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# Identification of Biological Samples in a Case of Contamination of a Cytological Slide Preparation*

**ABSTRACT:** Here we report a case where a discrete section of the cytological slide preparation of a female individual was obviously contaminated with pleura liquid of a female tumor patient. Analysis of the cancerous pleura liquid and the healthy cells of the slide preparation showed different DNA profiles, indicating that the material originated from two different female individuals. The DNA profile of the cell mixture revealed a heterogenous pattern whereby the alleles could be assigned to the healthy and the tumor patient. Loss of heterozygosity (LOH) was observed in four of eight short tandem repeat systems for the pleura liquid and the cell mixture. Despite the low amount of DNA on the slide preparation and the occurrence of LOH, it was possible to clarify the case and to support the assumption that a drop of cancerous pleura liquid contaminated the cytological slide.

**KEYWORDS:** forensic science, cytological preparation, cell mixture, short tandem repeat analysis, loss of heterozygosity

Short tandem repeat (STR) systems are well validated and useful markers for identification and paternity testing. In general, DNA typing shows robust and reliable results, but in some cases interpretation of the DNA profiles is complicated by preferential amplification, loss of heterozygosity (LOH), or microsatellite instability (MSI) because of degradation processes, or the existence of cancerous tissue samples. Several reports have been published either on the identification of swapped or contaminated histological slides (1–3) or the usage of histological specimens as reference samples in body identification by means of the application of STR analysis. Histological preparations may not only contain healthy but also malignant tissue. It is known that STR analysis of tumor DNA shows the phenomenon of LOH and MSI, which are known to play an important role in cancer research and diagnostics. Numerous examples of LOH in cancer have been described and many have been mapped to areas located in close proximity to markers employed in human identity testing (4–7). Rubocki et al. (8) described the STR analysis of normal and cancerous biopsy material from one individual for medicolegal purposes and pointed out the problems concerning LOH in STRs usually used in human identification. Here, we report a case of contamination of a cytological slide preparation from an obviously healthy woman with pleura liquid of a female tumor patient with an ovarian carcinoma. On behalf of an institute of pathology, we carried out STR analysis on the cytological slide preparation of the healthy female person and on pleura liquid of the female tumor patient in order to clarify the question of the origin of the distinct area of cell mixture in the slide preparation.

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## Materials and Methods

### *DNA Extraction*

DNA was extracted from 4 mL of pleura liquid with the All-tissue DNA kit (GEN-IAL, Troisdorf, Germany) according to the manufacturer's instructions. DNA extraction of the slide material was performed using 80 μL of 5% Chelex100 solution (Biorad Laboratories, Hercules, CA) (9) and 2 μL of proteinase K (20 mg/mL). From the cytological slide preparation that was obviously contaminated with tumor cells, two samples were prepared: one from the section containing the mixture of tumor and healthy cells and one as the reference sample from the remaining area containing only healthy cells.

### *DNA Quantification*

Extracted DNA was quantified using the Quantifiler Human DNA Quantification Kit (Applied Biosystems, Darmstadt, Germany) according to the manufacturer's instructions. The analysis was performed on the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Darmstadt, Germany) and the ABI Prism 7000 SDS software v.1.1.

### *PCR Amplification*

PCR amplification was carried out using the multiplex kit MPX2 (SERAC, Bad Homburg, Germany) according to the manufacturer's instructions. The amount of template DNA for amplification ranged from 10 to 4 ng. DNA was amplified in a TRIO-Thermoblock (Biometra, Göttingen, Germany).

### *Electrophoresis*

Electrophoresis was carried out on an ABI Prism 310 Genetic Analyzer (Applied Biosystems) using a 47 cm × 50 μm ID capillary with the polymer POP6. Electrophoresis was performed using the module GS STR POP6 (1 mL) D. From each sample, 1 μL was mixed with 12 μL HiDi-formamide (Applied Biosystems) and

0.5 µL of the internal standard genRES LS 500ROX. After denaturation (5 min, 95°C), the samples were cooled on ice and immediately transferred to the autosampler. The data were collected using the ABI Prism 310 collection software (3.0.0) (Applied Biosystems, Darmstadt, Germany) and analyzed with the 310 Genescan (3.7.1) and Genotyper (3.7 NT) software.

## Results and Discussion

Microscopic examination of the slide preparation revealed that a discrete section of the preparation was obviously contaminated with tumor cells of a papillary carcinoma (Fig. 1). The remaining area contained only epithelium cells of the portio cervicis and endocervix (Fig. 2).

Prior to PCR analysis, each DNA extract was quantified using a real-time quantitation system in order to determine the optimal amount of template DNA for amplification. The knowledge of the DNA concentration in the PCR amplification is essential for optimized peak balance and overall performance especially in cases of critical biological material such as tumor tissue samples where the phenomenon of LOH can occur. In our case, the DNA concentration was determined to be 8.8 ng/µL for the pleura liquid, 1.8 ng/µL for the mixture of healthy and tumor cells, and 13 ng/µL for the section of healthy cells. The amount of template DNA for amplification ranged from 10 to 4 ng so that it could be excluded that imbalanced DNA profiles resulted from low DNA content in the samples.

Analysis of the electropherograms showed reproducible results for all samples. The section of the healthy cells of the cytological slide preparation and the tumor cells of the pleura liquid showed different DNA profiles, indicating that they came from two different female individuals (Figs. 3 and 4). Analysis of the cell mixture revealed a complex DNA profile that showed a mixture of alleles from the healthy patient and the tumor patient (Fig. 5) and the different peak heights indicated an unequal mixture ratio. For the STR systems D21S11 and FGA where the two individuals did not share one or more alleles and where the phenomenon of LOH did not exist, the peak heights of the DNA of the tumor cells exceeded the peak heights of the healthy cells by three to four times. Of the eight STR systems examined, four showed the phenomenon of LOH, which is known to play an important role in cancer research and diagnostics (Fig. 3). The proportion of minor/major allele was determined to be 0.29 (D8S1179), 0.35 (ACTBP2), 0.54 (VWA) and 0.63 (TH01). The extent of LOH can range from unbalanced peak heights where one of the alleles of the STR system is the minor component (partial LOH) and to complete allelic drop-out of one of the alleles. Additionally, it depends on the proportion of cancerous/healthy cells in the examined tissue. The phenomenon

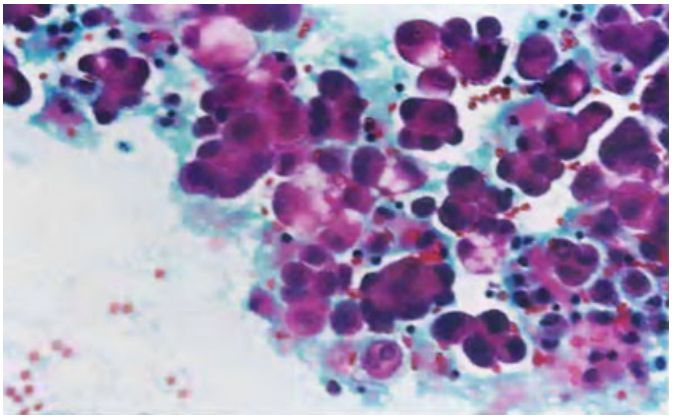

FIG. 1—*Papillary adenoid carcinoma, tumor cells present hyperchromatic cores and polymorphism, erythrocytes and inflammatory cells—exactly like an ovarian carcinoma with papillary structures (Papanicolaou, magnification ×400).*

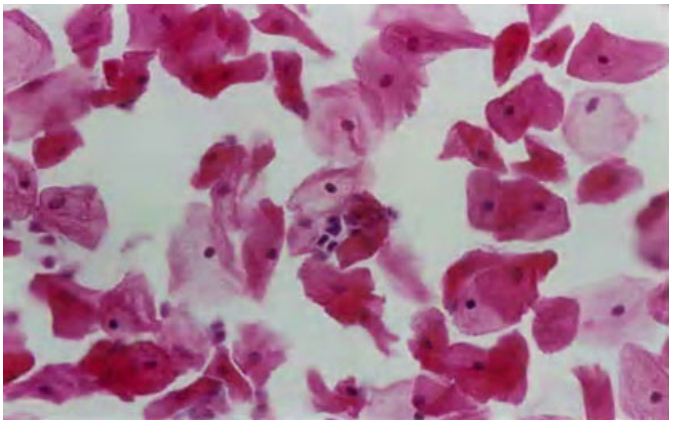

FIG. 2—*Same slide as Fig. 1 with typical superficial squamous epithelium with small cores in the centers, no erythrocytes and no inflammatory cells (Papanicolaou, magnification ×400).*

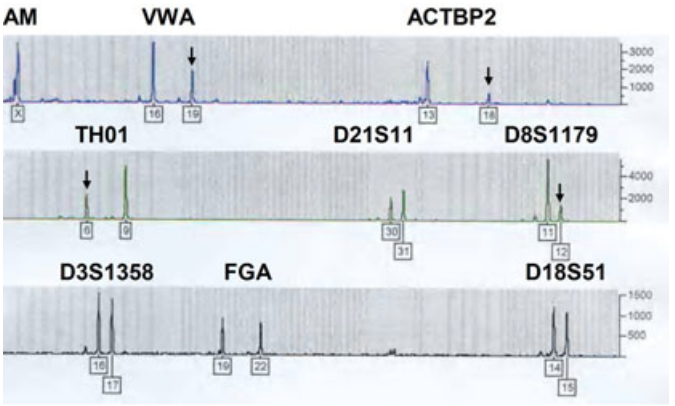


FIG. 3—*Electropherogram generated with the MPX2 kit: DNA profile of the pleura liquid of the female tumor patient. Arrows indicate LOH (loss of heterozygosity).*

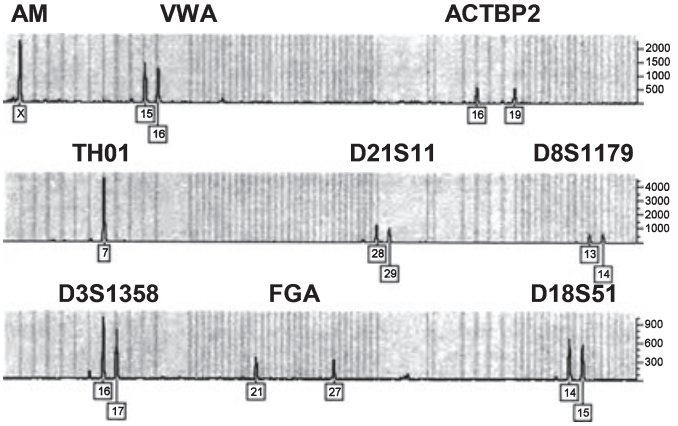


FIG. 4—*Electropherogram generated with the MPX2 kit: DNA profile of the cytological slide preparation containing only healthy cells.*

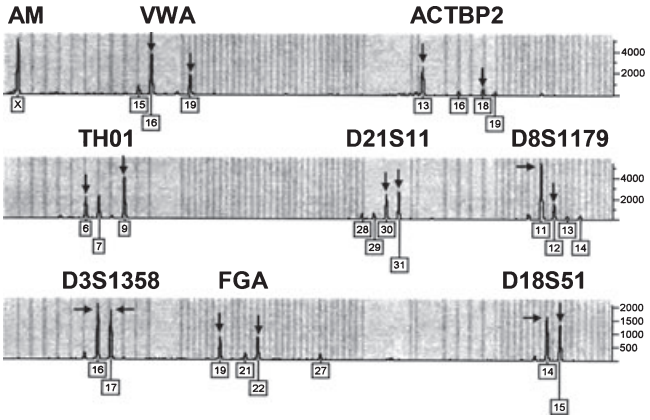


FIG. 5—*Electropherogram generated with the MPX2 kit: DNA profile of the cytological slide preparation containing the mixture of healthy and tumor cells. Arrows indicate the DNA profile of the pleura liquid.*

of LOH can play an important role in the interpretation of DNA profiles where cancerous material is used for human identification and where no healthy and fresh material is available. In general, histological preparations are formalin-fixed and paraffin-embedded tissue samples, which can cause problems in the interpretation of DNA profiles because of degradation processes during fixation (10,11). Preferential amplification and allelic drop-out caused by degradation processes cannot always be distinguished from LOH in cancerous material and interpretation rules would be useful (12).

Despite the critical parameter listed above concerning cancerous tissue samples, the application of STR analysis clearly verified the contamination of the cytological slide preparation studied here. The assumption of the pathologist that the contamination of the slide had taken place during the simultaneous preparation of the slides for the automatic staining process and that a drop of the cancerous pleura liquid contaminated the cytological slide preparation of the healthy woman was confirmed.

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