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Malignant Tumors and Forensics – Dilemmas and Proposals

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Aim To evaluate the effect of genetic instability and degradation in archived histology samples from cancerous tumors and to investigate the validity of short tandem repeat (STR) typing of these samples and its potential effect on human identification.

Methods Two hundred and twenty eight slides of archival pathology tissues from 13 different types of malignant tumors were compared with healthy tissues from the same individuals. DNA analysis was performed using standard techniques for forensic STR analysis, PowerPlex®16 and Identifiler® on 2 distinct sample sets. Genetic instability was assessed by comparing reference tissues with cancerous tissues derived from the same individual. Loss of heterozygosity, a ≥50% reduction in heterozygosity ratio between healthy and diseased samples, and microsatellite instability, the presence of an additional allele not present in reference tissue, were assessed. The quality of profiles obtained with respect to completeness among the archived samples and degradation using the 2 platforms were also compared.

Results Profiles obtained using the Identifiler® system were generally more complete, but showed 3-fold higher levels of instability (86%) than those obtained using PowerPlex® 16 (27%). Instances of genetic instability were distributed throughout all loci in both multiplex STR systems.

Conclusion After having compared 2 widely used forensic chemistries, we suggest individual validation of each kit for use with samples likely to exhibit instability combined with fixation induced degradation or artifact. A "one size fits all" approach for interpretation of these samples among commercially available multiplexes is not recommended.

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The analysis of human DNA polymorphisms is a valuable tool in genetic research and diagnostics and has become routine in clinical and forensic laboratories. It has aided in diagnosing diseases, determining paternity, identifying human remains, and attributing biological specimens to a source. However, DNA analysis for identity testing is only useful if there is a reference sample to compare with the sample in question. In cases where no obvious reference is available, it is necessary to find an alternative source. Difficulty can arise when the authenticity of the reference sample is questionable, or when the sample is taken from tissues with possible genetic variability, such as a malignant tumor (1-7). Since a known characteristic of malignant tumors are permanent alterations in the DNA molecule, DNA from these cells can display numerous anomalies including microsatellite instability (MSI), characterized by the presence of an additional allele or a size shift in an existing allele, deletions, or loss of heterozygosity (LOH) (8-13). Short tandem repeats (STRs; microsatellite DNA) commonly examined in forensic identification are not thought to be linked to visible traits or pathologies and are considered relatively stable and well distributed in the population. To that effect, some profiles obtained from nuclear and mitochondrial DNA of malignant tissues have been proven to be consistent with their normal counterparts. However, given the potential for pathological variability through genetic instability, it is necessary to evaluate its effect on the validity of the nuclear DNA analysis for identity determination and if steps can be taken to abrogate its impact.

Clinical tissue samples are not usually employed in forensic casework, but can play a role in the identification of unknown bodies and paternity testing, as well as aid in resolving issues stemming from pathology mix-ups and contamination (1,2). Archival pathology specimens are carefully cataloged and widely available, given their use in diagnostics and are a potentially large source for forensic STR testing. STRs are relatively stable, but degradation may have an effect, especially when analyzing tissues which have been treated with highly aggressive chemicals (eg, formaldehyde, xylene, etc.) during typical histological processing.

This research, conducted over a period of several years, analyzed and reviewed characteristics of DNA profiles obtained by means of 2 different commercially available kits, which are widely used in forensic identification. These kits both contain the widely accepted "core loci," recommended by the Federal Bureau of Investigation for use in forensic DNA databases (http://www.promega.com/geneticidproc/eusymp2proc/17.pdf). The aim of the study was to determine STR profile differences between healthy and pathologically altered tissues from the same source using 2 widely used forensic multiplex polymerase chain reaction (PCR) tests. These genetic differences arise due to disease and can complicate the use of malignant tissues as reference samples in forensic biology. We also sought to determine if variations occur in the indicators of genetic instability (LOH and MSI) among different tumor types and to assess possible variations in genetic instability in the same tumor type. This information allows us to recommend interpretation guidelines for the use of archived potentially diseased, and thus genetically unstable, material for human identification.

MATERIALS AND METHODS

Selection and collection of samples

A total of 228 samples, in the form of blocks of formalin fixed paraffin-embedded malignant tissues were examined and compared with healthy tissues from the same individuals. Thirteen different types of malignant tumors were represented in the study material: adenocarcinomas of the stomach, uterine endometrium, ovaries, lung, prostate, cecum, colon, pancreas, breast, and rectosigmoid colon; leiomyosarcomas of the uterus; ovarian papillary serous carcinomas from the uterine endometrium; endometrial carcinomas; malignant melanomas from the skin; and hepatocellular carcinomas.

All tissues of interest were gathered from archival and waste formalin-fixed paraffin-embedded tissues and underwent identical tissue fixation and paraffin embedding. The samples dated from 1 to 17 years before the study. Tissues had been taken from the patients as part of routine, necessary diagnostic procedures in concordance with Institutional Review Board approvals from the original institutions conducting the tests (Department of Pathology, University of Pennsylvania Presbyterian Medical Center, Philadelphia; Department of Pathology, NYU School of Medicine, New York; and NYC Department of Health and Mental Hygiene, New York).

The samples for this study were chosen according to tumor type and no identifying information was retrieved in any step of the study. The tissue donors were not contacted in any manner. Identifying and personal information was not used in the data collection and

analysis with the exception of the type of tumor the patient was diagnosed with and the organ from which the tissue sample was obtained.

DNA and subsequent bio-statistical analysis was conducted at the Office of Chief Medical Examiner in New York City, Department of Forensic Biology.

Experimental methods of analysis

Distribution of tumor types. Slides (n = 228) of healthy and tumor tissues were made from the formalin-fixed paraffin-embedded tissue blocks, prepared with 5 µm thick sections (stained with hematoxylin-eosin or unstained), and separated so that the healthy tissues were never analyzed alongside their tumor counterparts to prevent contamination.

One hundred and sixteen malignant tumor tissues and their healthy counterparts were analyzed, representing 13 different tumor types (Table 1)

All tissues were submitted to DNA extraction, quantitation, PCR of STR regions using the indicated system, separation of amplified products by capillary electrophoresis, and comparative and statistical analysis.

Phenol-chloroform isoamyl alcohol (PCIA) extraction. This method of extraction was chosen based on previous experience and was proven as effective when a low amount of poor quality DNA was expected (2). Using a sterile scalpel, one slide for each sample was scraped into a tube (Eppendorf, Hamburg, Germany) tube containing extraction buffer (10mM Tris [pH 8.0], 50mM EDTA [pH 8.0], 100mM NaCl, and deionized $H_2 0$), 20% SDS, 0.39M DTT, and Proteinase K (20 mg/mL) and incubated overnight in a shaking 56°C heat block. Appropriate extraction negatives were also included, without tissue. After incubation, samples were centrifuged for 5 minutes at 1000 rpm. The clear supernatant, along with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1), was transferred to an Eppendorf® Phase Lock Gel Tube (Eppendorf) and centrifuged for 2 minutes at 13 000 rpm under a fume hood. The aqueous top layer was carefully transferred to a Microcon 100 column (Millipore, Bedford, MA, USA) pre-moistened with TE^{-4}. The columns were centrifuged at 2500 rpm for 30 minutes. Additional centrifugation was added as necessary to filter the entire volume. After the liquid was discarded, TE^{-4} was added and the columns were centrifuged again. To elute the DNA, 40 µL of TE^{-4} was added to each column and the column was inverted into a new tube and centrifuged at 3500 rpm for 3 minutes. The column was discarded and the final sample volume was brought to 100 µL in TE^{-4}.

TABLE 1. Tumor samples and healthy counterparts analyzed in PowerPlex® 16 and Identifiler®

STR platform	Tumor type	Number of cases
PowerPlex®16	Adenocarcinoma*	38
	Leiomyosarcoma	10
	Papillary serous carcinoma	9
	Melanoma	2
	Hepatocellular carcinoma	2
	Endometrial carcinoma	9
Identifiler®	Papillary serous carcinoma	9
	Endometrial carcinoma	11
	Mixed adenocarcinoma:	15†
	component 1	14
	component 2	12

*Samples were taken from tumors of the stomach, uterine endometrium, lung, prostate, cecum, colon, rectosigmoid colon, pancreas, and breast.

†15 total cases were analyzed consisting of components 1 and 2.

DNA quantitation. The Quantiblot® Kit (Applied Biosystems) was used as per manufacturer's instructions and validated by Office of Chief Medical Examiner protocols for quantitation of all samples amplified in PowerPlex®16. For quantitation of samples amplified in Identifiler®, an *Alu*-repeat based real-time PCR was used (14).

PowerPlex® 16 STR amplification. Extracts were amplified using the PowerPlex® 16 multiplex system in the GeneAmp® PCR System 9700 (Applied Biosystems), following manufacturer's protocol. The amount of template DNA added to the reaction mixture was dependent upon the QuantiBlot results, with an optimal concentration of 1 ng and a volume of 20 µL. A negative control was made with 20 µL of TE^{-4} and a positive control was made with 10 µL of TE^{-4} and 10 µL of Positive Control DNA (Applied Biosystems). The amplification reaction was run in the following conditions: 95°C for 11 minutes; 96°C for 1 minute; 95°C – 30 seconds/60°C – 30 seconds/70°C – 45 seconds for 10 cycles; 94°C – 30 seconds/60°C – 30 seconds/70°C – 45-second for 22 cycles; 70°C for 45 seconds; 60°C for 30 minutes; and 4°C until the end of the reaction.

Identifiler® STR amplification. Identifiler® multiplex PCR system was used for STR analysis of 11 endometriod carcinoma, 9 papillary serous carcinomas, and 15 mixed adenocarcinoma samples, paired with histologically normal samples. A range of 500 pg/µL to 1000 pg/µL of DNA, as determined

by real-time PCR, in 5 μL total volume was used in each 13-μL reaction. Positive and negative controls were included in each amplification as for PowerPlex®16. The amplification reaction was run in the following conditions: 95°C for 11 minutes; 94°C – 1 minute/59°C – 2 minutes/72°C – 1 minute) for 28 cycles; 60°C for 60 minutes; 4°C until the end of the reaction.

Separation of amplified products. The amplified products were separated using the ABI Prism® 3130 Genetic Analyzer (Applied Biosystems), following the manufacturer's protocol.

DNA fragment length was determined by ABI's Genescan® software (Applied Biosystems) by comparing samples with the ILS 600 (PowerPlex® 16), or LIZ500 (Identifiler®). Electropherograms were generated and allelic designations were assigned using ABI's Genotyper® software (Applied Biosystems).

Assessment of genetic instability. Heterozygote ratio (HR) was determined using the formula: HR = A low/A high, where [A] is the allelic intensity in relative fluorescence units (RFU) (2,5,6). Microsatellite instability was identified by detection of abnormal alleles (allelic insertion, expansion, or contraction) at the STR loci compared with the healthy tissue (5). Losses of heterozygosity (LOH), defined by a ≥50% reduction in HR between healthy and diseased samples, and in reference tissue, were assessed. Expressed mathematically, LOH ratios were calculated according to the formula: $[A_1/A_2]_{diseased}/[A_1/A_2]_{healthy}$, where a ratio between 0.51 and 1 indicates no change from the reference tissue. Ranges between 0.0-0.5 and ≥2 indicated LOH. Comparisons were made with respect to the intensity of the signal peak and the size of the fragments, as well as to peak balance/imbalance. LOH is especially an interesting phenomenon, due to the fact that it could be a result of degradation/alteration of DNA.

Statistical analysis

Pearson correlation test was used to compare allelic instability rates at STR loci with sample age, using Microsoft Excel (Microsoft, Redmond, WA, USA).

RESULTS AND DISCUSSION

Working with archival, formalin-fixed paraffin-embedded tissues of malignant origin, presents potential challenges with respect to distinguishing sample degradation and procedural chemical alterations from genetic instability. Genetic instability can manifest as expansion, contraction, or loss of microsatellite DNA repetitive sequences.

With these issues in mind, samples were analyzed by 2 separate approaches, the first assessing the quality of profiles obtained using allele size and overall profile quality as indicators, and the second assessing genetic instability known to occur in tumors. The potential presence of LOH is of particular concern, as it can mimic the allelic drop-out commonly seen due to sample degradation.

Distribution of profile quality

With respect to the amount of DNA obtained from samples, no difference was observed between healthy and tumor tissues. Each sample was amplified once using the amount of DNA validated for the specific multiplex system. Similar to regular casework, in most instances there was not sufficient material to conduct multiple testes. Out of the 70 healthy samples tested by PowerPlex®16 multiplex system and the 1050 expected STR loci retrieved, 690 (67.50%) yielded profiles. Among them, 89.42% were from loci ≤350 bp and 10.58% from loci >350 bp. Of the 70 tumor samples tested by the same platform, out of the 1050 expected loci, 722 (68.76%) yielded profiles. Among them, 88.36% were from loci ≤350 bp and 11.64% from loci >350 bp. The healthy samples had 21 loci (3.04%) with HR≤0.7 (3 with HR≤0.45), while tumor samples had 122 loci (16.90%) with HR≤0.7 (36 with HR≤0.45). Most of these instances were from loci <350 bp.

In contrast, data obtained from the set of the samples amplified in Identifiler® did not show a strong association between locus size and HR (data not shown). Furthermore, 350 bp would not be a useful size distinction for assessing results from this kit, as the allele range for Identifiler® loci is designed to be <360 bp and very few alleles are ≥350 bp.

For empirical analysis of profile quality, the following definitions were used: complete profile (all 15 loci), good profile (8-14 loci), poor profile (1-7 loci), and negative profile (no loci) (15). Amelogenin was not considered in this analysis, as Amelogenin-based sex determination is based on a 6-bp intron insertion and not a microsatellite repeat.

For samples analyzed using PowerPlex®, of the 70 healthy profiles 29 were complete (41%), 18 were good (26%), 13 were poor (19%), and 10 were negative (14%). Of the 70 tumor profiles, 31 were complete (44%), 21

were good (30%), 10 were poor (14%), and 8 were negative (11%). These results indicate that amplification quality is not affected by the presence of histologically abnormal tissue, and therefore tumor tissues may provide a comparable number of loci for forensic analysis compared with their histologically normal counterparts.

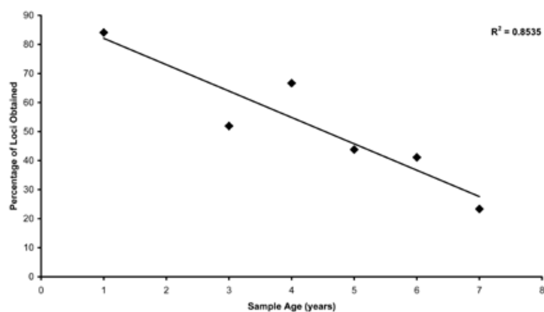
Given this determination, additional 86 samples, distinct from those amplified with PowerPlex®, were amplified using Identifiler® and assessed considering both normal and tumor samples together. Fifty four samples gave complete profiles (63%), representing a 20% increase over samples amplified using PowerPlex®, 22 were good (26%), 4 were poor (5%), and 6 gave no profile (7%).

Relationship between profile quality and sample age

The percentage of loci obtained for all the tissues stored for the 6 time intervals (1 year and 3-7 years) were assessed. The correlation coefficient between percent loci obtained and sample age was -0.9239, where a value of >0.9 was considered significant (Figure 1). This suggests a strong negative correlation between sample age and amplification success using the PowerPlex® system. Therefore, where possible, samples which have been collected more recently should be given preference over older tissue samples when using this kit.

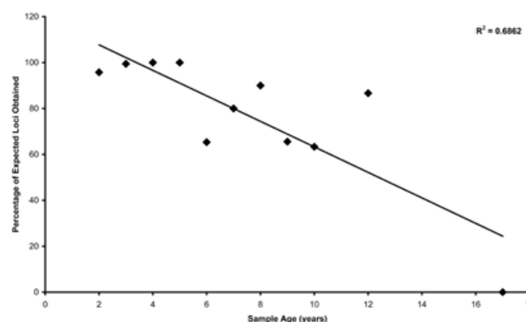
Conducting Identifiler® testing on samples with a storage time interval of 2 to 17 years post-collection, no significant linear relationship was observed correlating sample age to profile quality (correlation coefficient: -0.8284). Samples in the range of 2 to 5 years old gave 100% of the expected loci. In samples between 5 and 12 years old, some decrease in profile quality was observed, with a range of 62% to 90% of loci obtained with a nonlinear relationship (Figure 2). Therefore, samples from 0-12 years old may be safely considered for forensic analysis in this system. Given that the single 17-year-old sample gave a negative profile, samples above 12 years of age should be considered more carefully in this system.

Figure 1.



Correlation between loci obtained using PowerPlex®16 Multiplex STR system and sample age. Each data point shows the percentage of loci obtained (y-axis) for all samples of a specific age (x-axis). A linear negative correlation (R = 0.92) exists between sample age and amplification success.

Figure 2.



Correlation between loci obtained using Identifiler® Multiplex STR system and sample age. Each data point shows the percentage of loci obtained (y-axis) for all samples of a specific age (x-axis). Very weak linear correlation (R = 0.83) existed between sample age and profile quality in the Identifiler® system.

Relationship between profile quality by locus

Figure 3 shows the percentage of profiles obtained from both healthy and tumor tissues at each STR locus using the PowerPlex® system. Profile quality per locus was assessed by dividing the number of loci obtained at each locus by the total number of profiles obtained for healthy and tumor tissues, respectively. The quality of the amplification was unaffected by the disease state of the sample. Furthermore, it is clearly demonstrated that the percentage of the obtained loci is much higher for those of smaller size. In contrast, samples amplified in Identifiler® (Figure 4) platform demonstrated a higher success rate for the larger loci, and less dramatic fall-off in quality with respect to locus size. Additionally, this set of samples did not show any significant difference in profile quality between healthy and tumor samples and the results are presented for all samples together. It was clearly shown that the success rate for the same locus using 2 diverse multiplex platforms was different (Figure 5). We speculate that these differences may at least in part be due to components of each individual kit (buffers and primer sequences). Furthermore, the Power-

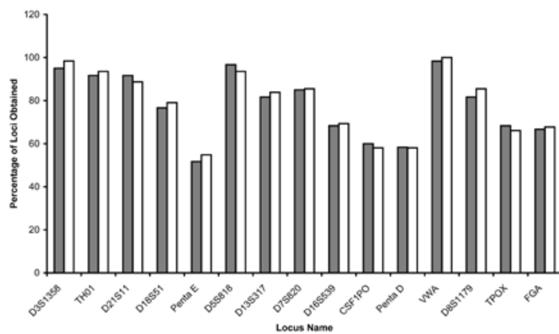
Plex® component of this study was conducted at least 1.5 years before the Identifiler® component. Differences in the success rate between the 2 kits may be reflective of chemistry optimization issues which have since been addressed by the manufacturer.

Genetic instability of tested tumor samples

Genetic instability includes any change in the tumor sample that was not present in the healthy sample including MSIs (an additional allele present in the tumor profile that was not present in the healthy profile [Figure 6] or a substitution of one allele for another [Figure 7]) and LOH (the loss of an allele that was present in the healthy profile but not in the tumor profile) (Figures 8 and 9).

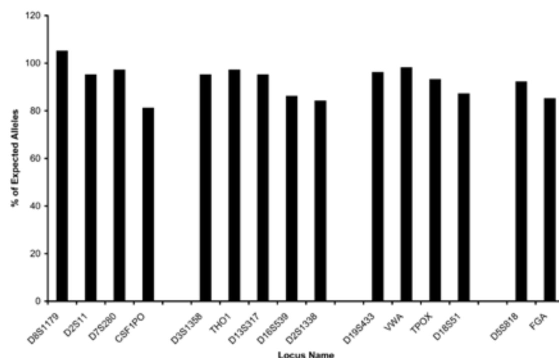
Genetic instabilities were observed in 29 out of 70 (41.4%) sets of profiles obtained by PowerPlex®16. Of the 38 adenocarcinoma profiles from the 383 loci obtained, there were 37 MSIs (9.6%) and 16 LOHs (4.2%). Of the 9 endometrial profiles from the 61 loci obtained, there were 7

Figure 3.



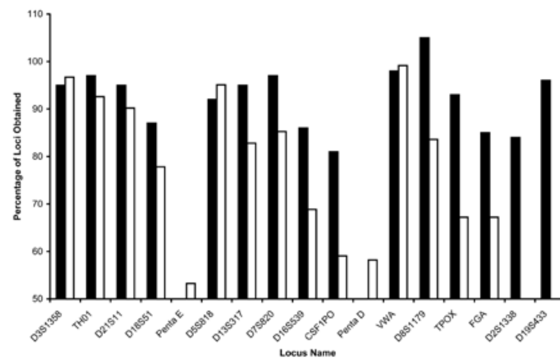
Percentage of loci obtained for healthy and tumor profiles using PowerPlex®16 Multiplex STR system. The percentage of loci obtained is shown as a function of the locus and is arranged in size order from the largest to the smallest by dye line (blue, green, yellow). Dark gray bars represent percentages for healthy tissue profiles and open bars represent percentages for malignant tissue profiles.

Figure 4.



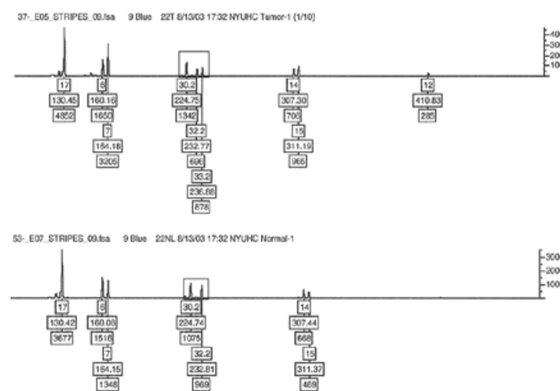
Percentage of loci obtained for profiles using Identifiler® Multiplex STR system. The percentage of loci obtained is shown as a function of the locus and is arranged in size order from the largest to the smallest by dye line (blue, green, yellow, red). Bars represent all tissue samples (both healthy and tumor) analyzed in Identifiler® Multiplex STR system.

Figure 5.



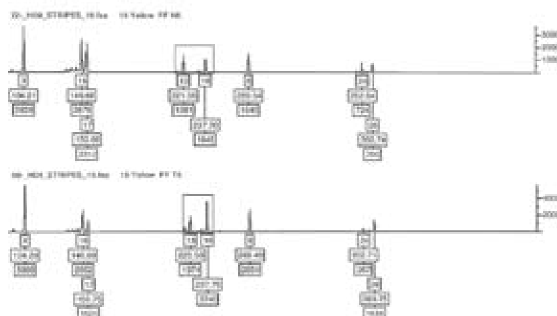
Comparison between percentages of loci obtained for all profiles amplified in Identifiler® Multiplex STR system (closed bars) vs PowerPlex®16 Multiplex STR system (open bars). The percentage of loci obtained is shown as a function of the locus and is arranged in size order from the largest to the smallest by dye line (blue, green, yellow, red). Loci not represented in a particular kit are not shown.

Figure 6.



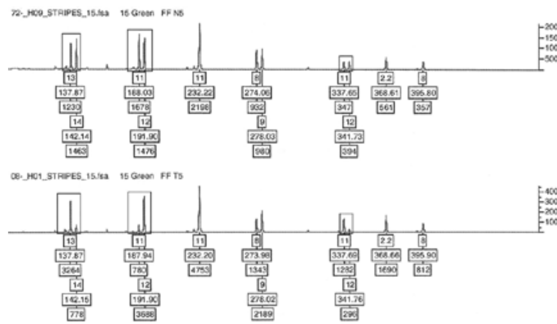
Example of microsatellite instability – the presence of an additional allele not found in the healthy reference (shown in boxed area). The electropherogram represents a sample amplified in PowerPlex®16 and separated on ABI Prism® 3130 Genetic Analyzer. Top line represents the healthy sample with corresponding tumor shown below. Peaks are labeled with allele call, size in base pairs, and height in relative fluorescent units.

Figure 7.



Example of microsatellite instability – a size shift in an allele present in the healthy reference (shown in boxed area). Electropherogram represents a sample amplified in PowerPlex®16 and separated on ABI Prism® 3130 Genetic Analyzer. Top line represents the healthy sample with corresponding tumor shown below. Peaks are labeled with allele call, size in base pairs, and height in relative fluorescent units.

Figure 8.

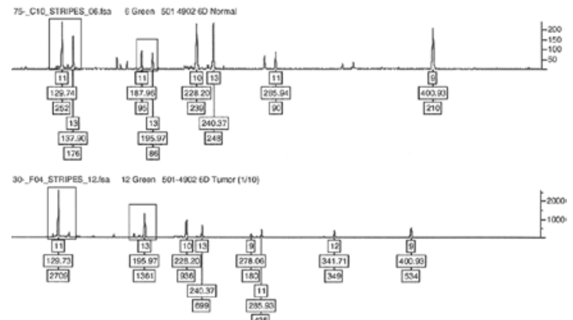


Example of partial loss of heterozygosity (shown in boxed area). Electropherogram represents a sample amplified in PowerPlex®16 and separated on ABI Prism® 3130 Genetic Analyzer. Top line represents the healthy sample with corresponding tumor shown below. Peaks are labeled with allele call, size in base pairs, and height in relative fluorescent units.

LOHs (11.5%). Of the 2 melanoma profiles from the 26 loci obtained, there were 4 MSIs (15.4%). Of the 9 papillary serous profiles from the 45 loci obtained, there were 3 MSIs (6.7%). Of the 10 leiomyosarcoma profiles from the 132 loci obtained, there was 1 LOH (0.8%). No specific tumor type showed a significantly higher rate and/or type of genetic instability over others. However, in examining a larger sample set, these differences may become evident.

Using either the AmpF*l*STR® SGM Plus or the AmpF*l*STR® SGM Profiler PCR amplification kits (Applied Biosys-

Figure 9.



Example of complete loss of heterozygosity (shown in boxed area). Electropherogram represents a sample amplified in PowerPlex®16 and separated on ABI Prism® 3130 Genetic Analyzer. Top line represents the healthy sample with corresponding tumor shown below. Peaks are labeled with allele call, size in base pairs, and height in relative fluorescent units.

tems) on 41 surgically removed gastrointestinal malignancies, Vauhkonen et al (5) observed allelic instabilities in 28 cases (68%). In our study, by means of PowerPlex®16, allelic instabilities were observed in 29 out of 70 sets (41.4%). Twenty-two sets (31.4%) could not be analyzed because one or both of the profiles were inconclusive. Thus, 19 sets (27.1%) of healthy and tumor tissues had consistent genotypes. This is consistent with the 32% of cases with identical genotypes observed by Vauhkonen et al (5).

In another study of solid tumors, Poetsch et al (6) also included a new category, partial LOH (pLOH), in addition to LOH and MSI. They defined pLOH as a >50% decrease in tumor DNA peak height when compared with the same allele in healthy DNA. Though they examined different types of tumors (solid tumors, lymph node metastases, and distant metastases), they observed 22% pLOH, 14% LOH, and 26% MSI.

Events of genetic instability were uniformly distributed throughout all loci, and no significant differences were found in a specific tumor type. Between 1.8%-8.5% of unstable alleles per locus through all analyzed tumor groups were found. Therefore, it is not possible to predict which loci may be more unreliable than others in determining a match between test and exemplar samples. In routine forensic testing, the profile obtained from an exemplar, regardless of LOH or MSI, would be compared with the test profile with the assumption no instability had taken place.

It is then vital to be conservative in interpreting samples derived from pathological material.

Since it was determined that in the PowerPlex® system the effect of degradation was more pronounced for alleles above 350 bp, it is recommended that alleles above this size not be considered when conducting statistical analysis to determine a forensic match. In alleles of this size, degradation effects cannot be distinguished from true instability, namely LOH. This cut-off provides a measure of reliability, as it allows the likelihood of degradation alone causing false instability determinations to be reduced. Therefore, in this system, severe peak imbalance may be indicative of instability, and the locus should be considered for exclusion from statistical analysis.

When considering samples amplified with Identifiler®, genetic alterations (cumulative for LOH and MSI) were very high in comparison with the same tumor type in the PowerPlex® system. Ninety percent of endometrioid carcinomas, 87.5% of papillary serous carcinomas, and 80% of mixed tumors exhibited at least one instance of genetic instability. Each component of the mixed tumors was considered separately, as histological differences could reflect differences in genetic stability and pathology. The lineage and pathway by which these mixed tumor components arise is unknown and is currently under investigation. This high rate of instability is in concordance with recently published results of Heaphy et al (16), who analyzed 239 different tumor tissues using Identifiler® and found that more than 99% of tissues which showed ≥2 instances of allelic instabilities were cancerous. Similar to those found by PowerPlex® system, instabilities found by Identifiler® in our study were not associated with a particular locus. Although intriguing, our results are representative of a small and specialized sample set. It is unclear whether this is due to the nature of these tumors or related to the sensitivity of the kit as evidenced in our quality analysis studies. Furthermore, unlike the PowerPlex® system, Identifiler® loci showed only weak association between locus size and profile quality, making setting a size cut-off for reliability problematic.

This increased sensitivity, if due solely to kit sensitivity and not the disease itself, presents a problem for blind interpretation (when no histologically normal sample is available for comparison) of pathology samples with the Identifiler® system. Since Identifiler® appears to be more sensitive to detecting genetic instability and the profiles are less sensitive to degradation, a false call due to instability may be more easily accepted as the true allele determination, thereby causing a false exclusion of a match. Therefore, a larger and more diverse sample set should be analyzed in this system to provide more specific guidelines.

The natural germline mutation rate of STRs is estimated to be between 10^{-3} and 10^{-4} per generation (15,17), which is low enough to provide the stability and reliability through generations for forensic identification and paternity testing. However, these and other STR markers are proven to be unstable in various tumor tissues (18,19). LOH and MSI are characteristic of many tumors. This instability has been demonstrated not only in mono- and di-nucleotide markers, but in tetra-nucleotide markers as well. Tetra-nucleotide STRs are commonly used in forensic and paternity testing, therefore it is vital to determine the genetic stability of the forensically relevant loci (20-23). There are mutations and alterations associated exclusively with tumors (24-33), which call the reliability of allelic typing with commercially available multiplex kits into question, especially in the cases of pathology samples.

In situations where direct comparisons between questioned and reference samples are made, mutation rates are sufficiently low that they may be of minor, although not insignificant importance. However, with comparisons between relatives in parentage testing and kinship analysis, which may be applied in mass disaster victim identification, mutational events can play a more significant role (34).

Based on the results of this study and other data regarding specific tumor types and its importance for the forensic community, we recommend the following procedure: whenever new primer chemistry is introduced and tested within forensic community, it is important that the stability and reliability of the alleles tested is confirmed in validation studies using genetically altered biological material, especially prior to the situations when it is to be used for the analyses of the archival tissues of pathological origin.

Disclaimer

Part of the study reported herein was presented in the form of preliminary data as a poster entitled: "Multiplex STR and Mitochondrial DNA testing for Paraffin Embedded Specimen of Healthy and Malignant Tissues: Interpretation Issues" at the 21st Congress of the International Society of Forensic Genetics (ISFG) held at Ponta Delgada, Azores, Portugal, September 13-17, 2005.

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