Specimen Provenance Complications Mitigated by Use of DNA Specimen Provenance Assignment (DSPA) Testing

August 2013

Strand Diagnostics, LLC.
5770 Decatur Blvd., Suite A
Indianapolis, IN 46241
1-888-924-6779

STRAND DIAGNOSTICS

TABLE OF CONTENTS

The Clinical Problem

Introduction

Each year in the United States, millions of patients undergo surgical biopsies to confirm the presence of cancer, the results of which routinely influence the diagnosis and subsequent patient treatment options. Because of the critical role these biopsy results play in the diagnosis and treatment of patients, it is essential to develop a system that ensures that samples are properly identified. The diagnostic testing cycle is not always free of mistakes, so from time to time, innovations emerge to improve this process. This paper examines the benefits of one such innovation—incorporating DNA Specimen Provenance Assignment (DSPA) into the diagnostic testing cycle for cancer.

Background

Cancer is a serious global health concern that currently accounts for one in every eight deaths worldwide. According to recent reports from the American Cancer Society, an estimated 12.7 million cases of cancer were diagnosed in 2008, and that number is expected to rise to more than 20 million by 2030 due to population growth and aging alone. The overall direct costs of treating cancer in United States in 2007 were $103.8 billion.[1]

The Diagnostic Testing Cycle

The process of collecting and evaluating the biopsy specimens used to render cancer diagnoses involves numerous steps and various medical professionals working in different locations. With such a complex process executed at a large scale, patient misidentification, specimen transposition or foreign cell contamination ("Specimen Provenance Complications") may occur in clinical or anatomical pathology and is an ongoing concern for clinicians, pathologists, and patients. Specimen Provenance Complications (SPCs) **detected** (as a result of quality assurance protocols) or **undetected** (occult) are an inherent by-product of the diagnostic testing cycle that, if left undetected, can lead to serious diagnostic mistakes, application of the wrong therapy and other adverse patient outcomes (e.g. one patient may receive unnecessary treatment that significantly alters quality of life, while the other patient's cancer remains undiagnosed and continues to advance).

A report from the Cleveland Clinic acknowledges that the potential for complications is ever-present. The article published by Cleveland Clinic's Dr. Mary Bronner, M.D., Section Head, Morphologic Molecular Pathology in the Department of Anatomic Pathology identifies 18 different steps in the diagnostic test cycle (from clinic thru the issuance of a histopathology report) :

"Considering the numerous medical professionals involved in processing a tissue sample from the patient to the pathologist reviewing it microscopically, it is truly a marvel that so few errors occur. Consider that from the patient, a biopsy sample or surgical resection specimen is initially (1) handled by the treating physician using carefully cleaned biopsy and surgical tools that have been used previously to obtain many other patients' samples. One or more nurses or other assistants assist the physician in (2) getting the tissue sample into a properly labeled specimen container. The specimen container is (3) batched with many other containers and (4) transported to the pathology laboratory. There, the specimen is (5) accessioned into the pathology computer system and is (6) assigned a unique surgical pathology identifier. Next, the paperwork and specimen container are (7) processed by a pathology assistant, resident, or pathologist who removes the specimen from the labeled container and examines and describes the tissue grossly. The tissue is (8) dissected by carefully cleaned instruments that also are used to dissect many other patients' samples. The specimen is (9) divided among a number of tissue cassettes, small plastic containers, which are (10) labeled individually with the surgical pathology number and a unique block number. The tissue cassettes have holes in them to permit flow of the various processing fluids required to process the tissue chemically into a final wax tissue block. Many hundreds of different patients' cassettes are (11) placed into a common chemical bath for this processing stage. Rarely, a tissue fragment from one patient can exit its cassette and enter through the cassette holes of another patient's block to become part of this second patient's block. A histotechnologist uses clean forceps, which are cleaned and used subsequently on many other patients' specimens, to (12) pick up the wax-infused tissue fragments from the processing cassettes and place them into the final wax tissue block that will be used to section the actual histologic slides. Another histotechnologist (13) sections 5 µm slices on a razor blade affixed to a microtome. These thin wax slices are (14) floated onto a carefully cleaned water bath, which has had many other patients' wax slices previously floating in it. Floating allows slices to flatten and be (15) transferred onto a glass slide, which has been hand-labeled by the histotechnologist. The slide later receives a permanent computer-generated slide label that is (16) affixed to the slide by another technician. Finally, all of the slides on any given patient's procedure are (17) assembled with the accompanying paperwork and (18) delivered to the pathologist for diagnostic interpretation. A mistake leading to inadvertent tissue contamination can occur at any one of the above logistically complex processes."[2] *(Numbers have been added by the author).*

For a visual representation of the biopsy evaluation process described above, please see the diagram in appendix A.

Evidence of Error

The existence of diagnostic mistakes due to Specimen Provenance Complications in the diagnostic testing cycle is documented in both medical research and real-life cases. One study from the College of American Pathology estimates that the annual biopsy switching error rate in the U.S. is approximately 0.33%.[3] While that figure may seem low, when applied to the 1.6 million breast biopsies performed each year,[4] the impact becomes much more significant. A 0.33% error rate indicates that more than 5,200 specimens may be subject to a Specimen Provenance Complication, and each situation usually involves more than one patient. Furthermore, the true incidence of both errors and the resulting adverse events is likely much higher than what is presently measured since research results are based only on errors that were actually detected and reported. The scientific papers summarized below provide further insight into the existence of SPCs in the diagnostic testing cycle.

Patient Identification Error Among Prostate Needle Core Biopsy Specimens–Are We Ready for a DNA Time-Out?[5]

This article discusses strategies for decreasing the occurrence of patient identification errors in surgical pathology—events that can lead to catastrophic outcomes for both patients and their physicians. Researchers performed root cause analyses following three cases of patient identification error involving prostate needle core biopsy specimens. It was determined that these events resulted from slips and lapses of automatic human action that may occur at numerous steps during the diagnostic testing cycle. Therefore, it may be difficult to entirely prevent identification errors solely through the optimization of work flow processes, and a DNA time-out to confirm patient identity is useful to eliminate identification errors prior to initiating treatment.

Rate of Occult Specimen Provenance Complications in Routine Clinical Practice[6]

This study evaluated a data set comprised of almost 13,000 prostate biopsies that were prospectively tested for otherwise occult (undetected) specimen provenance errors using DNA Specimen Provenance Assignment (aka “DNA fingerprinting”) as part of routine clinical practice. The frequency of occult Type 1 errors (a complete transposition between patients) and Type 2 errors (contamination of the patient’s tissue with one or more unrelated patients) was 0.26% and 0.67%, respectively. It is important to note, however, that for each complication event, (at least) two individuals are implicated, specifically the target patient and the foreign patient (or patients) whose tissue was misidentified as originating from the target patient. Thus, the 0.93% combined error rate underestimates the percentage of patients potentially impacted by provenance errors by at least a factor of two. The Type 1 rate showed no correlation with surgical pathology laboratory setting or urologic practice group setting; the Type 2 rate correlated solely with surgical pathology laboratory setting. It is noteworthy that every urology practice setting and pathology laboratory type with a representative sample size (at least 1000 specimens included in the data set) experienced at

least one Type 1 and one Type 2 error during the study period. The occult SPC rate in this limited data set provides an estimate of the scope of the problem of potential misdiagnosis due to occult specimen provenance errors in routine clinical practice.

The Changing Spectrum of DNA-Based Specimen Provenance Testing in Surgical Pathology[7]

This article documents the usefulness of DNA-based specimen provenance testing for resolving specimen source contamination and identity problems in surgical pathology. Researchers' investigation of a series of consecutive cases during a five-year period revealed that this analysis can be applied in virtually any setting in which specimen source confirmation is requested. In addition, it can also be performed in the absence of any direct indication that an error has occurred, primarily when pathology findings are unexpected or the clinical setting is atypical. By identifying errors that may go undetected using current laboratory protocols, DNA-based specimen provenance testing represents an important step in the ongoing mission to improve patient safety.

Biopsy Misidentification Identified by DNA Profiling in a Large Multicenter Trial[8]

This article discusses the REDUCE trial, a multi-site, double-blind study designed to determine whether dutasteride 0.5 mg daily decreases the risk of biopsy-detectable prostate cancer compared with a placebo during a four-year period (known as the REDUCE trial). When three biopsy mismatches were discovered at the end of the second year, a mandatory biopsy identity testing program using DNA profiling was implemented. Various other process changes and educational efforts were made at this time as well to improve sample handling and chain of custody. Year two biopsies were evaluated retrospectively, while year four biopsies were evaluated prospectively. Testing revealed a sample mismatch rate of .4% during the first two years (13 sample handling errors) and .02% during the second two years (one sample handling error). The authors concluded that the potential for biopsy misidentification should be a key concern for both large clinical trials and routine clinical practice. Accordingly, more detailed attention to chain of custody and widespread use of DNA testing of patient samples should be considered.

Reported Cases of Specimen Provenance Complications (SPCs)

There are several reports of adverse patient outcomes due to occult Specimen Provenance Complications are published in consumer and trade media. These reports not only highlight the harm to patients, but often identify the serious legal implications for the healthcare providers responsible for the error. Three such cases involving breast and prostate biopsies follow.

Med-Mal Claim Brought for Complications After Surgery[9]


VERDICTS & SETTLEMENTS

Med-mal claim brought for complications after surgery

$1,050,000 Settlement

Man died 18 months after anoxic brain injury

$1,375,000 Settlement

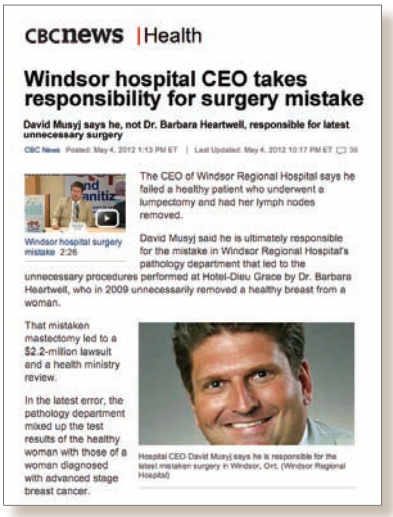
Virginia Medical Law Report
January 2012

An article in the *Virginia Medical Law Report* discussed a case in which the prostate tissue samples of a healthy patient were switched with the tissue samples belonging to another patient who had cancer. The mix-up resulted in an unnecessary prostatectomy and subsequent urinary leakage and erectile dysfunction for the 60 year-old plaintiff. The other patient, referred to as "John Doe" in the article, presumably had a delay in treatment of his cancer. The switch was uncovered after routine post-surgical pathology tests revealed no cancer. An investigation discovered that the samples were mistakenly switched at some point during the diagnostic testing cycle with the urology clinic and the pathology lab pointing fingers at each other as the source of the mistake. Ultimately, the case was settled out court with both the urology clinic and the pathology lab contributing to the $1,050,000 settlement.

'False Cancer' Lawsuit[10]

According to an article in the *New York Post*, a New York woman who was undergoing surgery for injuries suffered in a car accident was misdiagnosed with Stage 4 metastatic breast cancer based on a lab's mishandling of her biopsy tissue. During routine post surgical tests, the woman's biopsy was contaminated with tissue from the sample of a patient who did have cancer. Since the diagnosis was Stage 4 cancer, her doctors recommended radiation treatment as soon as possible. However, after doctors were unable to pinpoint her type of cancer, they asked for a second opinion from a different lab. The new test revealed the misdiagnosis but not before she had already undergone six radiation treatments. A lawsuit against the hospital involved in the incident quickly ensued, but at the time of this paper's publication litigation was still pending.

Windsor Hospital CEO Takes Responsibility for Surgery Mistake[11]


CBCnews | Health

Windsor hospital CEO takes responsibility for surgery mistake

David Musyj says he, not Dr. Barbara Heartwell, responsible for latest unnecessary surgery

The CEO of Windsor Regional Hospital says he failed a healthy patient who underwent a lumpectomy and had her lymph nodes removed.

David Musyj said he is ultimately responsible for the mistake in Windsor Regional Hospital's pathology department that led to the unnecessary procedures performed at Hotel-Dieu Grace by Dr. Barbara Heartwell, who in 2009 unnecessarily removed a healthy breast from a woman.

That mistaken mastectomy led to a $2-million lawsuit and a health ministry review.

In the latest error, the pathology department mixed up the test results of the healthy woman with those of a woman diagnosed with advanced stage breast cancer.

CBC News
May 2012

This article from *CBC News* documents the story of a biopsy misidentification occurring in the Canadian healthcare system. The CEO of a large hospital in Windsor, Canada has accepted responsibility for an administrative error that resulted in a healthy female patient being falsely diagnosed with breast cancer and undergoing unnecessary procedures, including a lumpectomy and removal of her lymph nodes. The error originated in the pathology department when the woman's test results were mixed up with those of another patient who had advanced stage breast cancer.

An Innovative System to Reduce SPCs

Human error is an inevitable component of all manual processes in healthcare, and despite the implementation of various quality improvement measures, the problem of Specimen Provenance Complications persists, especially occult SPCs. Developing a system that not only reduces the number of SPCs in the diagnostic testing cycle, but also detects otherwise undetected SPCs improves diagnostic accuracy and is essential to preventing adverse outcomes associated with misdiagnosis. Key elements of such a system are described in the sections below.

Bar Coding

Probably the most common identification error reduction strategy in laboratory use today is simply to replace or supplement hand-written identification with computer-generated and computer-read bar codes. Though still not nearly as pervasive in laboratory settings as other industrial, retail and logistics arenas, bar coding is a very cost effective means of tracking specimens and applying a degree of scientific precision to identification not possible from manual methods. Bar codes are dramatically less susceptible to human error than any form of hand-written and visually interpreted identification. Applying a unique patient identification bar code to all components involved in the collection of a patient's specimens (i.e., biopsy jars, requisition, etc.) is an excellent way to help prevent Specimen Provenance Complications that can occur during the collection, handling and processing of biopsy samples.

Forensic Chain of Custody Principles

Forensic chain of custody principles commonly used in the criminal justice system have valuable applications in the healthcare setting. These serve as a means of maintaining the integrity of biopsy samples by providing documentation of the control, transfer and analysis of patients' specimens. Specific examples include having patients self-identify on key paperwork associated with their biopsies, implementation of security seals on biopsy collection kits and taking photographs of samples as they are received at the lab for evaluation.

DNA Specimen Provenance Assignment (DSPA) Testing

Although tactics such as those mentioned above have the effect of reducing errors of specimen provenance, there is still a possibility that some SPCs will remain undetected. Therefore, implementing additional measures to establish specimen provenance is an essential part of addressing the problem. Incorporating DNA Specimen Provenance Assignment (DSPA) testing into the diagnostic testing cycle allows for absolute confirmation of patient identity at the molecular level, and significantly reduces the opportunity for adverse outcomes due to Specimen Provenance Complications, especially those that are otherwise undetected. Like chain of custody principles, DNA testing is most commonly performed in forensic settings for the purpose of

crime scene analysis and/or parentage. However, numerous researchers have acknowledged the potential benefits of leveraging this proven technology in the healthcare setting.

Suba et al. concluded in their article published in the *Journal of Urology* that the medical community may be ready for a "DNA time-out" to address the problem of biopsy switching errors. "Patient identification errors among prostate needle biopsies may be difficult to entirely prevent through the optimization of work flow processes. A DNA time-out, whereby DNA polymorphic microsatellite analysis is used to confirm patient identification before radiation therapy or radical surgery, may eliminate patient identification errors among needle biopsies."[5] Further, a January 2013 Washington University study published in the *American Journal of Clinical Pathology* confirms that the average frequency of occult Specimen Provenance Complications is 1.9% across all clinical settings.[12]

In DSPA testing, a panel of 16 microsatellite Short Tandem Repeat (STR) markers that recognizes highly variable loci of human DNA is used in a PCR-based assay and analyzed by capillary gel electrophoresis. The 16 STR loci range from approximately 75 to 400 base pairs in length, and are highly polymorphic in the human population. The collective data from this panel result in a genetic profile, or "DNA fingerprint," representing the individual. DSPA testing can be used to compare the profile from the malignant specimen(s) with that determined from DNA isolated from a patient's reference sample (taken via cheek swab at the time of biopsy). DSPA helps ensure each patient is assigned the appropriate diagnosis prior to initiating treatment.

The Diagnostic Use of DSPA Testing by Clinicians

Using these error reduction techniques coupled with a DSPA test on a prospective basis as part of routine clinical practice ensures that surgical biopsy samples belong exclusively to the patient being diagnosed, thus arming clinicians with one more point of concordance and providing the critical information they need to proceed confidently with treatment recommendations. Patients can then receive the care they require in a prompt manner to facilitate the best possible outcomes. This prospective use of these techniques helps overcome the problem of otherwise undetected (occult) specimen provenance complications (which occur at the rate of 1.9%) prior to affecting patient outcomes.

The Quality Assurance Use of DSPA Testing by Pathologists and Lab Personnel

Laboratory personnel who suspect that a specimen provenance complication (switch or contamination) has occurred can benefit by the use of DSPA testing. Examples of complications that can resolved by the use of DSPA testing include: specimens arriving at the lab without proper identification; slides are suspected of being switched among patients; cancerous tissue fragments discovered in equipment (e.g. microtome, water bath, Ventana® system); unexpected/inconsistent tissue-type on a slide. DSPA can be used to match tissue to tissue or tissue to a reference sample (e.g. buccal swab, blood card) in order to help sort out problems and resolve concerns about specimen provenance complications prior to issuing final histopathology reports.

Benefits of Using DSPA Prospectively

Implementing a system consisting of the bar coding, forensic chain of custody principles and DNA Specimen Provenance Assignment testing offers numerous benefits for both patients and the healthcare providers involved in their care.

Enhancing Diagnostic Accuracy and Patient Safety

Incorporating a system that utilizes patient-specific bar coding, forensic chain of custody principles, and DSPA is a means of reducing SPCs during the collection, handling and processing of biopsy samples. Using this system prospectively as part of the routine clinical practice can significantly reduce SPCs, thus bringing enhanced levels of diagnostic accuracy and patient safety to the diagnostic testing cycle.

Mitigating Risk for Healthcare Providers

Significantly reducing the potential for Specimen Provenance Complications helps prevent the risk of costly litigation and negative publicity associated with the administration of incorrect treatment that could seriously threaten a practice or hospital system's viability. This is particularly important for those centers with physician-owned laboratories that bear full liability for the occurrence of errors. The minimal operational changes required for implementation of a system like the one described above can prevent a physician from administering inappropriate treatment, resulting in a significant cost-benefit to the practice and the healthcare system as a whole.

Cost-Benefit of Prospective DSPA Testing in Routine Clinical Practice

While it is well documented that adding DNA testing to the diagnostic testing cycle is useful in identifying misidentified samples and preventing adverse patient outcomes, researchers are just now beginning to investigate the cost-effectiveness of this approach.

Recently, a team from Washington University in St. Louis, Missouri, developed the first economic model for evaluating if this common type of DNA testing should be utilized in the diagnosis of cancer. Their paper entitled "Development of a decision-analytic model for the application of STR-based provenance testing of transrectal prostate biopsy specimens"[13] offers the following conclusion:

"Given the rapidly declining pricing of STR-based identity testing, it is likely that testing to confirm the identity of positive prostate biopsy samples will be a cost-effective method for preventing treatment errors stemming from misidentification."

Conclusion

Whether used prospectively as part of routine clinical practice to diagnose occult specimen provenance complications or used as a quality assurance tool to sort out suspected provenance complication, DNA Specimen Provenance Assignment testing has been shown to be a cost-effective way of ensuring that surgical biopsy samples being evaluated belong exclusively to the patient being diagnosed.

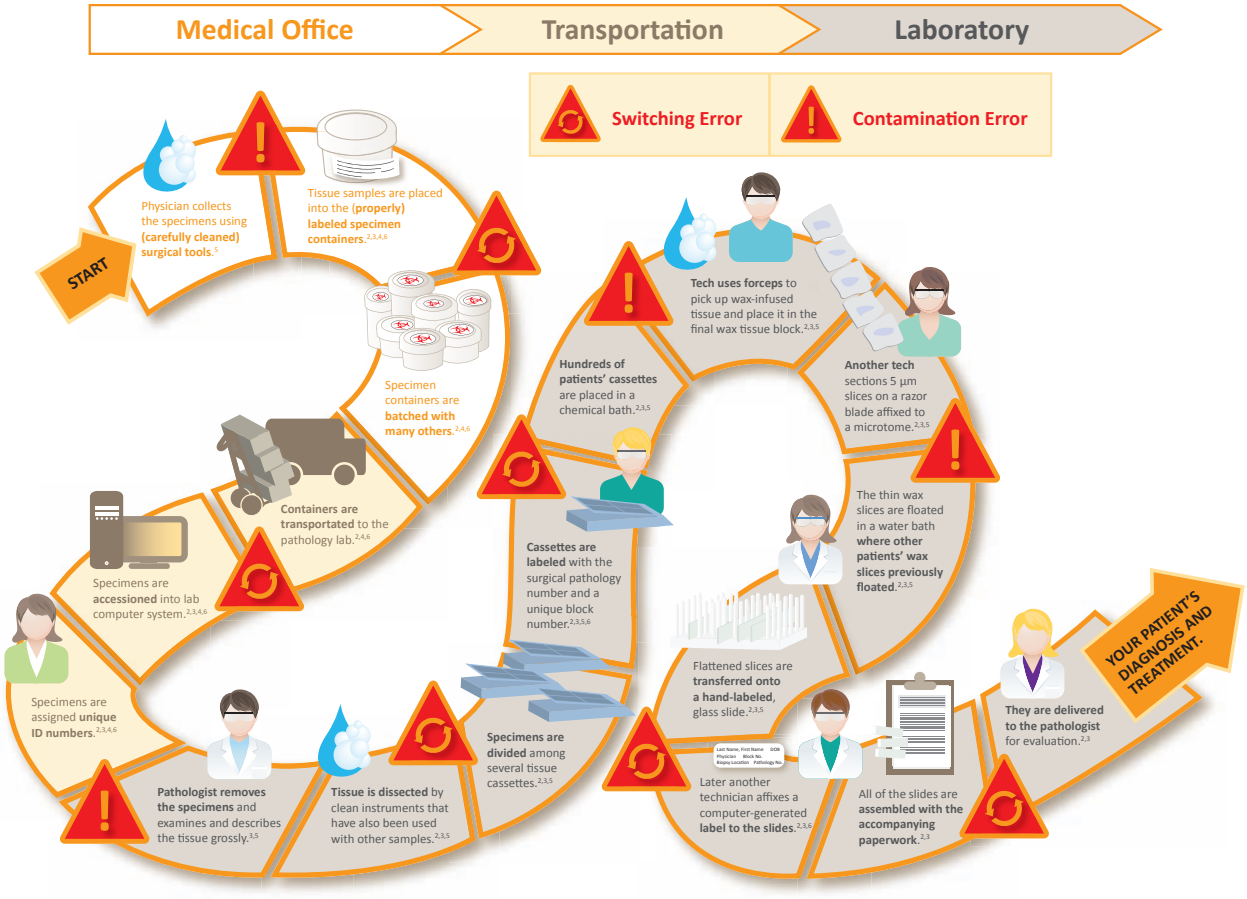
It is important to note that the authors of this paper intentionally excluded any medical-legal costs associated with any adverse patient outcomes stemming from misdiagnosis. However, the authors did experiment with this in their model and concluded that their experimentation "indicates that legal costs would have a significant effect on the model if more concrete data could be obtained." Additionally, the paper did not consider the number of patients that might have been impacted from receiving the "complementary" false-negative, nor the costs associated with eventually treating those patients with their disease in a more advanced stage (i.e. not providing treatment at the earliest opportunity).

SOURCES

1. American Cancer Society. Cancer Facts and Figures 2012. Atlanta: American Cancer Society; 2012.
2. Bronner M. DNA fingerprint analysis for specimen identification. *Cleveland Clinic Clinical and Translational Pathology Research.* 2006; Fall: 5-7.
3. Nakhleh RE, Zarbo RJ. Surgical pathology specimen identification and accessioning: College of American Pathology Q-Probes study of 1,004,115 cases from 417 institutions. *Arch Pathol Lab Med.* 1996;120:227-233.
4. Data provided by Thomson Reuters In-Patient and Out-Patient Views.
5. Suba EJ, Pfeifer JD, Raab SS. Patient identification error among prostate needle core biopsy specimens–are we ready for a DNA time-out? *J Urol.* 2007;178:1245-1248.
6. Harada S, Gocke C. Specimen identity testing using deoxyribonucleic acid analysis in clinical and surgical pathology setting. *Pathol Case Rev.* 2010; 15(4):116-120.
7. Pfeifer JD, Zehnbauer B, Payton J. The changing spectrum of DNA-based specimen provenance testing in surgical pathology. *Am J Clin Pathol.* 2011;135:132-138.
8. Marberger M, McConnell JD. Fowler I, et al. Biopsy misidentification identified by DNA profiling in multicenter trial. *J Clin Oncol.* 2011; 29(13): 1744-1749.
9. Virginia Medical Law Report. Med-mal claim brought for complications after surgery. ©Virginia Lawyers Media, January 2012.
10. Boniello K. 'False cancer' lawsuit. New York Post. http://www.nypost.com/p/news/local/manhattan/false_cancer_lawsuit_15twqx6rOl0W1c8sY2YU0J. Published Dec. 21, 2011. Accessed May 13, 2012.
11. CBC News. Windsor hospital CEO takes responsibility for surgery mistake. http://www.cbc.ca/news/canada/windsor/story/2012/05/04/wdr-mistaken-windsor-surgery.html. Published May 4, 2012. Accessed May 13, 2012.
12. Pfeifer JD, Liu J. Rate of Occult Specimen Provenance Complications in Routine Clinical Practice. Am J Clin Pathol 2013;139:93-100.
13. Pfeifer JD, Singleton M, Lambert DL, Kymes SM. Development of a decision-analytic model for the application of STR-based provenance testing of transrectal prostate biopsy specimens.

APPENDIX A

Complications within the Diagnostic Testing Cycle[1]



1. Bronner MP. DNA fingerprint analysis for specimen identification. Clinical and Translational Pathology Research. Division of Pathology and Laboratory Medicine, Cleveland Clinic. 2006;Fall:5-7.
2. Pfeifer JD, Zehnbauer B, Payton J. The changing spectrum of DNA-based specimen provenance testing in surgical pathology. *Am J Clin Pathol*. 2011;135:132-138.
3. Suba EJ, Pfeifer JD, Raab SS. Patient identification error among prostate needle core biopsy specimens–are we ready for a DNA time-out? *J Urol*. 2007;178:1245-1248.
4. Marberger M, McConnell JD. Fowler I, et al. Biopsy Misidentification Identified by DNA Profiling in Multicenter Trial. *J Clin Oncol*. 2011; 29(13):1744-1749.
5. Harada S, Gocke C. Specimen identity testing using deoxyribonucleic acid analysis in clinical and surgical pathology setting. *Pathol Case Rev*. 2010;15(4):116-120.
6. Ford A. With AP specimen identification, the hero is zero. *CAP Today*. 2007.

