DNA analysis for prostate specimen verification: How I Do It

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Prostate cancer is the most common malignancy affecting men. Prostate biopsy remains the key clinical tool for selecting appropriate treatment options. The process of specimen collection and diagnosis is multistep and vulnerable to human error along every stage. Specimen provenance testing (SPT) aims to provide certainty that biopsy results can be trusted when recommending life changing treatments and has emerged as a necessary tool in medicine to counteract human error and specimen contamination. In this study we report our practice's experience using the Know Error test to verify prostate biopsy specimens. In this study, we retrospectively reviewed the results of a specific SPT known as Know Error which is used in our institution for specimen

verification during prostate biopsy. Over a period of 16 months, we identified 445 patients with a total of 921 specimens. The percentage of patients who had 1, 2 or 3 specimens analyzed was 29%, 38%, and 30%, respectively. Our cohort's rate of specimen verification was 92.8% with a 2.8% contamination rate. The pathology reports for 445 patients were then examined to determine Gleason Grade Group (GG) showing 180 GG1 and 148 GG2 patients. Cross reference of pathology reports and Know Error reports showed 8 GG1 and 9 GG2 patients had contaminated biopsy specimens. Specimen provenance complications such as contamination can negatively impact patient counselling and treatment modalities leading to unnecessary intervention and detrimental patient outcomes.

Key Words: DNA analysis, specimen contamination, prostate biopsies, specimen provenance complications.

Introduction

Prostate cancer is the most common malignancy and second most common cause of cancer death affecting

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men in the United States. Urologists rely on the validity of prostate biopsy and genomic testing of biopsy tissue to counsel men on their best treatment options for this disease. As such, results of prostate biopsy can significantly impact the lives of patients and alter their course of treatment. Unfortunately, the results are subject to human errors and contamination from the moment the specimen is collected. Beyond the collection process by the practitioner performing the biopsy, there are 17 distinct steps each with the possibility of switching or contamination errors.[1] Such

errors have been publicized in the news with examples ranging from patient misdiagnosis to unnecessary surgeries and interventions.[2,3]

Errors of assigning the correct specimen to the correct patient have been termed specimen provenance complications (SPC) and are classified into three categories. Type 1 is the transposition of samples, or the switching of samples and patients. Type 2 is contamination of the sample with external DNA and type 3 is insufficient specimen quantity for diagnosis.[4] Type 1 and 2 tend to be the most consequential.

To counteract the detrimental consequences of misdiagnosis and specimen contamination, DNA analysis techniques have been implemented. Short tandem repeat (STR) analysis is one such technique which was first used in the field of forensics and is now utilized by pathology labs to verify specimen identity. STR analysis relies on DNA variations which are amplified using polymerase chain reaction (PCR) to match the patient to the sample.[5]

In this article, we discuss our experience with the commercially available STR analysis tool "Know Error" (P4 Diagnostix, Beltsville, MD, USA) to verify prostate biopsy samples. We outline our protocol for specimen handling and verification as well as discuss the rate of specimen provenance complications identified.

Materials and methods

Prior to transrectal ultrasound guided prostate biopsy, informed consent is obtained from the patient. Using the commercially available Know Error test kit, a buccal swab is collected and placed in a sterile container that is pre-labeled with a unique barcode. This sample is sent separately to the P4 Diagnostix laboratory. Next,
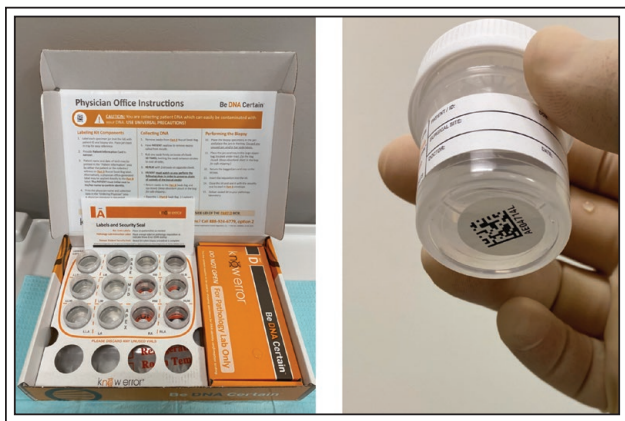

Figure 1. Know Error clinical setup for prostate biopsy specimen collection. The kit contains components needed for biopsy collection and DNA testing buccal swab.

TABLE 1. **Total number of patients analyzed per number of specimens**

Number of specimens	Number of patients
1	131
2	167
3	135
4	10
5	1
6	1
Total	445

a standard 12-core prostate biopsy is performed and placed in separate formalin containers each with the same unique barcode and sent to our institution's pathology lab for evaluation, Figure 1.

If a specimen is positive for a malignancy, a piece of the tissue is sent to P4 Diagnostix for DNA cross verification to the buccal swab and to check the specimen for genetic contamination. In the event of a discrepancy, the clinician is notified of the error. A sample of the report generated is presented in Figure 2.

If the prostate specimen is positive for malignancy, P4 Diagnostix offers DNA sequencing known as Uroseq that tests for common germline mutations in prostate cancer tissue such as BRCA1, BRCA2, ATM, CHEK2, HOXB13, PALB2, RAD51D, MLH1, MSH2, MSH6, PMS2, and EPCAM. The final pathology reports of the prostate tissue are shared with P4 Diagnostix, so recommendations can be made to which patients would benefit from DNA sequencing in accordance with 2019 NCCN guidelines based on risk criteria and family history.[6]

All results from patients undergoing prostate biopsy at our institution who consented to specimen provenance with Know Error from a 16 month period were analyzed for specimen provenance complications. We share our incidence of SPCs and contamination of specimens as well as evaluate incidence where genomics testing would be impacted from DNA contamination.

Results

Our cohort included 447 patients with a total of 922 specimens. Even though the recommended number of specimens for analysis is two, patients had a variable number of specimens sent by the pathologist. Table 1 shows that the proportion of patients who had 1, 2 or 3 specimens sent was 29%, 38%, and 30%, respectively.

Figure 2. DNA specimen provenance assay report.

TABLE 2. **Number of specimens in relationship to specimen results**

Specimen results	Number of specimens
Verified	855
Contaminated	26
Insufficient	40
Total	921

TABLE 3. **Analysis of Gleason grade per patient**

Gleason grade	Number of patients
1	180
2	148
3	29
4	39
5	43

Table 2 shows the frequency of each result. In our cohort, we had a 92.8% rate of specimen verification, 2.8% rate of contamination, while 4.4% of specimens were insufficient for DNA verification analysis. Figure 3 shows the rate of verified and contaminated specimens found relative to the number of specimens analyzed. For patients with 1-3 specimens the rate of specimen verification was 97.7%, 93.1%, and 92.3% respectively with a significant drop in verification rate for those with 4 or more specimens (87.5%, 80%, 50%). The rate of contamination showed an inverse relation with lower contamination rates in 1-3 specimens (0.76%, 2.4%, 2.7%) versus 4 or more specimens (7.5%, 20%, 33.33%).

Patient's charts were also examined for pathology results to determine the Gleason Grade Group (GG). Out of the 445 patients, 2 patients had negative biopsies, 4 patients were biopsies after radiation without recurrent disease, leaving 439 patients for analysis. In our cohort, 180 patients were GG1, 148 GG2, 29 GG3, 39 GG4 and 43 GG5, Table 3. Some of these patients with GG1 and GG2 cancers would have been candidates for genomic testing to determine their eligibility for active surveillance and we found 8 GG1 patients and 9 GG2 patients who had contaminated biopsy specimens which could have altered the accuracy of their results.
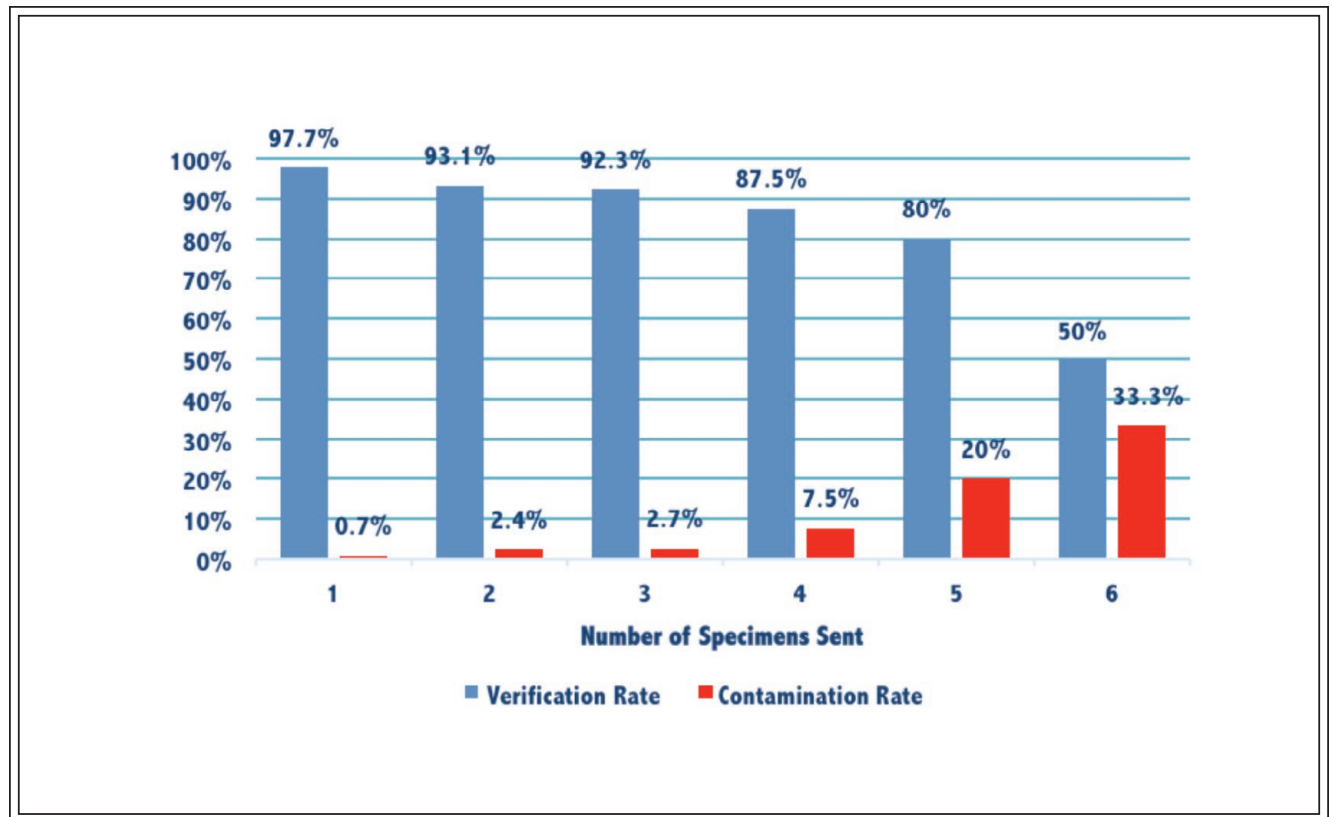

Figure 3. Rate of specimen verification and contamination per number sent.

Discussion

Prostate cancer is the most common solid tumor malignancy in men in the United States with diagnosis dependent on pathological analysis of biopsy specimens.[7] The accuracy of the collection and the handling of the specimens ensures the correct diagnosis and treatment. However, the process of tissue collection and analysis is vulnerable to human error during all the steps of specimen handling.[8] Suba et al performed a root cause analysis of 3 cases of prostate cancer where type I errors occurred.[8] The authors introduce the idea of "DNA timeout '' similar to a surgical timeout prior to procedures; a DNA timeout allows the verification of the pathology specimen using SPT to ensure the correct diagnosis is made for the correct patient.

SPT can also play a role in the post-diagnosis stage. Pfeifer et al examined 24 cases where SPT was done to verify pathology specimens. The study describes the following scenarios where DNA testing is needed to confirm the initial diagnosis: retrospective evaluation of pathology after definitive treatment when the clinical course is atypical, unexpected diagnosis, and "peace of mind" for patients prior to aggressive treatment.[9] Harada et al, discussed a case of an unexpected diagnosis of laryngeal cancer, where the biopsy included both benign and malignant tissue. DNA testing of the tissue discovered that the malignant tissue was a contaminant secondary to a labeling error.[10]

The rate of verification in our cohort was 92.8% with a type II error rate of 2.8% which is similar to the rate published by large cohort studies.[5,11] The rate of verification was similar for specimens 1 through 3 with a decline in the rate after 4 or more specimens are evaluated along with an increase in the contamination rate. Our results show that the recommended 2 specimens are sufficient to have accurate results and the increasing probability of specimen contamination with increasing exposure to personnel.

While the type II error rate is relatively low, it may have detrimental effects on patient care which can vary from misdiagnosis to mistreatment with unnecessary surgery or radiation. SPCs not only adversely affect patients, but also causes significant financial burden on the healthcare system. Wojno et al performed a systematic review of studies evaluating SPC to calculate the economical burden of incorrect diagnosis. The study evaluated medical cost and legal cost of a total of 20,322 SPCs which totaled to $145.8 million for medical costs and $694.8 million for legal costs.[1] DNA provenance tests do add to the cost of biopsy, however its significantly lower than the cost of SPCs. Pfeifer et al analyzed the cost effectiveness of DNA testing and found that DNA testing is cost effective at the price of $618 per person and becomes cost saving at $290 per person.[12] The cost of the test is significantly lower than the cost of complications as calculated by Wojno et al to be $3,776 per patient.[1]

Contamination may also interfere with genomic tissue testing which is becoming a common tool for predicting the aggressiveness of prostate cancer and extending active surveillance eligibility.[13] Wojno et al examined the effect of SPC on cell cycle progression score. The study found that specimen contamination can significantly alter risk score and patients' calculated 10 year mortality.[5] Specimen contamination can also affect other RNA seq based tests such as Prolaris, ProMark, and Decipher which are used to predict the aggressiveness of prostate cancer and guide future treatments in patients.[14] In our patient population a total of 17 patients (8 GG1 patients and 9 GG2) might have been counselled about active surveillance using contaminated results.

Our results also show an increasing incidence of Type II contamination errors with increasing number of specimens prepared and sent for Know Error testing. We interpret this correlation to illustrate the increasing probability of contamination with increased human handling of the specimen. Consequently when genomic testing is performed, the specimen should likely be sent for this and then directed to Know Error to be verified free from contamination before results are released. To SPT the biopsied tissue either before or in tandem from a different cut of the original paraffin embedded tissue will either increase human handling and/or eliminates assurance that the tested tissue has not been contaminated.

Conclusion

Our experience using the Know Error platform to ensure specimen provenance has demonstrated the incidence of DNA contamination within our own population and has helped provide peace of mind for many patients that their treatment recommendations are accurate and relate to their own biopsy material with a low rate of contamination. The Know Error kit is fully compatible with the standard 12 core prostate biopsy template and does not significantly impact patient flow through the procedure clinic. □

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