

# ULTRARAPID CYTOKERATIN IMMUNOHISTOCHEMISTRY FOR INTRAOPERATIVE ASSESSMENT OF SENTINEL LYMPH NODES

Visa Manni<sup>1</sup>, Mika Tirkkonen<sup>2</sup>, Leo Paljärvi<sup>3</sup>, Teppo Haapaniemi<sup>4</sup>, Taneli Tani<sup>4</sup>, and Jorma Isola<sup>1</sup>

<sup>1</sup> Institute of Biosciences and Medical Technology (BioMediTech), University of Tampere, Tampere, Finland.

<sup>2</sup> Fimlab Laboratories, University Hospital of Tampere, Tampere, Finland.

<sup>3</sup> Kanta-Häme Central Hospital, Department of Pathology, Hämeenlinna, Finland.

<sup>4</sup> Päijät-Häme Central Hospital, Department of Pathology, Lahti, Finland.



UNIVERSITY OF TAMPERE

## Background

- Frozen section diagnosis of sentinel lymph nodes is often requested intraoperatively. Micrometases are known to be missed in a significant fraction of cases when using morphologic stain (TB, H&E) only.
- We studied the feasibility of ultrarapid cytokeratin immunohistochemistry (U-IHC) and compared the results with standard intraoperative histology and final postoperative paraffin section histologic findings.

## Sample preparation

- One to four sentinel nodes (average 2) were studied for each patient. Each sentinel node was sliced at 2 mm intervals. Slices were embedded into a single cryoblock and cut at 5-7  $\mu$ m sections (Fig 1). Altogether N=1013 clinical breast cancer cases were studied.



Figure 1. Slicing of sentinel node and embedding technique into a cryoblock. Modified from Krogerus et al. *Histopathology* 2004; 44: 29-34.

## Ultrarapid Immunohistochemistry

- Two peroxidase-conjugated monoclonal antibodies (pan-CK or CK19) as part of ready to use reagent kits: Cytonel-Plus or Cytonel-19 (Jilab Inc., Tampere, Finland) were used.
- U-IHC protocol consisted of acetone fixation (2 min), followed by incubations with anti-cytokeratin-HRP conjugate (3 min at +42C), DAB chromogen (1.5 min at +42C), and hematoxylin counterstain (10 sec.) using Cytonel-Plus and Cytonel-19 kits (Jilab Inc., Tampere, Finland). In total, ultrarapid intraoperative IHC took ~8-9 min to perform. Example stainings are shown in Figs. 2 and 3.
- Human appendix and colon were used as positive controls.

## Results

- Study sets from three pathology laboratories consisted of 60, 120 and 833 consecutive sentinel node operations. The rate of late positives (=micro or macrometastases missed) without intraoperative IHC ranged from 6-13%, and dropped to 3-8% when the information of U-IHC was taken into account (late positives reduced by 38-55%) (Table 1).

## Further reading and literature

Holm M et al. Intraoperative immunohistochemistry staining of sentinel nodes in breast cancer: clinical and economical implications. *Breast* 2008;17:372-5.

Krishnamurthy S et al. A prospective study comparing touch imprint cytology, frozen section analysis, and rapid cytokeratin immunostain for intraoperative evaluation of axillary sentinel lymph nodes in breast cancer. *Cancer* 2009;115:1555-62.

Krogerus LA et al. Towards reasonable workload in diagnosis of sentinel lymph nodes: comparison of two frozen section methods. *Histopathology* 2004;44:29-34.

Leikola JP et al. Rapid immunohistochemistry enhances the intraoperative diagnosis of sentinel lymph node metastases in invasive lobular breast carcinoma. *Cancer* 2005;104:14-9.

Table 1. Study set comparison. The rate of late positives (=micro or macrometastasis missed) was reduced by 38-55 % with intraoperative IHC. \* including 20 % with further nodal positivity. \*\* Year 2010 cases. Study sets in 3 different laboratories:

Affiliate hospital / time span	Number of cases, N	Metastasis in PAD	Late positives	Late positives without U-IHC
Hospital 1 2010-2012	833	23 %*	4.3 %	9 %**
Hospital 2 2010	60	62 %	8 %	13 %
Hospital 3 2012	120	33 %	3 %	6 %

Hospital<sup>1</sup> Fimlab Laboratories, University Hospital of Tampere, Tampere, Finland.  
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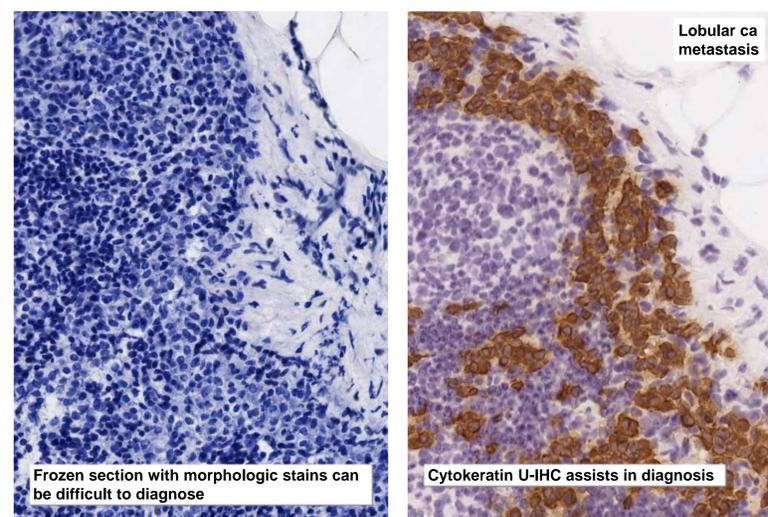


Figure 2. With morphologic stains (TB, H&E) only it is many times difficult to pinpoint micrometastasis. Cytokeratin U-IHC staining assists in detecting carcinoma.

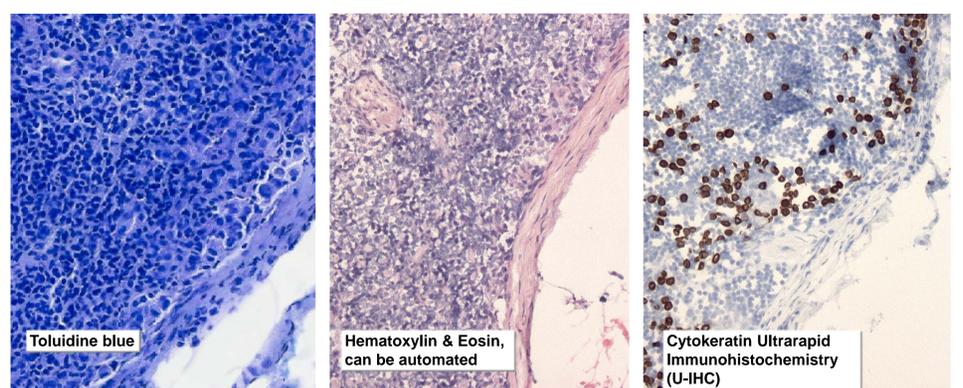


Figure 3. Series of breast cancer sentinel node adjacent sections stained with different staining methods.

## Conclusions

- Ultrarapid cytokeratin immunohistochemistry was found useful in the intraoperative examination of sentinel lymph nodes of breast cancer patients. The method is currently routinely used in several laboratories.