Molecular Auditing
An Evaluation of Unsuspected Tissue Specimen Misidentification
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Context.—Specimen misidentification is the most significant error in laboratory medicine, potentially accounting for hundreds of millions of dollars in extra health care expenses and significant morbidity in patient populations in the United States alone. New technology allows the unequivocal documentation of specimen misidentification or contamination; however, the value of this technology currently depends on suspicion of the specimen integrity by a pathologist or other health care worker.

Objective.—To test the hypothesis that there is a detectable incidence of unsuspected tissue specimen misidentification among cases submitted for routine surgical pathology examination.

Design.—To test this hypothesis, we selected specimen pairs that were obtained at different times and/or different hospitals from the same patient, and compared their genotypes using standardized microsatellite markers used commonly for forensic human DNA comparison in order to identify unsuspected mismatches between the specimen pairs as a trial of “molecular auditing.” We preferentially selected gastrointestinal, prostate, and skin biopsies because we estimated that these types of specimens had the greatest potential for misidentification.

Results.—Of 972 specimen pairs, 1 showed an unexpected discordant genotype profile, indicating that 1 of the 2 specimens was misidentified. To date, we are unable to identify the etiology of the discordance.

Conclusions.—These results demonstrate that, indeed, there is a low level of unsuspected tissue specimen misidentification, even in an environment with careful adherence to stringent quality assurance practices. This study demonstrates that molecular auditing of random, routine biopsy specimens can identify occult misidentified specimens, and may function as a useful quality indicator.


Errors in the Diagnostic Laboratory
One of the most important interfaces between patients and the health care system is the medical laboratory. Medical laboratory testing provides information to help maintain the wellness of patients, as well as to assist with the diagnosis of disease, the assessment of progression of a disease, and the identification of attributes of the disease and patient that can help select optimal treatment and decrease treatment morbidity. Approximately 70% of all medical decisions are influenced by the results of a patient’s medical laboratory test.1,2 Because of improvements in both testing methods and the usefulness of information generated from medical testing, laboratory medicine—or pathology—is growing rapidly in both patient use and technical complexity. Medical laboratory testing can be arbitrarily divided into 2 main areas: clinical pathology, which is typically concerned with testing of nontissue specimens, such as blood or urine, using analytical equipment, and anatomical pathology, which is typically concerned with the testing of tissue specimens or material aspirated from tissues using microscopy and direct examination of the specimen by a pathologist.

As with any interface between patients and a health care system, there is the potential for error. Errors may be preanalytical, occurring during specimen acquisition, transport, submission to the laboratory, and/or accessioning of the specimen into the laboratory; analytical, due to problems performing or interpreting the analysis; or postanalytical, which may be due to errors in generating and/or disseminating a report of the test. A useful review of these classifications has been published by others.3 Laboratory errors at any stage can be catastrophic for the patient, resulting in inappropriate treatment or lack of treatment. In fact, laboratory errors are also impactful in the veterinary pathology laboratory.4 Misidentification of a specimen that results in the assignment of a correct test result to the wrong patient is a type of laboratory error that has one of the greatest risks for adverse impact.

There are a large number of steps in the medical laboratory evaluation of human tissue specimens, many of which can lead to a misidentification error and subsequent misdiagnosis.5 A common source of tissue received by a medical laboratory for analysis is small tissue biopsies, which are obtained from outpatient procedures in doctor’s...
Furthermore, specimens that had a cancer diagnosis were preferentially not selected for analysis because of the potential for false interpretation of misidentification due to microsatellite instability. The major geographic sites of Calgary Laboratory Services were each represented in the specimens to approximate fair sampling of the different geographic areas and practice concentrations over the Calgary Health region (Calgary, Alberta, Canada). Table 1 illustrates the descriptive statistics of the specimens.

Paraffin blocks corresponding to the specimen pairs were obtained, and 4 × 20 μm sections were pooled into a single tube. DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit (No. 69506, Qiagen Inc, Toronto, Ontario, Canada) as per the manufacturer’s instructions. DNA concentrations were measured using a Nanodrop spectrophotometer (Fisher Scientific, Ottawa, Canada), and DNA was diluted in water for microsatellite analysis. Microsatellite markers were determined using the AmpFLSTR Identifier Plus PCR Amplification Kit (No. 4427368, Life Technologies Inc, Burlington, Ontario, Canada) using half-size (10-μL) reactions otherwise, as per the manufacturer’s instructions. Briefly, reactions were set up to a volume of 10 μL, and then polymerase chain reaction was performed on an Applied Biosystems thermal cycler (Fisher Scientific) as: denaturing at 94°C for 11 minutes, then 28 cycles of denaturation (94°C × 20 seconds) and annealing/extension (59°C for 180 seconds). Reactions were extended at 60°C for 10 minutes, then stored at 4°C prior to analysis. Microsatellite alleles were visualized using an Applied Biosystems Inc 3130 capillary electrophoresis instrument (Fisher Scientific).

**RESULTS**

Table 1 summarizes the characteristics of the specimens that were sampled during this study. It was hypothesized that the most commonly evaluated biopsy specimens would be the most likely to be misidentified. In our practice area, these are GI, skin, breast, and prostate biopsies. The Calgary Laboratories Services diagnostic laboratory network evaluates specimens in the city of Calgary (Calgary, Alberta, Canada) from 1 large quaternary care hospital (Foothills Medical Center), 2 midsize secondary care hospitals (Rockyview Hospital and Peter Lougheed Hospital, Calgary, Alberta, Canada), and a dedicated pediatric hospital (Alberta Children’s Hospital). The community laboratory site (Calgary Laboratory Services, Diagnostic and Scientific Center, Calgary, Alberta, Canada) receives specimens from non–hospital-based outpatient clinics and physician offices. Several years ago, health care administrators determined that efficiency in the Calgary Health Region could be improved by concentrating specific surgical services in specific hospitals. Thus, Rockyview Hospital is the only site that receives urologic surgical specimens (eg, prostate), and the community lab—Calgary Laboratory Services, Diagnostic and Scientific Center—receives the bulk of specimens from outpatient dermatology patients; hence, there are distortions in the frequency of specimen types associated with the different laboratory sites.

For this study, a total of 1944 specimens, or 972 alphabetically selected specimen pairs, were satisfactorily evaluated by genotyping. When possible, 1 specimen of a patient specimen pair, which was sampled on a different date, was preferentially paired with another specimen from 1 of the 3 major biopsy groups. Because GI biopsies are the most commonly evaluated tissue specimens, they represent the largest group of specimens evaluated by genotyping. In addition to the 1944 specimens used for the genotype study, 261 additional biopsy specimens did not yield at least 6 unequivocal genotype markers and were rejected from the study as insufficient for analysis. In addition, a further 186
specimens were requested, but the paraffin blocks were either unable to be located or were unavailable, or inspection of the block revealed a specimen that was likely to be completely destroyed by the analysis. In addition to the specimens studied, 2 deliberately mismatched specimen control pairs (4 unrelated specimens selected and paired randomly) were surreptitiously introduced into the specimen pool to evaluate the quality of the analysis. Genotype traces from the specimen pairs were evaluated manually. Both pairs of mismatched specimen controls were unequivocally identified by investigators blinded to their presence, and the traces were censored from further analysis. From analysis of the 972 acceptable patient pairs, 1 unanticipated, mismatched pair of specimens was detected in the initial survey (Figure 1, specimen pair X1 and X2). The 2 formalin-fixed, paraffin-embedded specimen blocks were submitted to the local clinical molecular diagnostic laboratory (Calgary Laboratory Services, Molecular Pathology Laboratory, Calgary, Alberta, Canada) for independent analysis. The 2 specimens were reextracted independently and evaluated using the AmpFLSTR Identifiler Plus via standard operating protocols in the clinical laboratory. The results confirmed that the 2 specimens that were documented as originating from the same patient did not share the Identifiler Plus.

Figure 1. Identification of biopsy specimen pair mismatch from the primary genotyping screen. Pairs of tissue specimens documented in medical records as originating from the same patient but submitted at different times and/or to different locations were collected, and genomic DNA was prepared. The DNA was evaluated for 13 different, standardized microsatellite markers that are commonly used for forensic genotype comparisons (AmpFLSTR Identifiler Plus) and compared between the sample pairs. A and B, A composite of the genotypes of the 13 markers obtained from evaluation of specimens X1 (A) and X2 (B), which were documented as originating from the same patient. C and D, The genotyping composite obtained from reevaluating the independently extracted specimens X1 (C) and X2 (D).
genotype markers (Figures 1 and 2). Several specimens that could have been the source of misidentification for each specimen (eg, biopsies received temporally similar to either specimen X1 or X2) were genotyped and compared; however, a matching specimen to either of the mismatched specimens was never identified, and hence the mismatch could not be resolved (data not shown). Fortunately, this case of specimen mismatch had no clinical consequence for either patient.

**DISCUSSION**

In spite of much attention being devoted to the error-free handling of tissue specimens, clinical suspicions of misidentification are not uncommon. Most of these may be resolved by a pathologist who may be able to determine physical mixups on the basis of morphologic examination (eg, liver expected and lung observed). More difficult cases can be resolved by molecular means, such as evaluation of microsatellite profile in comparison with a known sample from a particular patient or patients. For example, in our diagnostic laboratory, for 2015 we evaluated 27 cases that were flagged by pathologists for potential mismatch or specimen contamination (Table 2) out of a total of 252,590 surgical or biopsy specimens from the Calgary region. We also received an additional 35 cases from other health care areas for evaluation. Cases for identification analysis can consist of 2 or more specimens. We have evaluated up to 12 specimens for a single case of potential
mismatch (biopsy specimens thought by a referring clinic to potentially be out of chronologic order). Although our diagnostic experience has shown that misidentification of surgical pathology specimens does occur and can be identified through careful pathologist surveillance, we were very curious to know the baseline frequency of unsuspected misidentification. Because most of our referred cases were associated with mismarking or loss of labels from small biopsy specimens, such as prostate cores, we decided to focus on those types of specimens because they may represent the most likely groups of specimens to be misidentified.

Our data show that there is, in fact, a low level of specimen misidentification even among the specimens submitted to an academic, quaternary care hospital. Our misidentification frequency of 1 specimen in 1944 analyses (0.0514%) yields a 95% CI of approximately 0.000013 to 0.003 depending on which of 4 approaches to calculate intervals in a binomial proportion is used.9

Laboratory errors can have catastrophic effects on patient care; hence, various approaches to study causes of laboratory errors have been undertaken. One retrospective study10 concerned the mismarking of patient specimens. The authors studied root causes of 253 adverse events and found that misidentification accounted for 182 of the 253 adverse events (72%). Of the 182 misidentification cases, 132 were attributed to preanalytical errors. Although this study, and others,11 clearly show the disproportionate impact of misidentification errors on adverse events to patients, the study was based on root cause analysis reports, and hence there was no denominator to determine error frequencies. Furthermore, errors across all aspects of laboratory medicine were included in the study.

Some attempts have been made to identify frequencies of misidentification errors in laboratory medicine. One group reported a longitudinal study of 6.6 million laboratory specimens from Los Angeles during a 26-week period and identified 16 632 specimen errors, of which approximately 1% were misidentification errors.12 This group evaluated all types of laboratory specimens, although these primarily consisted of phlebotomy specimens, which have a higher level of scrutiny to prevent misidentification errors than other types of specimens, and errors were identified and reported by members of the health care team. Implementation of 3 new patient safety projects significantly reduced the frequency of specimen misidentification errors.12

Another study of laboratory errors used a large database developed and maintained by the College of American Pathologists (CAP).13 The author reviewed data collected from 130 CAP quality studies and, while evaluating several performance measures, found that failure to identify patients properly prior to specimen collection had a surprisingly high frequency of 6.5%. These were errors that were detected and reported, and the specimens studied were all blood specimens.

Misidentification errors in blood specimens may be due to the misidentification of patients by wristband reading errors. One older, large study evaluated errors from 712 US hospitals.14 Wristband errors due to a variety of causes occurred with a frequency of approximately 2.2%. A subsequent evaluation showed a reduction of the initial error rate of 7.4% to 3.1% following continuous monitoring and educational initiatives.15 Similarly, in 14 Australian laboratories, errors transcribing a patient’s name from the pathology requisitions to the computer system were reviewed. In this study, an institution made transcription errors involving patient identity in a median 1% of cases, whereas the worst performer made identification errors in 9% of cases.16 Similarly, in a CAP Q-Probes study performed in 660 institutions, a total of 5514 of 114 934 outpatient requisitions (4.8%) were associated with at least 1 type of order entry error, including 1 or more discrepancies in the identity of patients or physicians.17 A study of preanalytical errors of blood specimens directed to a clinical laboratory used direct observation techniques to identify typical operating procedures, then recorded nonconformities and errors from 3 clinical wards during a 6-month period.18 Preanalytical error frequency was 29.916 ppm, which included a variety of causes. Aside from technical errors, such as underfilling blood collection tubes, or hemolysis, the frequency of patient misidentification was 352 ppm. Interestingly, prelabeling blood collection tubes was associated with 2 of 3 episodes of specimen identification errors. In other studies on this topic, laboratory errors due to misidentification ranged from 1% to 2% for inpatients and from 0.2% to 6% for outpatients.19

Other studies have shown that the error rate is likely much higher for general laboratory specimens than transfusion specimens (around 1%), although this number can vary widely. For example, 2 studies have reported that the frequency of specimen misidentification in a stat laboratory might be as high as 8.8%.20,21 In contrast, a large study of patient and specimen identification errors detected at 120 institutions identified an overall rate of patient identification errors of only 55 per 1 000 000 billable tests.22 In this study, the errors were detected and reported by members of the medical care team, and the vast majority of laboratory tests were blood tests.

The CAP studied specimen accessioning accuracy in anatomic pathology in 1995.23 A total of 417 laboratories participated in the study. Identification and accessioning deficiencies were found in 60 042 of 104 115 cases accessioned (5.9%). Errors related to specimen identification accounted for 9.6% of these deficiencies, for an overall identification error rate of 0.57%.

Surprisingly, there are relatively few studies addressing the important area of surgical pathology specimen misidentification. The most definitive studies have evaluated prostate biopsies, possibly because of the well-known

<table>
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<th>Test</th>
<th>Mismatch Identified</th>
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<tbody>
<tr>
<td>Specimen mixup</td>
<td>2</td>
<td>15b</td>
</tr>
<tr>
<td>Specimen contamination</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Confirmation of specimen</td>
<td>Not applicable</td>
<td>4</td>
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a This table documents the 2015 requests for specimen genotyping received by the Calgary Laboratory Services Molecular Pathology laboratory to evaluate when a specimen mixup or contamination was suspected by a pathologist. Each case may consist of 1 (different areas of a histology slide tested for contamination) or multiple (up to 12) specimens that could be potentially misidentified. Also, confirmation of specimen identity (with a fresh blood specimen, usually, or a previous pathology specimen, occasionally) was requested by either a physician or a patient who did not believe the results of the pathology report.

b One specimen provided insufficient DNA quality/quantity to allow testing.
phenomenon of “vanishing cancers,” first defined with prostate cancers in 1995.24 “Vanishing cancer” is the term used to describe discordance between an initial sampling of a tumor diagnosed as cancer, and the inability to identify malignancy in a subsequent resection of the site of biopsy. Of course, another reason for discordance between biopsy and resection histology is laboratory error, and hence the focus on study of these types of specimen.

A French study used molecular genotyping to compare prostate biopsies with resection specimens.8 All pT0 prostate cancer biopsies accrued during a 20-year period at a single institution were compared with resection specimens. A total of 18 eligible biopsies were available, and 12 were able to be compared with the resection tissue. Of these, molecular genotyping did not identify any errors in identification. In an Austrian study, prostate biopsy specimens were compared to blood specimens accrued within the context of a large clinical trial.25 After 3 specimens were initially found to be misidentified during the analysis for the original objectives of the trial, it was decided that all year 2 and year 4, protocol-mandated biopsy specimens should be evaluated. Thus, of 4777 year 4 specimens, 1 (0.02%) was found to be misidentified, and of 6458 year 2 specimens, 13 specimens (0.4%) were found to be misidentified. Furthermore, of 6735 reference blood specimens evaluated, 31 (0.5%) were found to be misidentified.

In addition, a large US study evaluated specimen provenance errors by compiling information obtained from routine molecular diagnostic provenance testing of 12947 prostate biopsy specimens26 where misidentification was not initially suspected. This study identified a frequency of occult specimen misidentification (type 1 error) of 0.26% and a frequency of specimen contamination (type 2 error) of 1.69%. None of these studies used randomly selected specimens submitted for routine analysis.

Mixup of surgical specimens can occur secondary to labeling misidentification. A large US study of 69 hospitals of varying size and care levels, including 34 rural and 35 urban settings, used 12 quality criteria evaluating both container and requisition characteristics.27 This study found that there were identification defects in 2.9% of cases (1780 of 60501 cases).

Unfortunately, it is difficult to compare studies because of differing definitions for errors, study methodology, and different quality assurance programs. Interestingly, it appears that no studies directly evaluating either blood or tissue for unsuspected misidentification of randomly selected surgical pathology specimens have ever been published. Given the potential for expensive liability in such cases, a voluntary, random review of tissue specimens looking for misidentification might be somewhat unpopular in jurisdictions with an overtly punitive legal system.

The identification of a significant, detectable frequency of errors in laboratory testing is contrary to public perceptions—“Public expectations regarding their own safety when entrusted to our care is: no preventable errors.”28 In Canada, confidence in the quality of laboratory testing was seriously eroded through examples of failures in the quality of the diagnostic process in several high-profile media cases.29-32 Unfortunately, misidentification of a laboratory specimen has to be suspected before the specimen is selected for scrutiny or more formal identification verification. If the pathologist is diligent or fortunate, he or she may identify a discrepancy between the clinical history and the microscopic appearance of a surgical pathology specimen, to generate the suspicion of misidentification. Nonetheless, it may be quite easy to miss such a discrepancy, especially if there is a mixup in a group of similar specimens, such as prostate or colon biopsies. Because the consequences of misidentification errors to patients are potentially very high, this information needs to be known in order to identify a need, if any, for improved safeguards in the process.

As the potential for patient morbidity and legal liability for physicians and hospitals is significant, quality assurance programs to decrease error in the diagnostic laboratory are continuously being studied and improved.33 Root cause analysis34,35 is a systematic method of investigating errors that may lead to changes in protocols which can ultimately reduce errors. Errors that are not uncovered through investigation of patient morbidity or death, however, are usually identified through voluntary reporting, which can be very capricious and likely does not achieve 100% compliance. Follow-up studies have shown that careful attention to reducing identification errors has a positive effect. For example, when phlebotomists refused to collect blood from patients with a wristband error, the rate of such errors decreased markedly.35 Likewise, identification of a common reason for cytopathology specimen misidentification at 1 institution resulted in a change in practice that markedly reduced these errors from 0.59% to 0%.36 Introduction of bar code specimen identification systems has resulted in the reduction of specimen misidentification errors in several hospital settings in the context of blood transfusions37 and pathology specimen tracking.38,39 Of course, one needs to know that there is a problem with a process, before that problem can be solved and the process monitored for recurrences.

The impact of specimen identification errors can be significant. Aside from anecdotal cases of severe therapeutic morbidity or even unnecessary amputations from cancer misdiagnosis, there are substantial economic impacts. A US-based study evaluated the economic implications arising from specimen provenance complications, such as misidentification or contamination in prostate biopsy specimens.40 This study used the frequency of type 1 and type 2 provenance errors identified in a published study of prostate biopsy specimens.26 Although it was based on US information, which included patient quality of life impact, as well as medical and medicolegal costs, which may not be completely applicable to other countries, a substantial cost exceeding $879.9 million was calculated—approximately $3,776 per cancer diagnosis. Extrapolated to other biopsy specimens, the economic costs could measure in the billions of dollars and would also predict an adverse quality of life impact to thousands of patients.

Errors in surgical pathology have a wide variety of causes (reviewed in Valenstein and Sirota41). Recently, members of the Initiative for Anatomic Pathology Laboratory Patient Safety evaluated systemic processes involved in the analysis of clinical laboratory specimens and developed a set of metrics determining which processes had high or low risks for contributing to adverse patient outcomes.41 Steps associated with the potential for specimen misidentification usually corresponded to the high-risk grouping and merit greater focus within quality management systems. Since the 1980s, molecular biology tools have been developed to assist with the identification of foreign DNA in forensic applications,32 but the use of such “genetic fingerprinting” techniques has only recently been commonly applied to

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resolve surgical and cytopathology specimen mixup or contamination concerns, as has been reviewed elsewhere.45 Such tools can also be used to confirm the origin of nontissue or acellular laboratory specimens, such as urine.44 In hospitals that are experienced in such techniques of molecular genetic analysis, resolution of these types of diagnostic dilemmas is quite feasible and should be solicited when appropriate as a basic risk management tool. These techniques also now allow for a quality review, or molecular audit, of the integrity of the surgical pathology specimen diagnostic process, which would evaluate the concordance between random tissue specimens supposedly obtained from 1 patient at 2 different times to measure the baseline rates of misidentification for either specimen, rather than relying on recognition of mixup, sometimes by good fortune. Although microsatellite allele analysis, such as that used in this study, is the gold standard, recent highly automated, platform-based techniques, such as single-nucleotide polymorphism genotyping by mass spectrometry,46 can offer improvements in throughput, robustness, and cost-effectiveness. Given the projected cost of misidentification errors in high-impact areas of laboratory testing, such as cancer diagnosis, perhaps the origin of definitive specimens leading to a diagnosis for which treatment is associated with a significant risk of morbidity (amputation, large resection, aggressive systemic chemotherapy, etc) should be evaluated by at least a minimal panel of genotyping markers if there is a perceived risk of mixup. For example, a prostate biopsy leading to a prostate resection might be selected for confirmation versus an incisional biopsy from a bone tumor where there may be a less likely chance of mixup in a typical laboratory. A more limited panel of markers, such as 6 microsatellite markers (versus the 13 that we used in this study) or a small panel of single-nucleotide polymorphism markers, could be compared with blood from the patient. Because it is not uncommon for patients to question the possibility of mixup resulting in an unexpected diagnosis of cancer, this practice could both allay some patient anxiety and be a reasonably inexpensive risk management practice for the hospital and/or laboratory. At the very least, molecular auditing of random surgical pathology specimens may be a useful quality assurance indicator, and/or may be helpful in identifying objective improvements in misidentification error rates after the implementation of new error reduction programs.

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