-oxidation of branched-chain fatty acids and their derivatives. Not specific for the prostate, but overexpressed in >90% prostate adenocarcinomas and PIN. Should always be interpreted with basal cell markers.

3 assessments; 89-90% pass rate; 171 participants

2-6 concentrated / 4 RTU

Optimal staining: rmAb 13H4 as well as pAb CP200/PP200 and pAb12498 alone or in cocktails and RTU products
P63 and P40 (ΔNp63)

Transcription factor, member of p53 gene family, present in several isoforms, which regulate growth and development of epithelial organs. Mostly used for demonstration of myoepithelial/basal cells, but also in subtyping of carcinoma. When confirming the line of differentiation the expression of P63 should be interpreted together with the staining for high molecular weight cytokeratins, while P40 expression seems to be more restricted to squamous, urothelial and myoepithelial cells.

Both are performing well as markers of myoepithelial cells.

Antibodies to P63 give also positive staining in nuclei of transformed B-lymphocytes and cross-react with epitope in the cytoplasm of striated muscle.

2-6 concentrated / 3-7 RTU

P63: 4 assessments; pass rate 20-95%; 688 participants

8-14 concentrated/ 6-7 RTU

P40: 2 assessments; pass rate 56-74%; 211 participants
Optimal staining for P63: mAb 4A4*, DAK-p63 and rmAb DBR16.1
Optimal staining for P40: mAb BC28 and rmAb ZR8
* Protocol settings recommended by vendor often had to be modified for optimal results
P63 in Lymphoma and striated muscle

Pleural fluid - DLBCL

Striated muscle
Cytokeratin Family: many different intracytoplasmic structural proteins - a dominating intermediate filament protein of epithelia and epithelial tumors, but present also in other cell types. Expression depends on cell type and differentiation status. Pattern of cytokeratin expression is helpful in differential diagnosis of tumors and defining primary location of CUP.

Antibodies to High Molecular Weight Cytokeratins reacted with epitopes on: CK1 (67 KD), CK5 (58 kD), CK6 (56 kD), CK10 (56.5 kD), CK14 (50 kD).
High Molecular Weight Cytokeratins

Run 38. Optimal staining for CK-HMW (CK5)

Squamous cell carcinoma of the lung

Prostate adenocarcinoma and PIN
High Molecular Weight Cytokeratins

5-10 concentrated / 9-15 RTU

**Optimal results:** mAb XM26 (CK5), D5/16 B4 (CK5/6), LL002 (CK14) and RTU with rmAb SP53 (CK5)

When the clone **34BE12 (called also CKHMW)** is applied with HIER and sensitive protocol settings, it reacts in addition to epitopes on CKs 1, 5, 10 and 14 also with other epitope probably on degenerated CK19, present in the same cellular compartment. **This may lead to false positive interpretation!**

This cross reactivity is reduced, but not completely eliminated by using proteolytic pre-treatment or a combined pre-treatment using both HIER and proteolysis.

**The clone 34BE12** is considered inappropriate as a general marker for High Molecular Weight Cytokeratins, but still may work well for demonstration of basal cells in prostate.
Run38. Optimal staining for CK-HMW (CK5)

High Molecular weight Cytokeratin

False positive staining for CK-HMW using clone 34BE12

Breast adenocarcinoma

Normal liver

Normal liver

Breast adenocarcinoma
Clone 34BE12 can sometimes be an inspiration
PAX8/PAX2

Transcription factor critical for development of eye, thyroid, urinary and reproductive organs. Nuclear staining detected in the tumors of thymus, thyroid gland, kidney/upper urinary tract and Müllerian system and some other organs. Antibodies raised to N-terminal of the PAX8 protein give positive staining in B-lymphocytes due to the high sequence homology of the N-terminal regions of with PAX5, resulting in cross reactivity.

No optimal results achieved with PAX2 (3 concentrated Abs and 2 RTU products) among 9 laboratories submitting staining for the Run 34.

8-13 concentrated / 5-6 RTU
PAX8: 2 assessments; pass rate 64-71%; 158 participants

Successful Abs: mAbs MRQ-50* and BC12, ILQ-150, rmAb ZR-1 as well as pAbs 10336-1-AP and CP379, NBP-32440 and 363A.
(mAb BC12 and rmAb ZR-1 against C-terminal do not cross-react with PAX5)
*Commonly used mAb MRQ-50 gives significantly lower proportion of optimal results on Ventana platform, but improves with high pH HIER and 3 step polymer.
PAX8

Run 42 2a. Optimal staining of the kidney
Run 42 2b. Insufficient staining of the kidney
Run 42 4a. Optimal staining in RCC
Run 42 4b. Insufficient staining in RCC
Multifunctional transcription factor regulating development and function of ductal epithelial cells of the breast, urothelium, epidermis, skin adnexa, and a subsets of T cells. GATA3 expressed in >90% of primary and metastatic ductal and lobular carcinomas of the breast, urothelial and skin carcinomas, trophoblastic and endodermal sinus tumors as well as many other tumor types. Rarely expressed in mesenchymal tumors. Serves as a useful positive marker in the characterization of mammary and urothelial, but also renal and germ cell tumors, mesotheliomas, and paragangliomas.

1 assessment; pass rate 72%; 124 participants

2 concentrated / 7 RTU
Optimal results: mAb L50-823 and corresponding RTU formats
GATA3

Optimal staining

Run 44 2a. Tonsil

Insufficient staining

Run 44 2b. Tonsil

Run 44 3a. Breast carcinoma

Run 44 3b. Breast carcinoma
PLAP, OCT3/4 and SALL4

- **PLAP** - membrane bound enzyme important for cell migration and transport through cell membranes. Marker of germ cell neoplasia but present also in other tumors.

- **OCT3/4** - transcription factor specifically expressed in cells with pluripotent capacity, i.e., embryonic stem cells and germinal cells. Specific for germ cell tumors except yolk sac tumor.

- **SALL4** - transcription factor a master regulator of embryonal pluripotency together with the other pluripotency-related transcription factors. Is a highly sensitive marker for germ cell tumors but it is not entirely specific.
**PLAP**

**Optimal**

Seminoma classic

**Insufficient**

5-8 concentrated / 5 RTU

**PLAP:** 3 assessments; pass rate 52-91%; 324 participants

**Optimal results:** mAbs 8A9*, NB10, PL8-F6** and rmAb SP15

* MAG and weak to moderate cross-reactivity in smooth and striated muscle cells

** MAG staining and cytoplasmic staining of satellite cells of peripheral nerves
Run **20 3b.** The epithelial cells in appendix (blood group A) - positive MAG reaction with mAb clone 8A9.

Run **14 3b.** Staining of smooth muscle in appendix and striated muscle cells in tongue with clone 8A9.

**Run 35 3a and 3b.** MAG reaction and lot to lot variation with mAb clone PL8-F6

Appendix mAb clone PL8-F6 lot MU22808

Appendix mAb clone PL8-F6 lot MU22612
Optimal staining of the intratubular germ cell neoplasia.

Insufficient staining of the intratubular germ cell neoplasia.

3-6 concentrated / 4 RTU
OCT3/4: 2 assessments; pass rate 77-83%; 61 participants

Optimal results: mAb clones MRQ-10*, C-10** and N1-NK
*Cross-reacts with epitopes in neuroendocrine cells, follicular dendritic cells and myofibroblasts.
**Less successful performance on Ventana platform
OCT3/4

Run 35. Photo 2a and 2b

Run 35. Photo 5a and 5b
OCT3/4

Run 36 3a. mAb clone MRQ-10 appendix.

Run 36 3b. mAb clone C-10 in the appendix

Run 36 4a. mAb MRQ-10 embryonal ca.

Run 36 4b. mAb C-10 embryonal ca.
Optimal results: mAb 6E3 as a concentrate and RTU
Concentrated Abs vs. RTU

The graph compares the concentrations of several proteins: PSA, NKX3.1, AMACR, P63, GATA3, PLAP, and OCT3/4, between Concentrated Abs and RTU. The y-axis represents the concentration levels, ranging from 0 to 80, while the x-axis lists the proteins.

Each protein is represented by two bars: one for Concentrated Abs (brown) and one for RTU (pink). The concentrations for each protein are visually compared to identify any differences between the two conditions.
Conclusions

• Increasing number of participants and products
• Increasing number of successful RTU products
• Some RTU products still need modifications for optimal results
• Increasing awareness of factors relevant for optimal results (platforms, clones, elements of protocol)

More details on www.nordiqc.org