Specimen Provenance Testing Identifies Contamination That Affects Molecular Prognostic Assay Results in Prostate Cancer Biopsy Specimens

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OBJECTIVE To determine if tissue contamination in histologic specimens can significantly affect the results of prognostic molecular markers that are routinely used as confirmatory tests to safely assign appropriate candidates to prostate cancer active surveillance protocols.

MATERIALS AND METHODS This study evaluates 2134 cases from a single, large urology practice that were successfully tested for DNA specimen provenance verification using short tandem repeat analysis for the presence of a significant level of contaminating DNA. After removal of the contamination, 5 of the samples were retested, and the results of the molecular diagnostic test were compared.

RESULTS Forty-nine of the 2134 cases (2.3%) sent for DNA provenance analysis were found to possess significant levels of contamination. Of these 49 cases, 7 were resent for a repeat molecular diagnostic test after being decontaminated. Five of these prostate cancer specimens had sufficient tissue and RNA to give a more accurate cell cycle progression (CCP) score. The average absolute change in these patients' CCP scores was 0.48, with a minimum of 0.1-unit and a maximum of 1.0-unit difference. These changes in CCP scores are significant enough to cause meaningful alterations in a patient's calculated 10-year mortality rate, as defined by their combined risk score.

CONCLUSION DNA contamination in unstained tissue sections sent for prognostic prostate cancer molecular diagnostic testing occurs in 2.3% of the cases, and can be of a magnitude that affects the results and subsequent clinical decision of appropriateness for active surveillance. UROLOGY 115: 87–91, 2018.

Specimen provenance testing (SPT), which utilizes the analysis of short tandem repeat (STR) in 16 unique loci, has become a common practice in urologic pathology laboratories that observe many specimens of the same type (ie, prostate biopsies). In recent years, STR analysis has continued to emerge as a way to ensure specimen identity through the comparison of the selected Combined DNA Index System loci, which is also used by the U.S. Federal Bureau of Investigation.[1,2] Specimen provenance complications (SPCs) can be classified as either type I or type II. Type I complications result from the transposition of specimens between patients. Type II complications, which were the focus of this study, result from DNA contamination by unrelated tissue due to various outside sources.[2] The surgical pathology workflow typically consists of 20 different steps that each has the potential to produce SPCs due to DNA contamination or false identification through human error or inattentiveness.[3] It has been reported that these complications will still persist even after meticulous review and revision of laboratory procedures, as shown in the large multicenter REDUCE trial. It is most alarming that although trials like this have showed that 13.3% of 11,235 biopsy cases submitted for DNA profiling were found to be contaminated at any level, SPT has not become a required step in the laboratory process that may significantly alter the results of routine molecular diagnostic testing performed on tissue specimens.[4] The histology laboratory was never designed to produce genetically pure samples for sophisticated and extremely sensitive molecular assays.

Type II complications, which contamination levels require tissue sample recuts to be sent to correctly match a patient's tissue with their extracted buccal swab DNA sample, are known to be present in anywhere from around 1% to 3% of all biopsy cases.[2,5] This is a significant number of cases that possess an adequate amount of contamination that

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could alter the result of a molecular diagnostic test on a prostate biopsy specimen. Molecular prognostic markers have had recent widespread acceptance in the confirmatory process of determining which men are eligible for active surveillance; therefore, impurities in molecular diagnostic testing could have a direct effect on patient treatment plan.[6,7] The biggest problem resulting from these SPCs are delayed or unnecessary treatments, which can be detrimental to patient health and outcome.[8]

The purpose of this study was primarily to determine the amount of error caused in molecular diagnostic testing that can be corrected by the use of SPT to increase accuracy of diagnosis and the resulting treatment. Current treatment options for low-risk prostate cancer include active surveillance protocols, which use molecular testing such as the cell cycle progression (CCP) score for risk stratification.[9] Moreover, this study has also highlighted the magnitude of the potential problem of SPCs in pathology laboratories that may go undetected by producing eye-opening statistics that continue to reveal the prevalence of significant type II complications as previously reported to be seen in 1.69% of all prostate biopsies in urology practices using DNA analysis within a surgical pathology laboratory setting.[2]

MATERIALS AND METHODS

Extraction

(Note: Specific details are provided because our SOP does not completely follow the kit instructions.)

Genomic DNA was extracted manually from formalin-fixed, paraffin-embedded tissues using the TrimGen Wax Free DNA Extraction kit (TrimGen, Sparks, MD). Microcentrifuge tubes containing the samples were centrifuged for 2 minutes, followed by a brief (approximately 15 seconds) incubation at 95°C. A mastermix containing 70 μL of resin and 7 μL of enzyme was added to each sample, and the sample was incubated at 56°C for 1 hour. The sample was then incubated at 95°C for 10 minutes, followed by centrifugation at 10,000 × *g* for 5 minutes, and the supernatant (approximately 30 μL) was transferred to a stock plate. DNA was quantified using PicoGreen (Invitrogen) and normalized to a concentration of 0.3 ng/μL.

Amplification and STR Analysis

STR analysis was performed on extracted genomic DNA (target of 0.75 ng) using the Identifiler Plus multiplex PCR kit (Thermo Fisher Scientific, Waltham, MA), following the manufacturer's recommended conditions reduced to one quarter reaction volume (6.25 μL). Fragment analysis was performed by capillary electrophoresis on an Applied Biosystems 3730 (Thermo Fisher Scientific), and the data were analyzed using GeneMapper ID v 3.2.1 (Thermo Fisher Scientific).

Data Set

Three years of SPT data was reviewed from a large urology practice. There were 2208 prostate biopsy specimens analyzed over this time period, of which 2134 specimens had SPT. Of the total amount of specimens that received DNA analysis, 49 cases were determined to possess a type II SPC due to an extraneous source DNA. Seven of the 49 cases were then resent for a second diagnostic test after the tissue had been recut and sequentially verified to solely contain the correct patient's DNA. The variation in the CCP scores was then analyzed for the individual cases to see any potential effects on the combined risk score (CRS) and the resulting potential to alter the considered treatment plan, with specific attention on the active surveillance.[9]

RESULTS

A total of 2134 prostate biopsy specimens from a single, large urology practice were reviewed using STR analysis to detect type II SPCs. Forty-nine of the 2134 cases were found to show contaminations. This is 2.3% of the total cases that were reviewed during the 3-year time period between January 2014 and December 2016. Seven of the 49 cases that were thought to possibly be clinically significant were resent for additional CCP testing after being decontaminated and recut for a new DNA analysis verification. This allows for the quantification of the effects that type II SPCs have on a patient's molecular test results and treatment plan. In the 7 cases retested, 2 cases were sent back showing insufficient quantities of DNA or tissue, but the remaining 5 cases showed an average change in CCP score of 0.48 or 4.8% due to the original contaminations (Table 1). This is greater than the published interassay variability of the CCP score for experimental replicates which shows a standard deviation of 0.1 unit.[10] No published data are available for retesting different areas of the tumor from a single patient to account for tumor heterogeneity. There was an average change of 1.14% in the CRSs of the 5 cases retested, which is a linear combination of the CCP score and the patient's current Cancer of the Prostate Risk Assessment (CAPRA) score.

Table 1. Seven of the 49 type II SPC cases that were resent for a new CCP score after contamination was removed

Patient Number	Initial CCP Score	Initial 10-Year Mortality Risk	Retested CCP Score	Retested 10-Year Mortality Risk	Change in CCP Score	Change in CRS
1	−0.8	8%	QNS	QNS	NA	NA
2	−0.4	4%	0.6	6%	1.0/10%	2%
3	0.1	8%	QNS	QNS	NA	NA
4	−0.2	7%	−0.1	8%	0.1/1%	1%
5	−0.6	6%	−0.9	5%	0.3/3%	1%
6	4.4	3.8%	4.0	3.1%	0.4/4%	0.7%
7	3.0	3%	2.4	2%	0.6/6%	1%
Average					0.48/4.8%	1.14%

CCP, cell cycle progression; CRS, combined risk score; NA, not applicable; QNS, quality not sufficient; SPC, specimen provenance complication.