

## HOPE – A NOVEL TOOL FOR THE PATHOLOGIST

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**Summary.** The growing demand for the detection of prognostic and diagnostic biomarkers by application of molecular-biological techniques has been slowed down to a large degree by the use of formalin and its influence onto antigenic structures and nucleic acids, although other reasons also exist. With regard to morphology a limitation in the possibilities, compared to frozen sections, takes place. With the introduction of the HOPE (Hepes glutamic acid buffer mediated Organic solvent Protection Effect) -fixation to date both have become possible; substantially enlarged possibilities concerning the detection of DNA, RNA and proteins in combination with excellent morphological results comparable to formalin-fixed tissues. We also highlight some aspects from our lung specific work during the last years. Immunohistochemical markers for differential diagnostics of malignancies in the lungs are discussed; their enhanced detectability using the HOPE-technique is pathbreaking for the future. The same holds true for the substantially enhanced detectability of germs (e.g. mycobacteria) in HOPE-fixed tissues.

**Key words:** HOPE, tissue fixation.

### INTRODUCTION

In general the pathologist obtains tissues for subsequent investigations fixed in formalin or other substances and less often in a fresh and non-treated state, mainly in cases where intraoperative frozen sections are needed or other than routine examinations are necessary and desirable (Figure 1).

Within the growing field of molecular diagnostics fixation of specimens and paraffin-embedding the common way represents an obstacle for the application of molecular assays, since the preservation of epitopes and nucleic acids is low when compared to frozen materials (Srinivasan *et al.*, 2002).

On the other hand, frozen sections have a comparably worse morphology. To overcome these disadvantages and to accomplish all of these demands, a novel fixation protocol has been established which is called the HOPE-technique.

Instead of formalin, a mixture of amino acids in a buffered solution is used, followed by dehydration and paraffin embedding in a very similar fashion to the conventional procedure of sample handling. HOPE-fixed materials then can be utilized for a wide range of molecular applications and have an excellent, formalin-like morphology (Olert *et al.*, 2001). Furthermore such materials can be stored for years thus fulfilling conventional demands of documentation purposes.

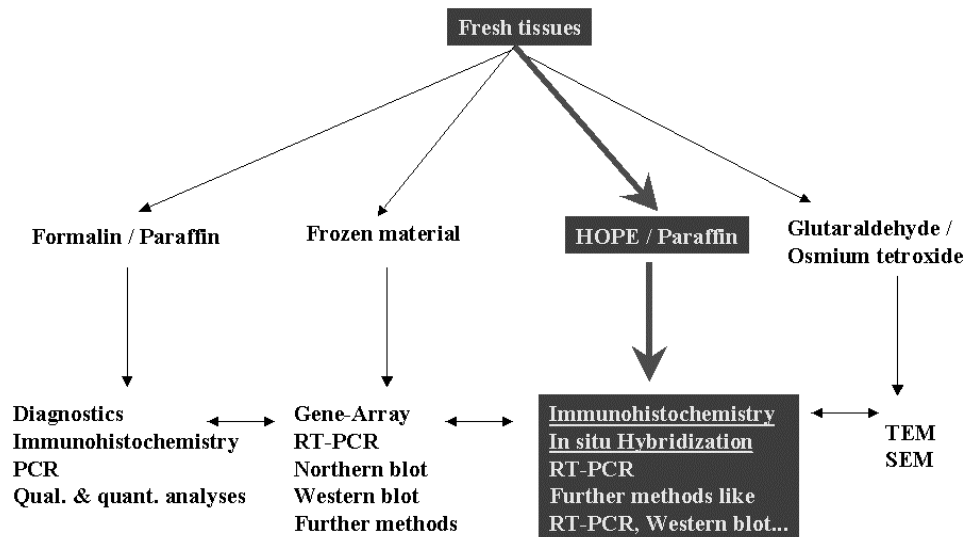


Figure 1 – Flowchart showing the different ways of tissue processing in pathology under addition of the HOPE-technique

#### APPLICATIONS AND EXPERIENCE USING THE HOPE-TECHNIQUE IN GENERAL

We demonstrated that the preservation of nucleic acids is comparable to frozen materials but over longer time intervals of more than six years, which is our practical experience up till now, probably even significantly longer (Wiedorn *et al.*, 2002).

This also results, among others, in a better detectability of infectious agents like mycobacteria by PCR in archived materials (Sen Gupta *et al.*, 2003) and other germs *e.g.* chlamydiae (Rupp *et al.*, 2004, in press).

One central part of our work has been the elaboration of techniques that enable the detection of transcriptional activity by *in situ* hybridization (Goldmann *et al.*, 2002; Pechkovsky *et al.*, 2002a; Pechkovsky *et al.*, 2002b).

This represents an ideal counterpart for classic molecular-biological approaches like RT-PCR or Northern blotting, since it enables to distinguish the certain cell types involved.

In a recent study we have demonstrated that the HOPE-technique is also applicable for cytospin preparation of single cells and enables *in situ* hybridization targeting even lowly transcribed genes to be successfully applied as well as immunocytochemical assays (Umland *et al.*, 2003).

In addition the HOPE-technique enables the use of antibodies of the so called cryotype in immunohistochemistry as well as the application of western blot analyses from paraffin embedded archived tissues with excellent results comparable to frozen tissues (Uhlig *et al.*, 2002; Droemann *et al.*, 2003; Uhlig *et al.*, 2004). Transcription microarrays with simultaneous analysis of numerous gene activities *e.g.* in breast cancer was also shown (Goldmann *et al.*, 2004, in press).

### IMMUNOHISTOCHEMISTRY OF TUMORS

Immunohistochemical results can be improved (quantitatively and qualitatively) by using the HOPE-technique. The detection of biomarkers by immunohistochemistry is a daily part of routine diagnostics in cancer and has crucial impact on diagnosis, prognosis, and therapy regimens. Immunohistochemistry with formalin-fixed tissues in a large proportion requires the application of thermic denaturation techniques like boiling of the specimens to recover the antigenic structures, which were masked by formalin.

This kind of approach, however, is hardly to be standardized, which is one of the major drawbacks in immunohistochemistry today. By using relevant biomarkers like Ki-67, Her2 and steroid hormone receptors as targets, which all require antigen retrieval techniques with formalin, we have shown that antigen retrieval can be completely left out if using the HOPE-technique instead of formalin due to better preservation of antigenic structures (Figure 2) (Goldmann *et al.*, 2003).

Moreover, this enables the application of non formalin-compatible antibodies, which have been used to describe the expression pattern of the recognition receptor Toll-like receptor 2 in a recent study (Droemann *et al.*, 2003).

The expression of surfactant protein-A (SP-A) by tumor cells of patients suffering from tumors in the lungs with a questionable origin is a helpful additional feature in the process of diagnosis by indicating primary carcinoma of the lung (Goldmann and Vollmer, 1999; Goldmann *et al.*, 2000; Goldmann *et al.*, 2001).

SP-A is expressed by the pneumocytes II in lung tissue and a portion of non-small cell lung carcinomas and has not yet been found to be expressed by any other tumors when detected immunohistochemically by use of the monoclonal antibody PE-10. During the last years we analyzed specimens of different malignancies with pulmonary location for the expression of SP-A by the use of the markers PE-10 together with TTF-1, another valuable marker for lung cancer.

The monoclonal antibody PE-10 used in this study provides high specificity when compared to the results obtained with polyclonal antibodies.

When staining for TTF-1 and SP-A in the same tumor specimens, either with subsequent sections or simultaneous immunohistochemistry, we found a heterogeneous expression of the respective epitopes (Figure 3). Up till now all extrapulmonary carcinomas stained for means of control, as already shown in previous studies, remained negative. A proportion of the cases had been negative for TTF-1 but positive for SP-A and *vice versa*.

We conclude that SP-A detected by immunohistochemistry using the monoclonal antibody PE-10 along with other parameters (especially TTF-1) represents a useful additional tool for individual diagnoses of malignomas located in the lungs with a questionable primary origin.

Especially if analyzing small biopsies and keeping in mind the well known heterogeneity of lung cancer specimens and the exclusion of thyroid metastases, we recommend to use both markers.

#### PCR FOR MYCOBACTERIA

PCR facilitates the amplification of enormous amounts of specific DNA-sequences in a simple way by utilizing very small amounts of template material. Detection of mycobacterial DNA in paraffin embedded materials employing PCR has opened up new possibilities in fast and sensitive diagnostics, especially if histopathological details imply the presence of mycobacteria but conventional stainings remain negative (Goldmann *et al.*, 1998).

But on the other hand, PCR like almost any other technique has its limitations, which are due to fragmentation of extractable DNA after formalin-fixation, paraffin embedding and de-paraffinisation.

This fragmentation inhibits PCR, since all DNA-strands bearing breaks inside the amplicons will not be amplified on the one hand, but, on the other hand still bind primers and taq-polymerase, so that efficiency of PCR is significantly reduced compared to non-fragmented DNA.

In addition the complex cell wall of mycobacteria is an obstacle to assess the DNA of these microorganisms making more rigid extraction methods necessary, which then contribute to further fragmentation of the extracted DNA (Goldmann *et al.*, 2001).

Comparison of PCR-results using DNA extracted from either HOPE- or formalin-fixed specimens in BCG-infected SCID-mice revealed a more than 100-fold enhanced sensitivity for the HOPE-fixed material.

Due to the preservation of DNA from degradation in HOPE-fixed tissues, even the differentiation within the *M. tuberculosis* complex was possible by spoligotyping (Sen Gupta *et al.*, 2003).

## CONCLUSIONS

HOPE-solution has been shown to be an excellent preservative for soft tissues, providing protection for proteins and nucleic acids in conjunction with well-preserved morphology.

It could be demonstrated that HOPE is not only suitable for tissue sections but also for single cells including the application of a vast repertoire of different immunological and molecular techniques and methods.

Taken together, the novel HOPE-technique is a substantial improvement making the archives of pathology a source of material well suited for a wide range of already available and forthcoming molecular–biological applications, which results in enhanced diagnostic and therapeutic possibilities.

## REFERENCES

- DROEMANN D., GOLDMANN T., BRANSCHIED D. *et al.*, *Toll-like Receptor 2 (TLR2) is expressed by alveolar type 2 cells and macrophages in the human lung*, *Histochem Cell Biol*, 2003, 119:103–108.
- GOLDMANN T., RICHTER E., DEUTSCHBEIN M.E. *et al.*, *(Mycobacteria in formalin-fixed, paraffin-1998) PCR-based detection of embedded tissues as a molecular tool for the pathologist*, *EJ Pathol*, 2003, 4: 98–301.
- GOLDMANN T., DEUTSCHBEIN M.E., WIEDORN K.H. *et al.*, *Review: surfactant apoprotein-A in adult-human's diseases of the lungs*, *EJ Pathol*, 2000, 6:2–02.
- GOLDMANN T., VOLLMER E., *Immunohistochemical detection of SP-A helps to manifest the diagnosis in a patient suffering from a moderately differentiated adenocarcinoma of the lung*, *EJ Pathol*, 1999, 4: 99–108.
- GOLDMANN T., GALLE J., DEUTSCHBEIN M.E. *et al.*, *Report of two cases: diagnostic value of immunohistochemically detected surfactant apoprotein-A in malignant tumors located in the lungs*, *Ann Diagn Pathol*, 2001, 2:84–90.
- GOLDMANN T., RICHTER E., GALLE J., VOLLMER E., *PCR-based detection of Mycobacteria in paraffin embedded specimens: a molecular technique on its way into routine-diagnostics*, *E J Pathol*, 2001, 7(3):013–03.
- GOLDMANN T., VOLLMER E., GERDES J., *What's cooking? Detection of important biomarkers in HOPE-fixed paraffin embedded tissues eliminates the need for antigen retrieval*, *Am J Pathol*, 2003, 163:2638–2640.
- GOLDMANN T., WIEDORN K.H., KÜHL H. *et al.*, *Assessment of transcriptional gene activity in situ by application of HOPE-fixed, paraffin-embedded tissues*, *Pathol Res Pract*, 2002, 198:91–95.
- GOLDMANN T., FLOHR A.M., ESCOBAR H.M. *et al.*, *The HOPE-technique permits Northern blot and microarray analysis in paraffin-embedded tissues*, *Pathol Res Pract*, 2004, in press.
- OLERT J., WIEDORN K.H., GOLDMANN T. *et al.*, *HOPE-fixation: a novel fixing method and paraffin embedding technique for human soft tissues*, *Pathol Res Pract*, 2001, 197:823–826.
- PECHKOVSKY D., ZISSEL G., GOLDMANN T. *et al.*, *Different pattern of NOS2 and NOS3 mRNA expression in human A549 cells and primary cultured alveolar epithelial cells type II*, *Am J Physiol*, 2002a, 282:L684–692.
- PECHKOVSKY D., ZISSEL G., STAMME C. *et al.*, *Human alveolar epithelial cells type II induce nitric oxide synthase-2 expression in alveolar macrophages by an interferon-gamma-dependent pathway*, *Eur Resp J*, 2002b, 19:672–683.

- RUPP J., DROEMANN D., GOLDMANN T. *et al.*, *Alveolar epithelial cells type II are major target cells for C. pneumoniae in chronic but not in acute respiratory infection*, FEMS Immunol & Med Microbiol, 2004, in press.
- SEN GUPTA R., HILLEMANN D., KUBICA T. *et al.*, *HOPE-fixation enables improved PCR-based detection and differentiation of Mycobacterium tuberculosis complex in paraffin-embedded tissues*, Pathol Res Pract, 2003, 199: 619–623.
- SRINIVASAN M., SEDMAK D., JEWELL S., *Effect of fixatives and tissue processing on the content and integrity of nucleic acids*, Am J Pathol, 2002, 161:1961–1971.
- UHLIG U., HAITSMA J.J., GOLDMANN T. *et al.*, *Ventilation-induced activation of the mitogen activated proteinkinase pathway*, Eur Respir J, 2002, 20:946–956.
- UHLIG U., FEHRENBACH H., LACHMANN R.A. *et al.*, *Phosphoinositide 3-OH kinase inhibition prevents ventilation-induced lung cell activation*, Am J Respir Crit Care Med, 2004, 169:201–208.
- UMLAND O., ULMER A.J., VOLLMER E., GOLDMANN T., *In situ hybridization and immunocytochemistry using HOPE-fixed, cultured human cells on cytospin preparations*, J Histochem Cytochem, 2003, 51:977–980.

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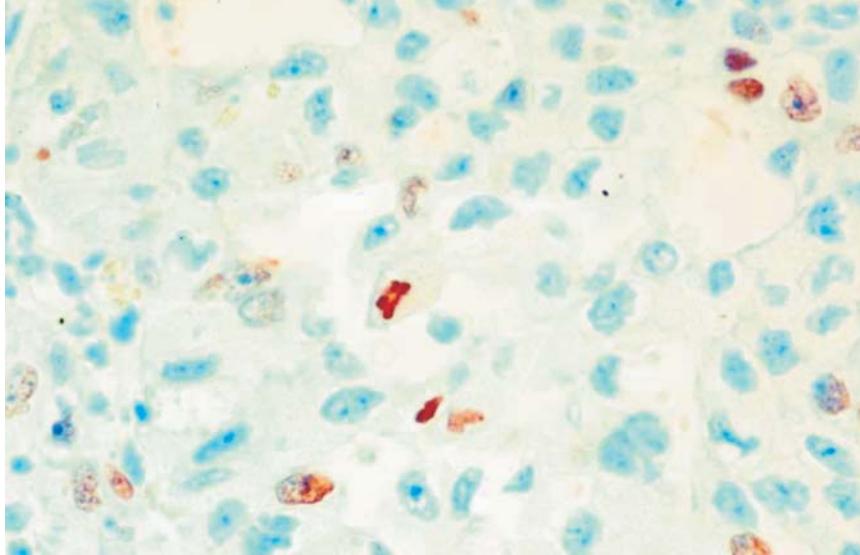


Figure 2 – Detection of Ki-67 in a HOPE-fixed non small cell lung carcinoma using the monoclonal antibody MIB-1 and the LSAB-technique without antigen retrieval. Note the nicely stained metaphase-plate in the center (×800)

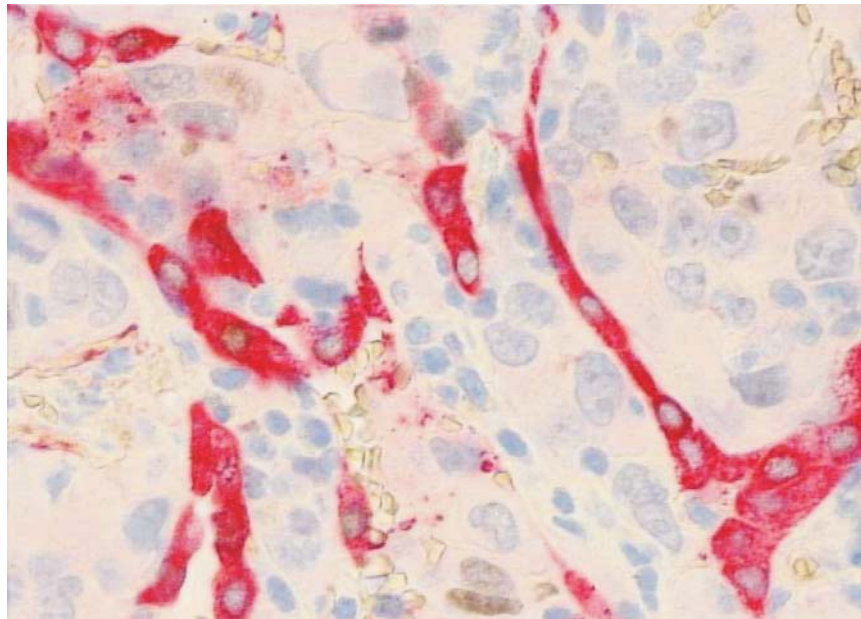


Figure 3 – Immunohistochemical double-staining of Surfactant protein-A (SP-A in the cytoplasm – red) and Thyroid transcription factor-1 (TTF-1 in the nucleus – brown) in a non small cell lung carcinoma revealing intratumoral heterogeneous expression of these two markers (×400)