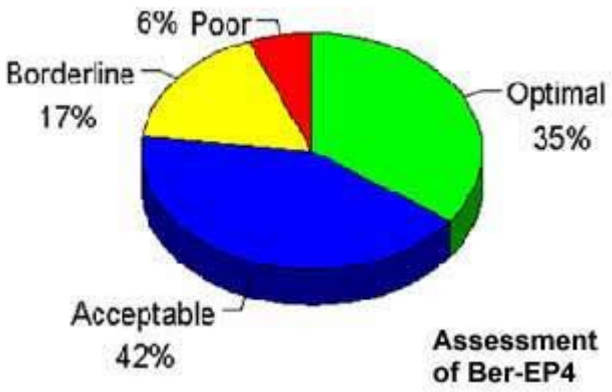
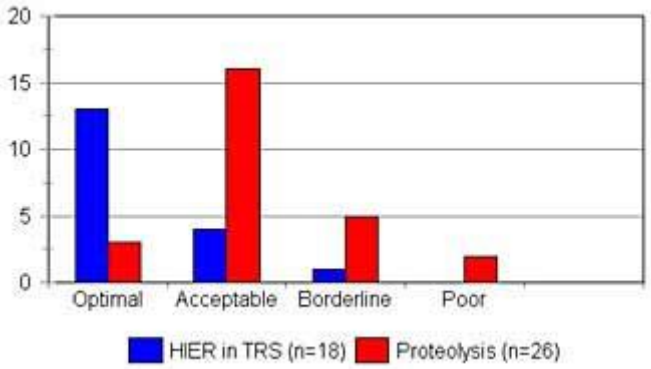
Assessment Run 6 2002

Epithelial antigen Ber-EP4 (Ber-EP4)

The slides to be stained for Epithelial antigen (EA) included an ovary (specimen 2; EA in epithelial inclusion glands and oocytes), appendix (specimen 3; EA in mucosal enterocytes), kidney (specimen 4; EA in collecting tubules and patchy in Bowman's capsule) and adrenal cortex (specimen 6; no EA), a testis/epididymis with malignant mesothelioma (specimen 1; EA in epididymis epithelium), and a lung adenocarcinoma (specimen 5; EA in tumour cells).

48 laboratories submitted stainings. All used mAb Ber-EP4 from DakoCytomation. At the assessment, 17 were deemed optimal, 20 acceptable, 8 borderline and 3 poor (see left diagram). The basis for an optimal result was an intense and distinct staining of cell membranes - particularly basolateral - and less pronounced diffuse cytoplasmatic staining of cells expected to stain.





The best pre-treatment appeared to be HIER in Target Retrieval Solution (TRS; S1699 or S1700, DakoCytomation). 13 out of 18 laboratories which obtained an optimal result used this pre-treatment. Antigen retrieval could also be obtained with proteolytic pre-treatment (Proteinase K or Protease type XXIV, Sigma). However, an optimal result was seen in only 3 out of 26 laboratories (see right diagram).

The most frequent parameters giving suboptimal reactions were:
- insufficient HIER (citrate buffer pH 6), and
- inadequate proteolytic digestion.

This was most evident in the lung carcinoma, whereas in the appendix the enterocytes stained well in almost all protocols. This emphasises the need for a careful selection of control tissue, e.g., kidney (confer Fig. 1a).

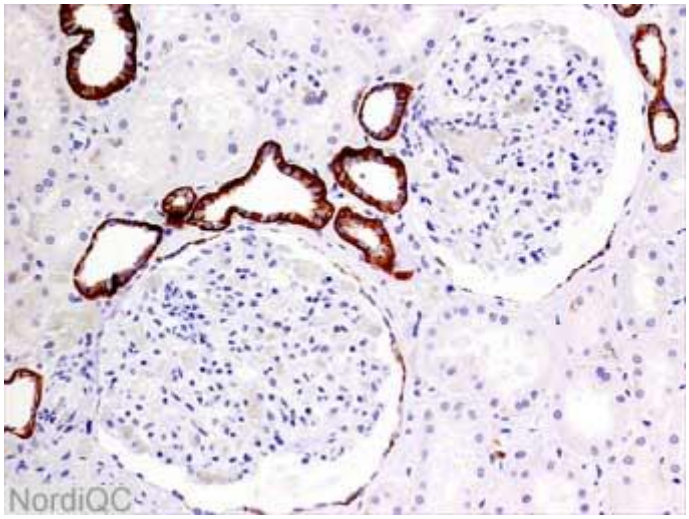


Fig. 1a
Optimal staining of kidney using mAb Ber-EP4. The collecting renal tubules show intense staining with basolateral memebrane enhancement. Also some cells of the Bowman's capsule are stained. The proximal tubules are unstained.

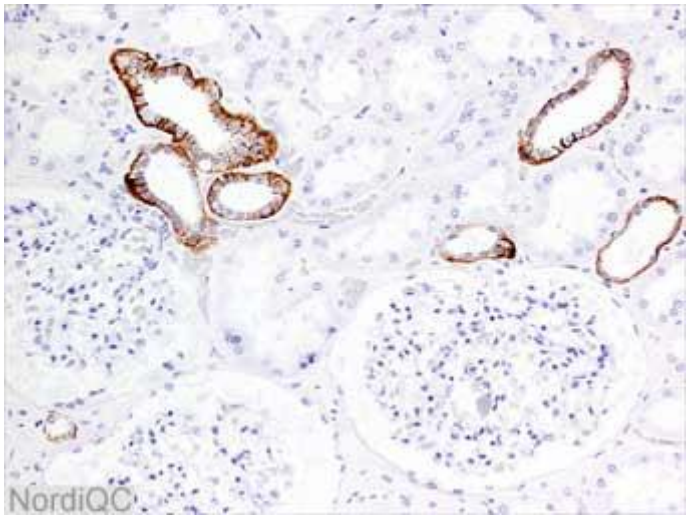


Fig. 1b
Acceptable staining. Same field and Ab as in Fig. 1a. The collecting renal tubules show weaker staining than in Fig. 1a, and the Bowman's capsule is unstained.

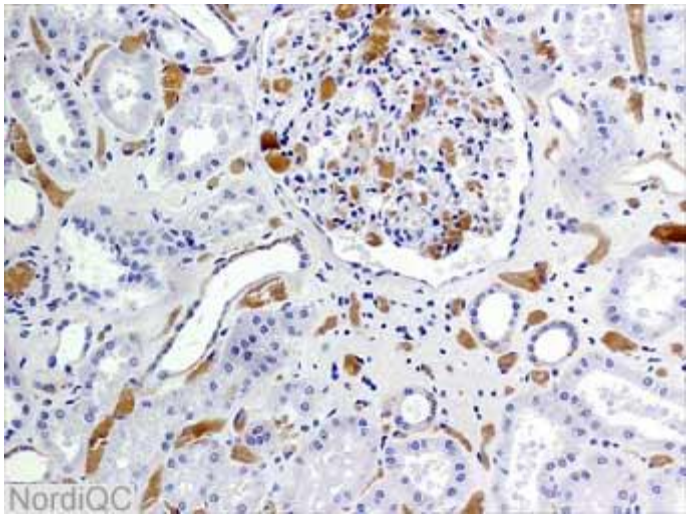


Fig. 1c
Insufficient staining. Same field and Ab as in Fig. 1a. The collecting tubules are unstained. False positive staining of erythrocytes (unsuppressed pseudoperoxidase).

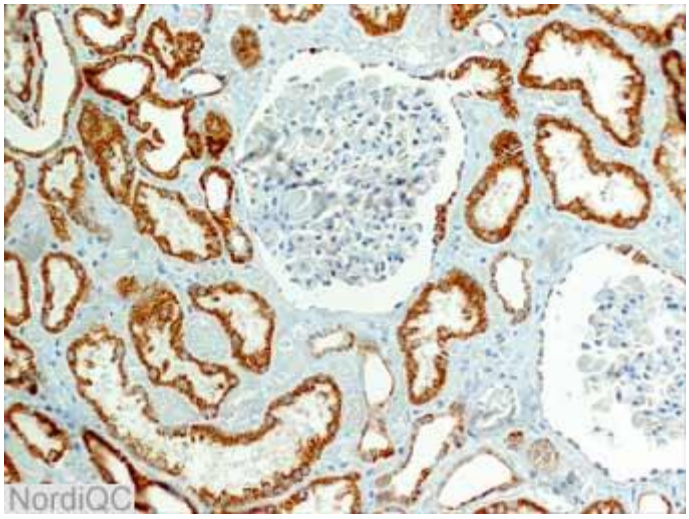


Fig. 1d
Insufficient staining. Same field and Ab as in Fig. 1a. The tubules (particularly the proximal) show false positivety due to endogenous biotin.

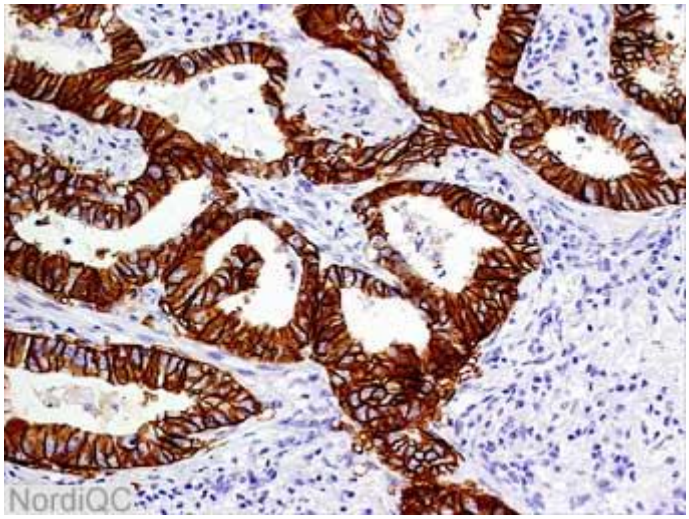


Fig. 2a
Optimal staining of lung adenocarcinoma using mAb Ber-EP4. Same protocol as in Fig. 1a. Intense staining of all tumour cells.

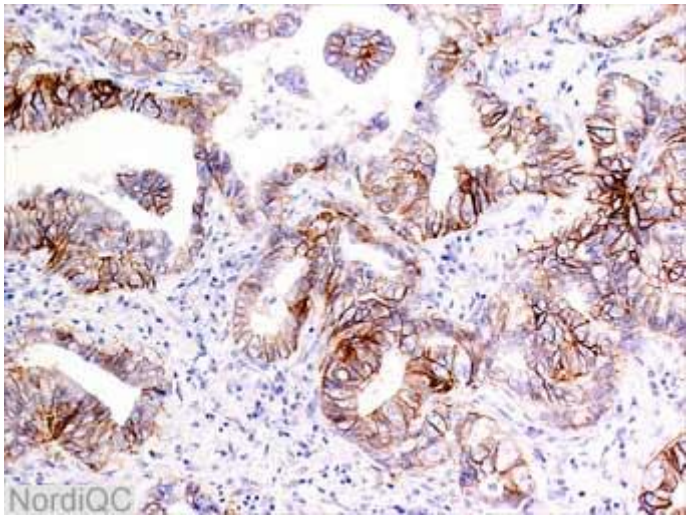


Fig. 2b
Acceptable staining of lung adenocarcinoma. Same field and Ab as in Fig. 1a. Same protocol as in Fig. 1b.

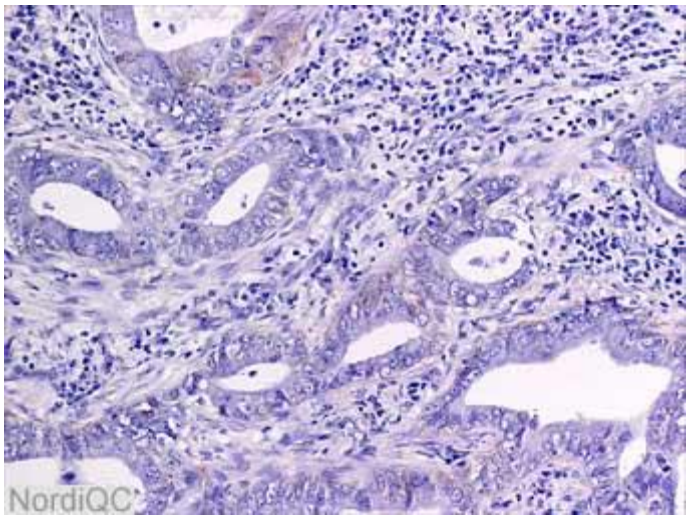


Fig. 2c
Insufficient staining of lung adenocarcinoma. Same field and Ab as in Fig. 2a. Same protocol as in Fig. 1c. The tumour cells are weakly stained or unstained.

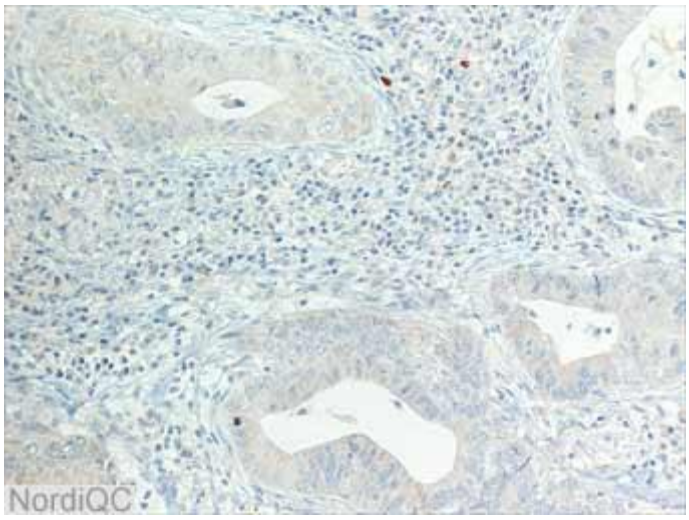


Fig. 2d
Insufficient staining of lung adenocarcinoma. Same field and Ab as in Fig. 2a. Same protocol as in Fig. 1d. The epithelial antigen of the tumour cells is unstained, but a weak false positive staining due to endogenous biotin is seen as a granular product in the cytoplasm.

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