Kaninjing E, Adeniji KA, Gachii AK, et al. Utility of formalin-fixed, paraffin-embedded prostate biospecimens from low-resource settings for use in next-generation sequencing studies in African-descent populations. *Journal of Global Health Reports*. 2023;7:e2023055. doi:10.29392/001c.84541

Research Article

Utility of formalin-fixed, paraffin-embedded prostate biospecimens from low-resource settings for use in next-generation sequencing studies in African-descent populations

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Keywords: biospecimens, low-resource, international, next generation sequencing, Prostate cancer

https://doi.org/10.29392/001c.84541

Journal of Global Health Reports

Vol. 7, 2023

Background

Men of African ancestry experience higher burden from prostate cancer compared to men of other ancestral backgrounds. Limitations in the availability of high-quality biospecimens hinder the inclusion of this population in genetic studies of prostate cancer. The use of formalin-fixed paraffin-embedded (FFPE) tissues represent a potential rich source of genetic material particularly in some international settings, where fresh frozen tissue is difficult to obtain. In this study, we investigate the feasibility of using FFPE biospecimens acquired from various international sites for utility in next-generation sequencing.

Methods

A total of 976 FFPE blocks were collected between 2002 and 2017 from six international sites in Africa and the Caribbean representing three consortia: Prostate Cancer Transatlantic Consortium; African-Caribbean Cancer Consortium; and Men of African Descent and Carcinoma of the Prostate. Genomic DNA was checked for quality and quantity. Differences in mean quality control (QC) for pre-and-post pathology training were assessed using t-test. Pearson chi-square with trend analysis examined association between time-category and QC success status. Association of continuous DNA quality (Q129/Q41 ratio) and time of specimen collection was estimated with linear regression. Samples with a DNA quantity >0.2µg and a Q129/Q41 ratio >0.00225 were submitted for whole exome sequencing (WES).

Results

There was a positive relative percentage change in DNA quantity from 2002 to 2017 for Jamaica, Kenya and Senegal. There was a decline in DNA quantity over the same time period for Nigeria. There was a statistically significant improvement in quality of samples

from Kenya (*P*=0.032), Nigeria (*P*<.001) and Senegal (*P*=0.043). There was a significant improvement in the collected DNA sample quality over time with an R^2 of 0.12.

Conclusions

FFPE samples from low-resource settings could potentially provide sufficient DNA for WES. Improvements in biospecimen collection processing and storage for research are needed in some of these settings.

Men of African ancestry (MAA) suffer disproportionately from prostate cancer (PC) compared to men of other races or ethnicities, globally.[1,2] In the United States (US), African American (AA) men have the highest risk of developing prostate cancer; tend to develop a more aggressive disease and are more likely to die from the disease when compared to other US-based race/ethnic groups.[3,4] Similar observations were made among MAA populations in the Caribbean, South America and the United Kingdom.[5,6] Prostate cancer remains the most commonly diagnosed cancer in men from sub-Saharan Africa (SSA), who experience the highest rates of PC mortality in the world.[7] Over the next two decades, the International Agency for Research on Cancer has projected an approximate 122% and 113% increase in SSA's prostate cancer incidence and mortality rates, respectively.[7] However, these numbers could be higher due to inadequate cancer surveillance.[2,6] The long-standing racial/ethnic differences in PC risk and mortality have yet to be explained, but presumably reflect both the differing prevalence of surveillance, access to healthcare, environmental and social factors as well as the frequency of underlying susceptibility alleles.[5]

PC has the highest effect of heritability of any of the major cancers as demonstrated by familial and twin studies.[8-13] Furthermore, having one or more first-degree relatives with PC is associated with a 2- to 3-fold increase in risk in family members and it is estimated that about 20% of the population possesses such risk.[10] To date, over 180 independent susceptibility loci have been found to be associated with the risk of PC.[14-34] However, the majority of these studies have been conducted in populations of European or Asian descent despite the urgent need for these studies in African descent populations. A few studies that included MAA populations have produced multiple lines of evidence to support genetic differences in the allelic architecture of PC within this population.[15,21-25,29,31] An admixture mapping study of PC in AA men by Freedman and colleagues was the first to implicate the 8q24 region of the genome as harboring risk alleles that may contribute to a greater risk of PC in men of African descent.[29] Further investigations from this research group identified two risk variants of low frequency in the region (<5%) which is only found among MAA.[24] In parallel, other studies have identified novel rare PC risk variants that are solely present in MAA populations.[25,27] These findings point to the importance of including this population in genomic studies of PC.

Exclusion of MAA populations in PC genomic studies creates deficit in scientific knowledge, which could limit conclusions and the translational impact of findings on this population. One reason for limited genomic studies for PC in African descent populations is the challenge of available high-quality PC biospecimens for genomic research. The use of formalin-fixed paraffin-embedded (FFPE) tissues in genomic studies represents a potentially rich source of genetic material, as FFPE tissues are one of the most widely available clinical specimens, particularly in international settings, where fresh frozen tissue is extremely difficult to obtain.[35,36] The potential utility of FFPE tissue as a source for genomic sequencing would greatly enhance population-based cancer studies, especially in research conducted in resource-limited regions, where cancer-related mortality rates are much higher and survival rates are poorer compared to the developed regions of the world.[36] However, use of FFPE samples present additional challenges for example, pre-analytic conditions can also contribute to variations in the quality and quantity of nucleic acid.[37,38] Therefore, we aimed to investigate the feasibility of using FFPE biospecimens acquired from six international sites for utility in next generation sequencing (NGS). Specifically, we used archival FFPE prostate samples from three international cancer epidemiology consortia supported by the Epidemiology and Genomic Research Program at the US National Cancer Institute https://epi.grants.cancer.gov/Consortia/ to investigate whether DNA obtained from archived prostate FFPE tissues is of sufficient quantity and quality for whole exome sequencing (WES) and to examine the effect of storage time.

METHODS

CANCER EPIDEMIOLOGY CONSORTIA

The African-Caribbean Cancer Consortium (AC3) was formed in May 2006 to further the study of viral, genetic, environmental and lifestyle risk factors for cancer in populations of African descent. With dedicated researchers based in the United States, Africa and the Caribbean, this consortium seeks to build knowledge, capacity and infrastructure to advance the science of cancer prevention and control in populations of African ancestry.

The Prostate Cancer Transatlantic Consortium (CaPTC) was formed in 2005 to address the disproportionate burden of prostate cancer among Black men globally. Its membership consists of prostate cancer scientists, clinicians, survivors, and advocates from North America, Europe, the Caribbean Islands, and West Africa. The consortium seeks to develop ethnically sensitive, and targeted approaches that will eliminate globally, the prostate cancer disparities of Black men.

Formed in 2007, the Men of African Descent and Carcinoma of the Prostate (MADCaP) Consortium focuses on epidemiologic studies to address the high burden of prostate cancer among men of African descent. MADCaP

Table 1. Passed DNA quality control and quantity control by consortia

Consortia	Total	Assay Passed	Assay Failed	% Passed	95% Confidence Interval
CaPTC					
DNA Quantity Control	755	464	291	61.5	57.9 to 64.9
DNA Quality Control	755	220	533	29.1	26.0 to 32.5
AC3					
DNA Quantity Control	189	56	133	29.6	23.5 to 36.6
DNA Quality Control	189	23	166	12.2	8.2 to 17.7
MADCaP					
DNA Quantity Control	32	26	6	81.3	63.7 to 91.4
DNA Quality Control	32	25	7	78.1	60.4 to 89

Prior to whole exome sequencing, FFPE samples were assessed for DNA quantity and quality. Samples with a DNA quantity >0.2ug and a Q129/Q41 ratio >0.00225 were classified as passing and submitted for sequencing.

includes investigators from the United States, United Kingdom, Africa and the Caribbean, who have cohort or case-control studies on prostate cancer among men of African ancestry. The MADCAP collaborative research is intended to improve prostate cancer prevention, detection, and treatment in men of the African diaspora.

SAMPLE PREPARATION AND GENOMIC DNA EXTRACTION

A total of 976 FFPE blocks were collected from six international sites representing the three consortia (189 from AC3: Bahamas, Cayman Islands, Jamaica & Kenya); (755 from CaPTC: Nigeria); and (32 from MADCaP: Senegal) with samples of PC, benign prostatic hyperplasia and normal tissue. Specimen collection year ranged from 2002 to 2017. All FFPE tissues in this study were reviewed and approved by individual Institutional Review Boards from their respective clinical institutions and by the Institutional Review Board at Tuskegee University. We employed similar sample preparation and genomic DNA extraction methods used in a previous study.[39] Subsequent to pathology review, five 10 µm-thick curls or cores were obtained from each block with >50% Tumor and ⩽50% Necrosis and shipped to Q^2 Solutions (Morrisville, NC) for DNA extraction, quantity and quality analyses. Following the manufacturer's protocol, genomic DNA and total RNA was purified using the Allprep DNA/RNA FFPE kit (Qiagen, Hilden, Germany). DNA quality and quantity were checked with Qubit 2.0 fluorometry (Life Technologies, Carlsbad, CA) and with KAPA hgDNA quantification and QC kit (Kappa Biosystems Roche, Basel, Switzerland). Samples (n=220) with a DNA quantity >0.2µg and a Q129/Q41 ratio >0.00225 were submitted for sequencing.

This study examined the feasibility of FFPE tissue for use in genomic research studies particularly in low-resourced settings. Additionally, we aimed to investigate whether DNA obtained from archived prostate FFPE tissue from international sites had sufficient quality and quantity for WES and the effect of storage time. Since Nigeria is the only country with samples from 2002 to 2017, we focused our analysis on the association between specimen collection time and quality of DNA solely on samples from this country.

STATISTICAL ANALYSIS

Test for trend in quality control (QC) success rate were performed using the Pearson's test for trend. Association of continuous DNA quality (Q129/Q41 ratio) and time of specimen collection was estimated with linear regression. The regression model uses the standard variance estimator for ordinary least-squares regression. Standard error was based on variance estimates given by the inverse of the negative Hessian (second derivative) matrix. Analyses were performed using Stata software 14.2. Statistical significance level was set at $P<0.05$.

RESULTS

DNA QUALITY AND QUANTITY ASSESSMENT

Of the 755 samples from CaPTC, 29.1% successfully passed quality control and 61.5% were successful at quantity control. Out of the 189 samples from AC3, 29.6% passed quantity control and 12.2% passed quality control. MADCaP had 81.3% of its specimens pass quantity control and 78.1% were successful at quality control. Table 1.

Trend analysis of DNA quality and quantity from six countries by sample collection year is depicted in Table 2. Of note is the positive relative percentage change in DNA quantity from 2002 to 2017 for Jamaica, Kenya and Senegal. There was a decline in DNA quantity over the same time period for Nigeria while the trend for Bahamas and Cayman Islands was inestimable due to paucity of data. The trend in DNA quantity was only statistically significant for Senegal ($p<.05$), with 36% improvement in 2014 relative to 2006. In terms of trend in the quality of samples collected from 2002 to 2017, there was a statistically significant improvement in samples from Kenya ($P<.05$), Nigeria ($P<.001$) and Senegal ($P<.05$).

As shown on Table 2, CaPTC was the only consortium with specimens collected in the entire range (2002 to 2017) from sites in Nigeria. Therefore, to further test for association between specimen collection time, DNA quality and

Table 2. Trends in DNA quantity and quality by country

		Proportion n (%) of success over time					P-value, trend+
Country	% Relative Δ 2002 -2017	2005 or earlier	2006-2008	2009-2011	2012-2014	2015-2017	
Bahamas							
Passed Quantity				(0)			Not estimable
Passed Quality				(0)			Not estimable
Cayman Islands							
Passed Quantity						8 (53.3)	Not estimable
Passed Quality						(0)	Not estimable
Jamaica							
Passed Quantity	100	(0)	3 (7.5)	(0)	(0)	1 (100)	Not estimable
Passed Quality	100	(0)	(0)	(0)	(0)	1 (100)	Not estimable
Kenya							
Passed Quantity	31.6				6 (37.5)	38 (69.1)	0.280
Passed Quality	15.9				3 (18.7)	19 (34.6)	0.032
Nigeria							
Passed Quantity	-14.5	10 (76.9)	48 (59.3)	41 (61.2)	89 (61)	275 (62.4)	0.718
Passed Quality	29.3	1 (7.7)	10 (12.4)	18 (26.9)	28 (19.2)	163 (37)	<0.001
Senegal							
Passed Quantity	36.4		7 (63.6)	10 (83.3)	9 (100)		0.020
Passed Quality	36.4		7 (63.6)	9 (75)	9 (100)		0.043

+P values based on non-parametric trend analysis using data from all years.

quantity, we focused the rest of the statistical analysis on CaPTC specimens from Nigeria.

A linear regression analysis was performed to further test the direction in the trend of quality in specimens from CaPTC, collected from 2002 to 2017. There was a significant improvement in the collected DNA sample quality over time ($F(1, 226) = 37.02$, $P < 0.001$), with an R^2 of 0.12. This suggests a positive association between quality of specimens and sample collection time (Figure 1).

DISCUSSION

Formalin-fixed paraffin-enabled (FFPE) blocks play an important role in oncology research as preserved tissue samples can be archived and studied for better insights about different tumor types and disease progression profiles. Preparation and storage of FFPE specimens are crucial to maintaining quality and utility of the specimen in research and therapeutic development. In low-and-middle income countries (LMIC), FFPE is more widely used compared to fresh frozen tissue as they require less resources to procure and store. While formalin treatment preserves tissue, it can also destroy nucleic acids making it difficult to identify true variants in tumor samples using NGS.[40] Our analysis of FFPE samples from six international sites show statistically significant improvement in quality of DNA samples from Kenya, Nigeria and Senegal. All samples from Senegal for example were from a major academic center with a long experience of research in prostate cancer. Positive relative change in DNA quantity over time was also noted in samples from Kenya and Senegal while paucity of data for Jamaica, Cayman Islands and Bahamas did not allow for any meaningful assessment of trend overtime. In Nigeria, there is a decline in quantity of DNA over time. There are significant challenges in tissue handling, sample processing and immunohistochemistry faced by pathologists in Nigeria and other LMICs. These include prolonged warm and cold ischemic times before tissue is immersed in fixatives which may result in hypoxic changes or autolysis. The other limitation is related to rarity of immunohistochemistry. Moreover, inadequate fixative is a major challenge, especially for specimens collected outside of University Teaching Hospitals (UTH) where resources are even more scarce.[41-43] While some are fixed in inadequate volumes of neutral buffered formalin (NBF), others are transported to the hospital in fluids such as normal saline and savlon. Furthermore, in remote clinical sites, it is not uncommon for specimen to be inadvertently kept in fixatives for several days or weeks before processing whereas at UTH specimens are processed on average within 6 to 24 hours of receipt in the laboratory. Unstable electrical supply is yet another challenge that disrupts tissue processing. The use of manual tissue processing and inadequate storage facilities for paraffin blocks are additional challenges in some clinical centers in Nigeria. Similar challenges were also observed in participating sites in the Caribbean. For example, in Jamaica most of the FFPE blocks had mold due to improper storage and were not suitable for inclusion in the study. These challenges need to be addressed to ensure sustained improvements in the quantity and quality of FFPE from low resource countries which will in turn enhance the potential utility of FFPE for NGS studies.

DNA and RNA were extracted and purified from the 755 FPPE samples from Nigeria with 464 possessing sufficient

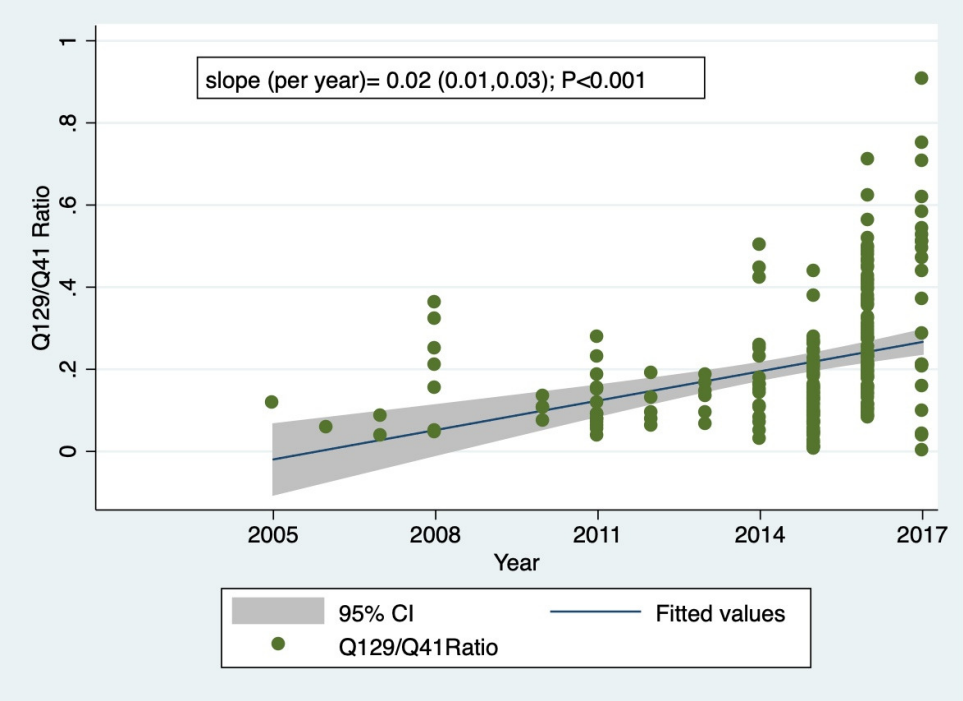

Figure 1. Association between specimen collection time and the Q129/41 Ratio.

The solid line indicates the estimated linear relationship between specimen collection year and Q129/41 ratio. The shaded area denotes pointwise 95% confidence intervals of the conditional mean. The dots denote specimens that successfully passed QC. *Q129/41 ratios for specimens collected in 2002-2003 were undetermined.

DNA quantity (0.2ug total DNA cutoff) while 220 samples met the quality threshold (0.00226 Q-ratio cutoff). We found a positive association between quality and specimen collection time and no statistically significant association between DNA quantity and time. This may be attributed to improvements made by pathologists and other clinical staff over the years in the preparation and storage methods of FFPE specimens.

This suggests that FFPE could potentially provide sufficient DNA quality and quantity for WES sequencing in low resource settings. Many medical centers in LMIC routinely store prostate cancer channel TURP or biopsy samples in formalin, due to the low cost and amenability to long-term storage. The vast majority of clinical samples are stored as FFPE tissue in which DNA and RNA necessary for NGS is often fragmented.[44] Additionally formalin causes protein crosslinking, further limiting the use of these tissues in research. Numerous studies have documented that the quality of FFPE samples varies depending on how surgical specimens have been prepared and preserved.[45]

Europeans make up more than three quarters of the available sequencing data for the world.[46] This underscores the need for more diversity within the sampling of genomic specimens from patients. Despite bearing a disproportionate burden of PC, men of African descent are underrepresented in biomedical research. To advance scientific knowledge and illuminate the etiology of PC, it is crucial that large numbers of tumor specimens from MAA are sequenced as they could inform diagnosis and stratification of patients for treatment[47] and help to reduce disparities in the outcome of PC. Consortia such as CaPTC, AC3 and MADCaP with a history of successful research projects among MAA could be important conduits to ensure diversity in our biospecimen and genetic studies. In the future, more efforts are needed to develop adequate bio banking networks for future studies promoting more racial and genetic diversity.

This study was not without limitations. As noted previously, challenges in collection, handling and processing of samples contributed to low amount of tissue in some of the FFPE blocks and resulted in many samples not passing the DNA quantity control test while others were over fixed. Additionally, sample storage and labeling of individual blocks made identification of some samples difficult. Paucity of data from some of the international sites in this study posed challenges in estimating trend of quality and quantity of samples over time.

CONCLUSIONS

Formalin-fixed paraffin-embedded (FFPE) tissue is a promising source for genomic sequencing that could diversify biomedical specimens and provide new lines of inquiries to tease apart factors that underlie disparities in PC outcomes. While there are significant challenges in the collection, handling, and storage of FFPE tissue in LMIC, this study shows there is some potential in biospecimens acquired from various international sites, under varying storage and handling conditions as helpful in genomic studies.

ACKNOWLEDGEMENTS

The abstract of this paper was presented at the 2020 American Association for Cancer Research Virtual Conference on The Science of Cancer Health Disparities as a poster presentation with interim findings. The poster's abstract was published in "Cancer Epidemiology, Biomarkers & Prevention". Abstract PO-135: Utility of formalin-fixed, paraffin embedded prostate biospecimens from low-resource international settings for use in next generation sequencing studies in African-descent populations | Cancer Epidemiology, Biomarkers & Prevention | American Association for Cancer Research (aacrjournals.org)

POSTER PRESENTATION (VIRTUAL)

2020 The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved (AACR), October 2020.

ETHICS STATEMENT

All FFPE tissues in this study were reviewed and approved by individual Institutional Review Boards from their respective clinical institutions and by the Institutional Review Board at Tuskegee University.

FUNDING

Leidos contract YT16-010 (Project ID# 001.050.0010) and NCI contracts HHSN261201600650P (Fox Chase Cancer Center) and HHSN261201600732P (University of Florida), respectively. U54-MD007585-26 NIH/NIMHD (Clayton Yates) and U54 CA118623 (NIH/NCI) (Clayton Yates), via Department of Defense Grant (PC170315P1, W81XWH-18-1-0589 (Clayton Yates).

AUTHOR'S CONTRIBUTIONS

DNM, FTO, CR, and CY were involved in project conception, study design, execution, and acquisition of data. ETK participated in study design, execution, data analysis and interpretation. KAA, AKG, PJ, JOB, CNO, MF, AAP, OAF, OPO, WA, MDJ, RAR, SKJ, CD, MJ, LN, SG, MN, contributed in data acquisition and analysis. JW, BK, DKF, performed data analysis and interpretation. DYG, KRB acquisition of data, analysis, and interpretation. All authors participated in drafting and reviewing the manuscript.

DISCLOSURE OF INTERESTS

Clayton Yates received consulting from QED Therapeutics and Riptide Biosciences. Clayton Yates owns stock in Riptide Biosciences. The other authors do not have any financial or other conflicts of interests.

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Submitted: March 13, 2023 GMT, Accepted: June 14, 2023 GMT

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