The Changing Spectrum of DNA-Based Specimen Provenance Testing in Surgical Pathology

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Abstract

Short tandem repeat (STR) analysis has emerged as the method of choice for testing to resolve specimen source contamination and identity problems that arise in surgical pathology. We studied a series of consecutive cases referred for STR typing during a 5-year period to document the usefulness of the approach and to describe the broadening scope of testing. The series demonstrates that STR-based typing can be applied in virtually any setting in which specimen source confirmation is requested, that STR-based typing is informative in 92% of cases, but that exceptions occasionally arise that complicate test interpretation. The series also demonstrates that in addition to traditional uses of STR typing, testing is now performed in the absence of any direct indication that a specimen mix-up or contamination may have occurred, namely, when the pathologic findings are unexpected or the clinical setting is atypical. The case series underscores the ability of STR testing to detect errors that cannot be captured by current laboratory protocols, a finding that has important implications for patient safety.

During the last decade, short tandem repeat (STR) analysis has emerged as the method of choice for DNA-based identity testing. The panel of STRs (also known as microsatellites) used in identity testing is based on the Combined DNA Index System (CODIS) loci originally selected by the Federal Bureau of Investigation of the United States.1 The CODIS loci feature extreme polymorphism and widespread distribution of the different alleles across different population groups, characteristics that not only make them useful in forensic settings, but also provide a very high power of discrimination for assigning the provenance of tissue samples in clinical settings.2-9 The clinical usefulness of STR-based testing using the CODIS loci is enhanced by the ease of testing (several commercial kits for analysis of the CODIS and/or related loci are available) and the availability of extensive technical resources to support test interpretation (including a large database of allele size distribution, largely generated by the forensic community).

Several diagnostic uses of STR-based identity testing in pathology are well established and include bone marrow engraftment analysis, diagnosis of hydatidiform moles, assessment of maternal cell contamination in prenatal specimens, and identification of uniparental disomy patterns of inheritance characteristic of some inherited disorders.10 In addition, it is well established that STR typing methods are of use in the resolution of specimen labeling/identification issues (which occur in about 6% of accessioned cases) and extraneous tissue contaminant issues (which can be identified in up to 2.9% of slides).11,12 It is noteworthy that, of the tissue contaminants encountered prospectively, approximately 30% are abnormal or neoplastic, and about 10% present some degree of diagnostic uncertainty.11
Given that STR typing is such a simple, relatively quick, inexpensive, and informative method of identity testing, it is not surprising that the clinical spectrum of cases referred for testing has evolved with time. To describe the broadened scope of testing and to study the usefulness of the approach, we studied the series of consecutive cases referred for STR typing at our institution during a 5-year period. Our series not only documents the technical aspects of STR testing in routine practice but also highlights that STR typing of tissue specimens is now performed in 3 novel settings in surgical pathology, ie, in cases for which the patient’s clinical outcome was unanticipated, in cases of an unexpected diagnosis, and by patient request. Unlike established uses of STR typing for resolution of specimen labeling/identification or tissue contaminant issues (in which typing is performed prospectively before diagnosis), these novel clinical settings share the common feature that testing is performed in the absence of any direct indication that a specimen mix-up might have occurred and highlight the ability of STR testing to detect diagnostic errors that cannot be captured by current laboratory protocols.

**Materials and Methods**

**Cases**

The files of the Lauren V. Ackerman Laboratory of Surgical Pathology, St Louis, MO, and the files of the Clinical Molecular Diagnostics Laboratory, Barnes-Jewish Hospital at Washington University Medical Center, St Louis, were searched to identify cases in which STR-based typing was performed on tissue samples to confirm sample provenance from 2005 (when STR typing was added to the surgical pathology test menu) through April 2010. All cases were included in the study cohort, including in-house cases and cases referred in consultation.

**Tissue Sample Preparation**

The patient sample was prepared for testing in 1 of 2 ways, depending on the type of tissue sample and the clinical context.

**Recuts Only**

For the cases in which the provenance ambiguity concerned all tissue in the block, 4-μm sections of the formalin-fixed, paraffin-embedded (FFPE) tissue block (up to 5 sections were used when the tissue fragment was ≤10 mm² in area) were placed into a new sterile microfuge tube. For these cases, a prior laboratory specimen, a buccal swab, or peripheral blood lymphocytes served as the reference sample.

**Microdissection From Glass Slides**

For cases containing a putative extraneous tissue contaminant (so-called floaters) and cases for which a putative mixture of tissue from more than 1 patient was present in the same block, the tissue in question was manually microdissected under direct microscopic examination as described briefly as follows. First, the relevant slides (whether H&E-stained sections of routinely processed FFPE tissue or frozen sections) were digitally imaged (ScanScope 2 with Spectrum software suite, Aperio Systems, San Diego, CA) to guide subsequent microdissection and to create a permanent record of the slide for archiving with the patient’s surgical pathology report. Second, the slides were decoyed by a xylene soak. Third, the relevant tissue fragments were collected by manual microdissection after thorough cleaning of the work area with 70% ethanol. The microdissection was performed by a pathologist wearing clean disposable gloves that were changed between each target, each target was collected using new sterile needles and surgical scalpels, and each target was placed into a new sterile microfuge tube. For these cases, a prior laboratory specimen, other tissue within the block, a buccal swab, or peripheral blood lymphocytes served as the reference sample.

**STR Typing**

DNA was extracted from the target samples according to established protocols. STR typing was performed via multiplex fluorescent polymerase chain reaction amplification using the AmpFISTR Profiler Plus ID amplification kit (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. Amplicons were separated by capillary electrophoresis on a 3130xl Genetic Analyzer (Applied Biosystems), and the STR marker profile was evaluated using the fragment analysis program GeneMapper v3.7 (Applied Biosystems).

**Regulatory Approval**

This study was approved by the Washington University School of Medicine Human Research Protection Office.

**Results**

Table I shows the relevant details of the cases in our prospective series. The series documents that STR-based typing can be applied in routine surgical pathology practice in which confirmation of specimen provenance is required, including tissue sections from FFPE tissue blocks, tissue microdissected from routinely prepared H&E-stained tissue slides, tissue microdissected from frozen section slides, bone marrow core biopsies, and cytology specimens.

In specific terms, Table 1 shows that testing was informative in 92% of cases (22/24). Both cases in which testing was not informative occurred when testing was performed to rule out a contaminant; in both cases, test failure was due...
to the lack of sufficient tissue to generate a marker profile in the putative floater. Of the well-established exceptions that can complicate identity determination by DNA typing,6,15-18 the presence of somatic mutation (specifically, microsatellite instability in tumor tissue) was present in a case in which an invasive ductal carcinoma of the breast was the tissue in question (Table 1, case 16), and the presence of a combination recipient and donor marker profile was noted in a case in which the tissue in question originated from a bone marrow transplant recipient (Table 1, case 22).

In more general terms, review of the case series shows that STR typing for resolution of specimen provenance questions now occurs in a broadened spectrum of clinical settings. Setting 2 encompasses cases in which there is, prospectively, concern for incorrect specimen labeling, a mismatch between the patient name on the container and the requisition, or a mismatch between the number of specimens indicated on the requisition and the number received by the laboratory. Setting 2 includes cases in which, prospectively, there is a question of an extraneous tissue contaminant (so-called floaters) on a frozen-section slide, a permanent section, or a cytology slide. Setting 3 consists of cases tested in retrospect because an atypical clinical course following definitive therapy has raised the possibility that a specimen mix-up may have occurred. Setting 4 encompasses cases in which there is no evidence or suspicion that a specimen identification error has occurred but that are nevertheless tested because the histopathologic findings are unexpected and/or suggest the need for a markedly different therapeutic regimen from that planned on the basis of clinical findings. Setting 5 consists of cases in which the testing is requested by the patient owing to anxiety over the clinical history, the diagnosis, the planned

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**Table 2.** Setting 1 encompasses cases in which there is, prospectively, concern for incorrect specimen labeling, a mismatch between the patient name on the container and the requisition, or a mismatch between the number of specimens indicated on the requisition and the number received by the laboratory. Setting 2 includes cases in which, prospectively, there is a question of an extraneous tissue contaminant (so-called floaters) on a frozen-section slide, a permanent section, or a cytology slide. Setting 3 consists of cases tested in retrospect because an atypical clinical course following definitive therapy has raised the possibility that a specimen mix-up may have occurred. Setting 4 encompasses cases in which there is no evidence or suspicion that a specimen identification error has occurred but that are nevertheless tested because the histopathologic findings are unexpected and/or suggest the need for a markedly different therapeutic regimen from that planned on the basis of clinical findings. Setting 5 consists of cases in which the testing is requested by the patient owing to anxiety over the clinical history, the diagnosis, the planned

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Description</th>
<th>Setting</th>
<th>Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Level 10R pulmonary lymph node; t/o extraneous tissue in actual frozen section</td>
<td>2</td>
<td>Presence of contaminant confirmed</td>
<td>Putative contaminant microdissected from actual frozen section</td>
</tr>
<tr>
<td>2</td>
<td>Endobronchial biopsy; t/o donor-acquired small cell carcinoma</td>
<td>3</td>
<td>Specimen switch confirmed</td>
<td>Example A in text</td>
</tr>
<tr>
<td>3</td>
<td>Cervical biopsy; t/o float in permanent sections</td>
<td>2</td>
<td>QNS; insufficient DNA in putative floater</td>
<td>Putative contaminant microdissected from recuts of FFPE tissue block</td>
</tr>
<tr>
<td>4</td>
<td>Endometrial curettage; t/o float in permanent sections</td>
<td>2</td>
<td>QNS; insufficient DNA in putative floater and endometrial tissue</td>
<td>Microdissection performed from recuts of FFPE tissue block</td>
</tr>
<tr>
<td>5</td>
<td>Intervertebral disk; t/o specimen switch</td>
<td>1</td>
<td>Specimen switch confirmed</td>
<td>Putative contaminant microdissected from recuts of FFPE tissue block</td>
</tr>
<tr>
<td>6</td>
<td>Endocervical curettage; t/o float in permanent sections</td>
<td>2</td>
<td>Presence of contaminant confirmed</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Large intestine biopsy; t/o specimen switch</td>
<td>1</td>
<td>Tissue origin from patient confirmed</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Liver biopsy; t/o contaminant as source of malignant tissue</td>
<td>2</td>
<td>Presence of contaminant confirmed</td>
<td>Putative contaminant microdissected from recuts of FFPE tissue block</td>
</tr>
<tr>
<td>9</td>
<td>Bone marrow core biopsy</td>
<td>1</td>
<td>Tissue assigned to correct patient</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Bone marrow core biopsy</td>
<td>1</td>
<td>Tissue assigned to correct patient</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Level 10L pulmonary lymph node; t/o extraneous tissue in actual frozen section</td>
<td>3</td>
<td>Presence of contaminant confirmed</td>
<td>Putative contaminant microdissected from actual frozen section</td>
</tr>
<tr>
<td>12</td>
<td>Vulvar biopsy; t/o specimen switch</td>
<td>1</td>
<td>Tissue origin from patient confirmed</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Prostate biopsy; t/o extraneous tissue in permanent sections</td>
<td>2</td>
<td>Presence of contaminant confirmed</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Brain biopsy; t/o extraneous tissue in permanent sections</td>
<td>2</td>
<td>Presence of contaminant confirmed</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Peritoneal washings; t/o specimen switch</td>
<td>1</td>
<td>Tissue origin from patient confirmed</td>
<td>Microsatellite instability of STR loci observed in tumor</td>
</tr>
<tr>
<td>16</td>
<td>Breast biopsy; t/o specimen switch</td>
<td>4, 5</td>
<td>Tissue origin from patient confirmed</td>
<td>Example B in text</td>
</tr>
<tr>
<td>17</td>
<td>Soft tissue biopsy; t/o specimen switch</td>
<td>5</td>
<td>Tissue origin from patient confirmed</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Identity of autopsied body</td>
<td>5</td>
<td>Patient identify confirmed</td>
<td>Example C in text</td>
</tr>
<tr>
<td>19</td>
<td>Prostate biopsy; t/o specimen switch</td>
<td>4</td>
<td>Specimen switch confirmed</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Bone marrow core biopsy; t/o specimen switch</td>
<td>1</td>
<td>Tissue assigned to correct patient</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Skull flap for cranioplasty; t/o specimen switch</td>
<td>1, 4, 5</td>
<td>Tissue origin from patient confirmed</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Esophageal and gastric biopsy specimens for Barrett esophagus surveillance; t/o contaminant</td>
<td>2</td>
<td>Presence of contaminant confirmed</td>
<td>Putative contaminant microdissected from recuts of FFPE tissue block; combined donor and recipient marker profile observed in patient sample</td>
</tr>
<tr>
<td>23</td>
<td>Small intestine biopsy; t/o specimen switch</td>
<td>2</td>
<td>Specimen switch confirmed</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Large intestine biopsy; t/o specimen switch</td>
<td>2</td>
<td>Specimen switch confirmed</td>
<td></td>
</tr>
</tbody>
</table>

FFPE, formalin-fixed, paraffin-embedded; QNS, quantity not sufficient; t/o, rule out; STR, short tandem repeat.

* Settings are described in Table 2.
medical or surgical therapy, and so on. Three cases illustrate the broadened spectrum of testing.

**Example A**

Case 2 in Table 1 is an illustration of STR typing when the clinical outcome is atypical or unexpected. A man underwent bilateral lung transplantation for chronic obstructive pulmonary disease. His initial clinical course was uneventful, but a routine right main-stem bronchus biopsy obtained 11 weeks after transplantation to rule out rejection demonstrated small cell carcinoma (high-grade neuroendocrine carcinoma) of presumed donor origin

An extensive workup for metastatic disease was negative; there was no evidence of malignancy on close follow-up, and the patient was alive and well 13 months after transplantation.

STR typing was performed on recuts of the main-stem bronchus biopsy specimen; recuts from blocks of tissue from the patient’s explanted lungs and from the lung allografts (specifically, from transbronchial biopsies) served as reference standards. Testing demonstrated a marker profile in the main-stem bronchus specimen different from both of the reference standards, interpreted as evidence that the main-stem bronchus specimen originated from a third person.

A search of laboratory records indicated that another patient known to have small cell carcinoma underwent transbronchial biopsy in the same suite of operating rooms the same day; owing to the lengthy time before the specimen switch error became apparent, no root cause analysis was attempted.

**Example B**

Case 17 in Table 1 is an illustration of STR typing for reassurance. A woman had a right buttock mass that rapidly developed at the site of recent trauma. The patient underwent incision and drainage of the mass after physical examination and imaging studies were consistent with a hematoma; microscopic examination of the material sent for pathologic examination showed scant fragments of pleomorphic high-grade sarcoma admixed with abundant necrotic debris and blood. The unusual clinical history, coupled with the planned aggressive surgical and radiation therapy, led the patient to seek confirmation that the scant tumor fragments were not a contaminant.

STR typing was performed on fragments of viable tumor microdissected from the H&E-stained sections of the mass; a buccal swab served as the reference standard. Testing demonstrated a marker profile in the tumor sample identical to the reference standard, interpreted as evidence that the high-grade sarcoma originated from the patient.

**Example C**

Case 19 in Table 1 is an illustration of STR typing to ensure appropriate therapy prospectively. Of 8 biopsy specimens from a man who underwent prostate needle core biopsy for an elevated prostate-specific antigen level, 2 showed adenocarcinoma, Gleason score 6; the other 6 biopsy specimens showed adenocarcinoma, Gleason score 8 or 9. Because risk
stratification for Gleason score 6 adenocarcinoma (moderate risk) is significantly different from Gleason score 8 or 9 adenocarcinoma (high risk) and, thus, the tumor grade would impact treatment recommendation and prognosis. STR typing was performed on recuts of all 8 needle biopsy specimens. Testing showed a marker profile in the 2 biopsy specimens with Gleason grade 6 adenocarcinoma that matched the reference standard from the patient but a different marker profile in all 6 biopsy specimens with Gleason grade 8 or 9 adenocarcinoma.

Discussion

The case series that we present demonstrates the broad clinical spectrum in which DNA-based identity determination is currently performed in routine surgical pathology practice. Specifically, our series confirms that, first, STR-based typing can be applied in virtually any setting in which confirmation of specimen provenance is requested, including sections from FFPE tissue blocks and tissue microdissected from routinely prepared H&E-stained tissue slides, frozen section slides, and cytologic specimens. Second, this unselected series demonstrates that, in routine practice, STR-based typing is informative in a very high percentage of cases. Third, the case series highlights the emergence of applications outside of the well-described uses of DNA-based testing to address specimen labeling/identification and extraneous tissue contaminant issues, ie, in cases for which the clinical outcome was unanticipated, to ensure appropriate patient management in cases in which the diagnosis was unexpected, and by patient request (Table 2). Unlike historic uses of STR typing, these latter 3 clinical settings share the common feature that testing is performed after the diagnosis has been reported in the absence of any direct indication that a specimen switch may have occurred and, thus, document an emerging role of STR typing to detect diagnostic errors. In this context, STR typing seems an appropriate method to help meet the Institute of Medicine laboratory quality measures for patient safety.

Even though testing was informative in 92% of cases in our clinical series, our results highlight the fact that rare test failures occur. Failures are most likely to occur in cases in which the volume of tissue is simply too small to produce an informative marker profile (eg, analysis of a putative tissue contaminant microdissected from a permanent section). Given that the CODIS loci were selected for (and commercial STR kits were optimized for) use in forensic settings, in which target nucleic acids are present in extremely small quantities, assay failures in surgical pathology owing to insufficient tissue substrate are rare. In our experience, 2 levels (each 4 μm thick)
of a tissue fragment less than 2 mm in greatest dimension will virtually always provide sufficient DNA to produce a definitive marker profile from frozen tissue slides or H&E-stained tissue sections of routinely processed FFPE tissue sections. In some cases, a sufficient quantity of DNA is available for testing, but degradation of the DNA produces an assay failure; since commercial STR typing kits use primers in the multiplex polymerase chain reaction amplifications designed to produce short amplicons, assay failures due to degraded nucleic acids are also rare. Nevertheless, the use of AmpFISTR Minifiler (Applied Biosystems), which features primers designed for even shorter amplicon length, makes it possible in some cases to recover a definitive marker profile when analysis using the AmpFISTR Profiler Plus ID amplification is not informative. Although exceptions that can complicate DNA-based identity determination exist, we encountered only the presence of somatic mutations in tumor tissue (specifically, microsatellite instability) and the presence of combination recipient and donor marker profiles in a bone marrow transplant recipient, both well-described confounding variables in forensic and identity-testing settings.6,15-18

Although our study does not specifically address the reasons for the broadened spectrum of settings in which STR typing is performed in surgical pathology, some general trends seem clear. First, as noted, testing provides a definitive result in most cases. Second, testing is rapid (maximum assay time of 4 days) and relatively inexpensive. Third, while surgical pathologists have long been aware of specimen identity issues and their impact on patient safety,11,12,21,22 increased awareness by other medical professionals, as well as the lay public, that STR-based approaches are available to address putative or occult specimen identity issues has undoubtedly led to more proactive testing. The fact that our series includes a number of cases in which testing was performed owing to patient self-referral indicates that this group of stakeholders is becoming increasingly informed of specimen identity and contamination issues and empowered to exclude them. Fourth, media reports of litigation resulting from high-profile specimen switches have likely engendered some testing merely to limit perceived medical-legal risk.21 Fifth, our use of digital whole slide imaging to create a record of the tissue sections destroyed by microdissection of problematic tissue fragments removes a significant barrier to testing; the digital image ensures that the original glass slide is not lost from the patient’s medical record.

In the context of medical-legal risk, it is difficult to overemphasize the role of DNA-based STR typing in documenting specimen mix-ups that were previously difficult to detect. Although other laboratory methods exist for detecting specimen switches,23-26 they all lack the power of discrimination, the sensitivity, and the almost universal applicability of STR-based typing. The emergence of STR typing of tissue specimens in surgical pathology in the absence of any direct indication that a specimen switch may have occurred (settings 3, 4, and 5 in Table 2) highlights the ability of the testing to demonstrate specimen identity errors and the subsequent indirect diagnostic errors that are not captured by current laboratory protocols. The magnitude of this category of specimen switches is currently unknown and cannot be estimated based on the case series we report herein; a prospective study designed to capture all occult errors is needed to address this issue. Nevertheless, our case series highlights the increasing use of STR typing in novel clinical...
settings to detect diagnostic errors relating to contamination or misidentification that would not otherwise be captured by current laboratory protocols.

Finally, it is worth noting that several emerging technologies provide the opportunity to perform identity determination in cases in which STR-based typing methods are uninformative. One such method is based on analysis of mitochondrial DNA. Studies have demonstrated that informative alleles of mitochondrial DNA loci can often be amplified from samples too small or too degraded for successful analysis of nuclear DNA,28 although the unique aspects of mitochondrial genetics introduce complications into the interpretation of the test results.1,18 Another novel test approach is based on analysis of single nucleotide polymorphisms. Forensically relevant single nucleotide polymorphisms have recently been identified that permit analysis of highly degraded samples, including in identity-testing settings.28

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References


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