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Full Length Research Paper

Effect of formalin fixation on DNA purity and quantity, nucleic acid, and amplicon size in cervical human papilloma virus detection

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Tissue fixation with 10% formalin and molten paraffin wax embedding (FFPE) is routinely used protocol for tissue preservation in histopathology laboratory. We therefore aimed at comparing the differences in DNA quantity, DNA purity, nucleic acid and its effect on primers (PCR) amplicon (bp) sizes between fresh cervical tissues and formalin fixed paraffin embedded (FFPE). The differences in DNA purity, quantity and nucleic acid were 2.02±0.42 versus 1.34±0.28, 47.73±37.45 vs. 21.84±25.52 (ng/µl) and 1.56±0.59 vs 0.49±0.46 between for fresh cervical tissue and FFPE respectively and were all statistically significant at p<0.005. The difference in amplification successes was higher for the 120 bp than for the 450 bp primers. The distribution of cervical human papillomavirus for fresh tissues and formalin-fixed paraffin-embedded tissues studied was 39% and 13% respectively. Although, the present results showed that PCR genomic DNA can be extracted from both fresh cervical smear and 8 years duration FFPE archived tissue blocks. Formalin fixed paraffin embedded tissue blocks is not recommended for epidemiological study for detection and typing of cervical human papillomavirus using high molecular weight base pair primers and conventional PCR.

Key words: Amplicon size, polymerase chain reaction (PCR), cervical tissues.

INTRODUCTION

Tissue fixation with 10% Formalin and molten paraffin wax embedding is a time saving and less expensive technique. It is the most widely adopted method of preserving human tissue biopsy and biological specimens. This has resulted in increased amount of formalin-fixed paraffin-embedded tissue blocks that represent both the pathology and pre-analytical handling of biological specimens. This reservoir of these specimens is increasingly being used for DNA, RNA, and proteomic studies. Moreso, clinical and molecular medicine are undergoing a revolution based on the accelerated advances in biotechnology such as DNA microarrays and proteomics. Quite a number of solution to critical questions such as how does the DNA sequence differs between individuals, what makes one individual more prone to a certain disease are being answered in

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the current post genomic era. Several government and non-profit organizations has now provided researchers access to human tissues for molecular studies and drug development. Excellent approach to studying genetic relatedness of species involving DNA sequence *via* comparisons which can be used to estimate branching order of phylogenetic trees as well as evolutionary distance between extinct taxa (Do and Dobrovic, 2015). The application of the polymerase chain reaction (PCR) and direct nucleotide sequencing has also accelerated efforts to examine a wide range of taxa with DNA comparisons (Florell et al., 2001).

Formaldehyde as a 10% neutral buffered formalin or otherwise formulated is the most widely used routine fixative in histopathology laboratories. This is because of its ability to preserve a wide range of tissues and tissue components. However, attempts to extract usable DNA from formalin-fixed tissues for molecular biological studies have been variably most likely successful due to different protocols used. (Kap et al., 2013). Also short-term treatment of tissue sections with formalin have shown significant reduction in the DNA solubility and quality (Korenkova et al., 2016). Although considerable evidence suggests that formaldehyde induces DNA degradation, few studies have reported yield of high-molecular weight DNA from formalin-fixed tissues (Lin et al., 2009).

Studies on chemical reactions between formaldehyde and nucleic acids have formulated the mechanism of reaction of formalin with macromolecules, formalin protein interactions. Do and Dobrovic (2015) stated that formaldehyde initiates DNA denaturation (inter chain hydrogen bonds break and bases unstack) at the AT-rich regions of double-stranded DNA creating sites for chemical interaction. There are four interactions of formaldehyde with DNA:

1). The first is an addition reaction. Formaldehyde is added to the nucleic acid base to form a hydroxymethyl (methylol) group (-CH2 OH).
2). The second is the slower electrophilic attack of N-methylol on an amino base to form a Methylene bridge between two amino groups.
3). Formaldehyde treatment can generate AP (apurinic and apyrimidinic) sites via hydrolysis of the N-glycosylic bonds, leaving free pyrimidine and purine residues. AP sites have a highly unstable cyclic carboxonium ion that hydrolyzes rapidly to yield 2-deoxy-D-ribose 36
4). Formaldehyde may also cause slow hydrolysis of the phosphodiester bonds leading to short chains of polydeoxyribose with intact pyrimidines (Bass et al., 2014; Sung et al., 2000). When compared to the DNA isolated from frozen tissues, formalin-fixed tissues exhibit a high frequency of non-reproducible sequence alteration. It has been speculated that the artefacts may be as a result of formalin cross-linking cytosine nucleotides on either strand. As a result, in PCR the Taq- DNA polymerase fails to recognize the cytosine and incorporates an adenine in the place of a Guanosine, creating an artificial C-T or G-A mutation (Do and Dobrovic, 2015).

Damaged DNA molecules known to promote jumping between templates during enzymatic amplification, permitting Taq-DNA polymerase to insert an adenosine residue at the end of a template molecule, then jump to another template and continue the extension of producing an artificial mutation that is subsequently amplified. Such mutations are more likely when fewer cells are used and isolated from a random mutation occurring in an early PCR cycle amplified to detectable level. Up to 1 mutation artefacts per 500 bases have been recorded (Kap et al., 2011). The actual frequency of errors is then a cumulative error including the reported Taq polymerase (normal) error frequency and the errors because of DNA damage and/or cross-linking depending on the degree of dilution (Bass et al., 2014). Although conformational sequencing of independent amplification can distinguish between artefacts and true mutations. It has been cautioned that non-recognition of such artefacts may have profoundly influenced mutation databases in formalin- fixed material (Do and Dobrovic, 2015).

MATERIALS AND METHODS

Study area

This study was a multicenter study carried out in Niger Delta University Teaching Hospital- Okolobiri (NDUTH) and Federal Medical Centre Yenagoa both in Bayelsa State, South-South geo-political zone of Nigeria. Both hospitals serve as referral centers in the state and training Institutions for Medical and Allied Medical Sciences Student of the State University. Bayelsa State shares hugely from poor health indicator WHO's ranking of the country health system of 187th position out of 191 countries in 2000 WHO country cooperation strategy (Ajay et al., 2008). The major occupation of the inhabitants is farming, fishing, palm oil milling, lumbering, palm wine tapping and local gin making.

Study design and sample size

The study was a retrospective cross-sectional design. The data on the relationship between cervical human papillomavirus (HPV) infection studied and other variables of interest as they exist in population was collected at a single point in time in accordance with the method of (Hennekens and Buring, 1987). The sample size of one hundred was used as determined by Naing et al. (2006), given a prevalence of cervical cancer Prevalence of 1.7% in Bayelsa State (Agba et al., 2016).

DNA extraction

The DNA was extracted from formalin fixed paraffin embedded cervical tissue and from cervical smears immersed in 5 ml of phosphate buffer. DNA extraction was done using the ZR FFPE DNA MiniPrep™ extraction kit manufactured by Inqaba Biotechnological, South Africa, following the manufacture's instruction.

Paraffin embedded cervical tissue blocks were first deparaffinized

using two changes of xylene. The tissue sections and transferred to a 1.5 ml micro centrifuge tubes. 1 ml of xylene was added to the sample, vortex and incubated at room temperature for 1 h with gentle rocking and centrifuged at 10,000 x g for 1 min. The supernatant was discarded and washed twice with 100, 95 and 75% ethanol for 5 min each with gentle rocking. The samples were washed once with 1 ml double distilled water for 5 min with gentle rocking and the supernatant was removed using a micropipette. To a deparaffinized tissue sample (≤ 25 mg) in a micro centrifuge tube, 45 µl of water, 45 µl of 2X digestion buffer and 10 µl of proteinase K were added and incubated at 55°C for 4 h. The micro centrifuge tube was transferred to an incubator set at 94°C and incubated for 20 min, 5 µl of RNase A was added to the mix and allowed to incubate for an additional 5 min at room temperature. 350 µl of genomic lysis buffer was added to the tubes and mixed thoroughly by vortexing. The mixture was centrifuge at 10,000 x g for 1 min to remove insoluble debris and the supernatant was transferred to a Zymo-Spin™ IIC Column and centrifuged at 10,000 x g for 1 min, 400 µl of g-DNA wash buffer was added and the spin column and centrifuged at 10,000 x g for 1 min. The Zymo-Spin™ IIC was transferred to a clean labelled micro centrifuge tube, ≤ 50 µl DNA elution buffer was added to the spin column and incubated for 3 min at room temperature, then centrifuged at top speed for 30 s elute the DNA. The eluted DNA was stored in ≤-20°C for use during molecular analysis.

Extraction of DNA from paraffin embedded cervical tissue and cervical smear

The disposable plastic cytobrush was used to collect the cervical scrapes and was immersed in 5 ml of phosphate buffer and stored in the refrigerator. The tissue was dislodged into the solvent and centrifuged at 14,000 x g for 2 min in a 1.5 ml micro centrifuge tube to concentrate the tissues in order to have enough mass of tissue to extract from. This procedure was repeated twice by adding double distilled water to the sample bottle in order to recover all the cervical tissue. The solvent was removed as much as possible, leaving the tissue mass in the 1.5 ml micro centrifuge tube. About ≤ 25 mg of tissue was introduced into a 1.5 ml micro centrifuge tube, 45 µl of water, 45 µl of 2X digestion buffer and 10 µl of proteinase K were added and incubated at 55°C for 4 h. The micro centrifuge tube was transferred to an incubator set at 94°C and incubated for 20 min, 5 µl of RNase A was added to the mix and allowed to incubate for an additional 5 min, room temperature. 350 µl of genomic lysis buffer was added to the tubes and mixed thoroughly by vortexing. The mix was centrifuge at 10,000 x g for 1 min to remove insoluble debris and the supernatant was transferred to a Zymo-Spin™ IIC Column and centrifuged at 10,000 x g for 1 min, 400 µl of g-DNA wash buffer was added and the spin column and centrifuged at 10,000 x g for 1 min. The Zymo-Spin™ IIC was transferred to a clean labeled micro centrifuge tube, ≤ 50 µl DNA Elution Buffer was added to the spin column. It was incubated for 2-3 min at room temperature, and then centrifuged at top speed for 30 s to elute the DNA. The eluted DNA was thus stored at – 20°C for use during molecular analysis (Table 1).

DNA quantification

DNA quantification was done using Nanodrop 1000 spectrophotometer. The Nanodrop 1000 (model no: ND 1000 UV/VIS- 1844, USA) spectrophotometer machine was connected to a system with the software. Two microliter of nuclease free water was used to calibrate the machine and the elution buffer was used to elute the DNA was used to blank the machine. Two microliter of the extracted DNA products were then placed on the pedestal of the machine and the quantity and purity of the extracted DNA, and nucleic acid were measured. The machine measures the DNA quantity in ng/µl and DNA purity at 260/280 mm absorbance and nucleic acid purity at 260/230 absorbance ratio. The separate absorbance were read as a ratio of both taken as the value for the variable measured. The values are then displayed on a monitor connected to the machine.

Detection of human Papillomavirus using nested PCR.

The method of Entiauspe et al. (2014) was adopted for detection of human papillomavirus. The MY09/11 and GP5+/6+ consensus primers where used to amplify the extracted DNA in a two stepped reaction. The MY09/11 consensus primer was used for the primary amplification while the GP5+/6+ consensus primer was used for the secondary amplification. The PCR mixture for the primary amplification reaction included; 1x master mix (which contains Taq polymerase, dNTPs, $MgCl_2$), forward and reverse primers at concentration of 0.2 µM and 5 µl of the extracted DNA was used as template. Nuclease free water of 6.9 µl was used to make up the PCR components to a final volume of 25 µl for the primary amplification, using the following PCR conditions; initial denaturing step at 94°C for 5 min, followed by 40 cycles of denaturation at 95°C for 60 s, annealing at 55°C for 60 s and initial extension at 72°C for 60 s, with a final extension at 72°C for 10 mins. The PCR mix for the secondary amplification was 1x master mix (which contains Taq polymerase, dNTPs, $MgCl_2$) forward and reverse primers at concentration of 0.2 µM and 1 µl of the amplified product of the primary amplification was used. Nuclease free water of 11.5 µl was used to make up the PCR components to a final volume of 25 µl. The PCR condition used included; initial denaturing step at 94°C for 4 mins, followed by 30 cycles of denaturation at 94°C for 60 s, annealing at 42°C for 60 s and initial extension at 72°C for 30 s, with a final extension at 72°C for 4 mins. The PCR products were analysed by electrophoresis on a 2% agarose gel stained with ethidium bromide and band sizes were estimated by comparison with 100 bp molecular weight marker (Quick-Load DNA molecular ladder, New England Biolabs Inc.). Ethical clearance (NDUTH/REC/0003/2015) was obtained from the ethical committee of the above mentioned hospital. The statistical analysis was done using Graph pad prism 5.

RESULTS

Macromolecules manipulation serves as potential tools for diagnosis and cure of certain cancers caused by dysfunctional macromolecules. Molecular biology has been applied in oncology for analysing the integrity of the macromolecules (DNA, RNA and proteins). A total of one hundred cervical samples were analysed, comprising of fifty fresh cervical smears and fifty formalin fixed paraffin embedded cervical tissue blocks retrieved from the pathology laboratory. The histopathology diagnosis revealed squamous cell carcinoma (24.0%), adenocarcinoma (06%), cervicitis (26.0%) normal (20.0%), CINI and II (4%) and others (20%). Results from the cervical smears revealed intraepithelial lesions, 28.0% low grade squamous intraepithelial lesion (LSIL) was (42.8%), ASCUS (21.4%) inflammatory smears (35.7%) and 72.0% negative for squamous intraepithelial lesions. The result was similar to previous reports documenting squamous cell carcinoma as the

Table 1. Primers synthesis.

Primer	Forward and revere	BP
MYO9/11	CGTCCMARRGGAWACTGATC GCMCAGGGWCATAAYAAGG	450
GP5+/6+	TTTGTTACTGTGGTAGATACTAC GAAAAATAAACTGTAAATCATATTC	150

Primary Source: NCBIgenebank.(http//www.ncbi.nih.gov/genbank).Inqaba biotechnical industries (pty) Hartfield South Africa.

Table 2. Comparison of DNA quantity, purity, and nucleic acid purity from different cervical sample types using Nanodrop technology.

Parameter	FFPE	Samples	Types	95cl	*t*	*p* value
		95%Cl	Smear			<0.05
DNA quantity(ng/ul)	21.84±25.52	14.85±22.09	47.73±37.45	29.09±58.01	4.13	0.01***
A260/A280 ratio	1.34±0.28	1.26-1.43	2.02±0.42	1.9-2.1	9.3	0.01***
A260/A230 ratio	0.49±0.46	0.30-0.60	1.56±0.59	1.23-1.57	8.4	0.01***

A260/280 ratio= DNA purity, A260/230 ratio = Nucleic acid purity. FFPE = formalin fixed paraffin embedded. *P* < 0.01**, Cl =confidence interval, t-student test, A=absorbance.

Table 3. HPV prevalence in normal and pathological women using MY09/11 and Nested Gp5+/6+ consensus primers.

Diagnosis	No. examined	MY09/11		Nested GP5+/6+		x^2
	n=100	Positive	Negative	Positive	Negative	
Normal	46(46.0)	09(69.23)	37(42.53)	30(57.69)	16(33.33)	**14.65**
Abnormal	54(54.0)	04(30.77)	50(57.47)	22(42.31)	32(66.67)	
Total	100(100.0)	13(13.0)	87(87.0)	52(52.0)	48(48.0)	

Statistically significant *p* <0.01****, MY09/11 and GP5+/6+ are consensus primers, normal = negative for intraepithelial lesion neoplasia for cytology/ negative for malignancy histopathology, Abnormal= positive for intraepithelial neoplasia for cytology and positive for malignancy for histopathology cases, x^2= chi square.

predominant type of cervical cancer (Serth et al., 2000). In the present study the DNA concentration and purity and nucleic acid purity of the samples studied were quantify spectrophotometricaly by measuring their absorbance at 260 and 280 nm using Nanodrop . DNA from fresh cervical smear and formalin-fixed tissues were extracted in all the cases using extraction kit as described previously. The difference in DNA quantity in fresh smear (47.73±37.45) compared with formalin fixed paraffin embedded (21.84±25.52) was statistically significant (p=0.01***, X^2 $X2$=4.13), DNA purity (A260/A280 ratio) from the smear samples was higher (2.02±0.42) compared to FFPE samples (1.34±0.28) (P<0.01***, X^2 =9.3). There also exits an increasing difference in the nucleic acid purity (A260/A230 ratio) of smear samples compared with FFPE (1.56±0.59vs0.49±0.46) respectively as shown in Tables 2 and 3, Figures 1 and 2.

DISCUSSION

Recent advances in molecular biology, proteomics and bioinformatics has cause archived histopathology specimen to become a valuable bank for molecular profiling and gene sequencing. The merit of these techniques is that small size sample is needed for providing a huge amount of detailed data. Due to the abundance in the availability of data, researchers have shifted their focus to macromolecules to investigate the mechanisms and fundamental principles of certain disease development and gene regulation (Srinivasan et al., 2002). Archived tissues blocks are used for a variety

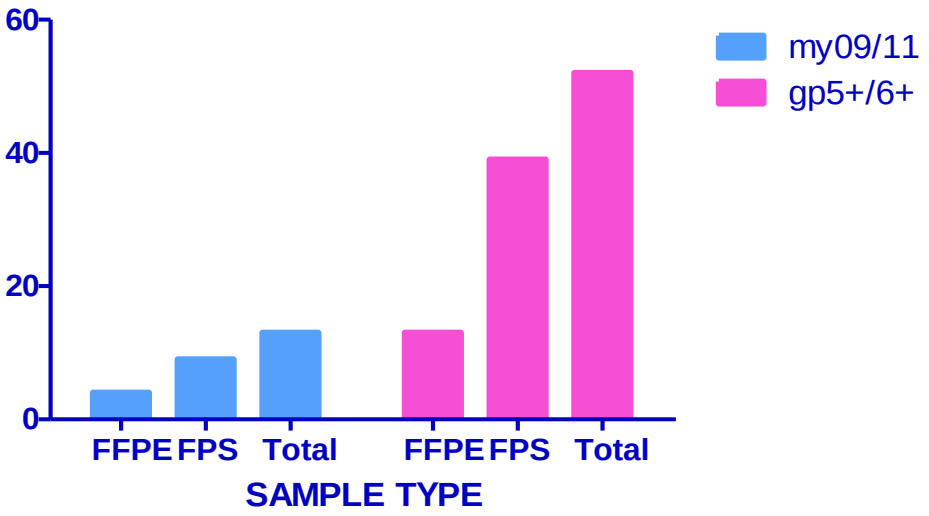

Figure 1. HPV Prevalence in Normal and Pathological Women Using MY09/11 and Nested Gp5+/6+ Consensus Primers by sample types, FFPE= Formalin fixed paraffin embedded, FPS= Fresh cervical smear.

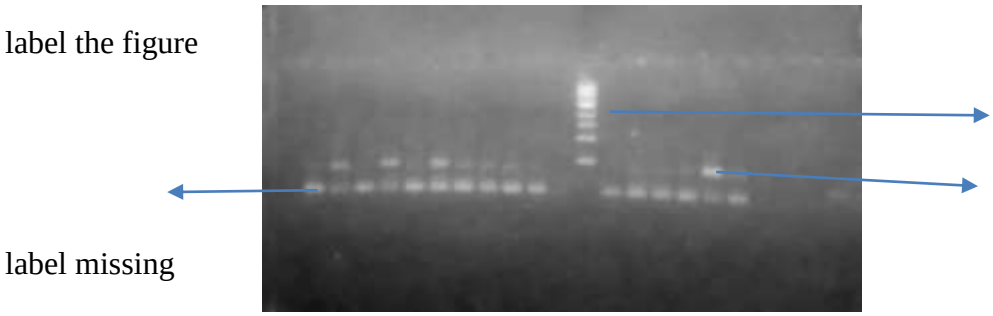

Figure 2. HPV detection using nested PCR MY09/11 and GP5+/ GP6+ consensus primer, Lane 1-14, 15- 35 and 36-44 represents the samples. Lane M represents the Quick-Load 100bp DNA molecular ladder.

of oncology research projects by screening for genetic markers from archived tissues (Koshiba et al., 1993; Olert et al., 2001). DNA extraction and polymerase chain reaction amplification are fundamental in DNA assays. The accessibility and quality of the DNA obtained is therefore important in macromolecules studies and thus for proper DNA extraction the macromolecules must be properly fixed. Fixatives used for preserving archived tissues can not only be used to maintain the tissues structures but also to protect macromolecules from degradative processes (Schander and Halanych, 2003) and understanding how fixatives affect the molecular quality and interference with results of the molecular assay is important (Srinivasan et al., 2002).

The present work is in agreement with previous studies that focus on the effects of certain fixatives on the quality and quantity of macromolecules. Their results showed that DNA obtained from archived tissues blood affect the sequencing results (Shibutani et al., 2000; DO and Dobrovic, 2015). Also previous studies of Bass et al. (2014) and Sung et al. (2000) has reported that formalin-fixed tissues exhibit a high frequency of non-reproducible sequence alteration which can as a result of lack of purity. It has been speculated that the artifacts may be because of formalin cross-linking cytosine nucleotides on either strand which is a major principle of formalin fixation. As a result, in PCR technique the Taq-DNA polymerase fails to recognize the cytosine and incorporates an adenine in the place of a Guanosine, creating an artificial C-T or G-A mutation (Ortiz-Pallardo et al., 2000). Damaged DNA from formalin fixed paraffin embedded samples are known to promote jumping between templates during enzymatic amplification permitting Taq-DNA polymerase to insert an adenosine

residue at the end of a template molecule, then jump to another template and continue the extension producing an artificial mutation that is subsequently amplified (Kocjan et al., 2016).

Furthermore, the extracted DNA was subjected to two consensus primer, 450 bp (MY09/11) and 120 bp (Gp5+/6+) respectively and result obtained showed that the 120 bp primer was more successful in the detection of the papillomavirus in the tissues and in the overall sample size. For the fixed tissues the short amplicon was more observed than the 450 bp amplicon. Previous research has reported amplification successes in shorter amplicon than longer and concluded that the DNA in the fixed tissues was damage caused by fixatives (Kocjan et al., 2016).

Also Ortiz-Pallardo et al. (2000) stated that DNA fragments of 200 bp could be amplified by PCR regularly from formalin fixed tissues, and the largest size that has ever been published was 959bp (Akalu and Reichardt 1999). The present study, result shows that fresh samples are the best for cervical human papillomavirus epidemiology studies compared with formalin fixed paraffin embedded archived tissue blocks. The differences in the current study prevalence by sample types (fresh samples and FFPE) have largely been attributed to DNA damage caused by the fixative use in processing the tissues, storage time and its corresponding actions on the purity and integrity of the macromolecules. Undoubtedly, there exists a tremendous need for human tissues for research and development and thus caution must be exercised in adopting the information on point mutation for diagnostic purposes and/or drug development.This is because it is possible that alterations in gene expression profiles during and or after the resection of the tissue.

Therefore, in order to obtain the maximum yield of PCR products, minimal DNA damage, and maximum utility for future clinical applications, the fixation temperature, pH, and duration must be carefully controlled during fixation. of samples in histopathology laboratory. Samples meant for macromolecules studies should be fixed in aldehyde-based fixatives and alcohol-chloroform-acetic acid-based fixatives at 4°C. In addition formaldehyde solution containing DNase-neutralizing EDTA could efficiently inhibit DNase degradation of DNA, compared to a formaldehyde solution alone (Kap et al., 2013).

Conclusion

The purity of DNA and nucleic acid were affected by tissue fixation with 10% formalin. The DNA quantity as well as the primers performances were fixatives dependent. Primers with short based pair between 120 to150 bp are better options for molecular studies in formalin fixed paraffin embedded tissues compared with those of 450 bp and above. It is important therefore to adjust the pH, temperature and formulation of fixatives in tissue for samples meant for macromolecules studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Agba E, Odutola M, Oga E, Adebamowo C (2016). Cancer in Nigeria-2009 - 2013: Cancer patterns in Nigeria states. Nigerian national system of cancer registries, Garki Abuja. pp. 21-22. https://nigeriacancerregistries.net/

Ajay T, Chistopher JL, Jeremy AL, David BE (2008). Measuring health system performance for 191 countries- GPE Discussion paper. World Health organization (30)EIP/GPE/EQC

Akalu A Reichardt JKV (1999). A reliable PCR amplification method for microdissected tumor cells obtained from paraffin-embedded tissue. Genetic Analysis 15:229-233.

Bass BP, Engel KB, Greytak SR, Moore HM (2014). A review of preanalytical factors affecting molecular, protein, and morphological analysis of formalin-fixed, paraffin-embedded (FFPE) tissue: how well do you know your FFPE specimen? Archives of pathology and laboratory medicine 138(11):1520-1530.

Do H, Dobrovic A (2015). Sequence artifacts in DNA from formalin-fixed tissues: causes and strategies for minimization Clinical chemistry 61(1):64-71.

Entiauspe LG, Silveira M, Nunes EM, Basgalupp SP, Stauffert D, Dellagostini OA,Seixas FK (2014). High incidence of oncogenic HPV genotypes found in women from Southern Brazil. Brazilian Journal of Microbiology 45(2):689-694.

Florell SR, Coffin CM, Holden JA, Zimmermann JW, Gerwels JW, Summers BK, and Jones DA, Leachman S.A (2001) Preservation of RNA for functional genomic studies: a multidisciplinary tumor bank protocol. Modern pathology 14:116-128

Hennekens CH, Buring JE (1987). Epidemiology in Medicine, Boston, little Brown 255(304):246-252.

Kap M, Arron GI, Loibner M, Hausleitner A, Siaulyte G, Zatloukal K, Murk JL, Riegman P (2013) Inactivation of Influenza A virus, Adenovirus, and Cytomegalovirus with PAX gene tissue fixative and formalin. Biopreservation and biobanking 11(4):229-234.

Kap M, Smedts F, Oosterhuis W, Winther R, Christensen, N, Reischauer B, Viertler C, Groelz D, Becker KF, Zatloukal K, Langer R, Slotta-Huspenina J, Bodo K, de Jong B, Oelmuller U, Riegman P (2011). Histological assessment of PAX gene tissue fixation and stabilization reagents. PLoS One 6(11):e27704.

Kocjan BJ, Hošnjak L, Poljak MJ (2016). Detection of alpha human papillomaviruses in archival formalin-fixed, paraffin-embedded (FFPE) tissue specimens. Journal of Clinical Virology 76(1):88-97.

Korenkova V, Slyskova J, Novosadova V, Pizzamiglio S, Langerova L., Bjorkman J, Vycital O, Liska V, Levy M, Veskrna K, Vodicka P, Vodickova L, Kubista M, Verderio P (2016). The focus on sample quality: Influence of colon tissue collection on reliability of qPCR data. Scientific reports 6:29023.

Koshiba M, Ogawa K, Hamazaki S, Sugiyama T, Ogawa O, Kitajima T (1993). The effect of formalin fixation on DNA and the extraction of high-molecular-weight DNA from fixed and embedded tissues. Pathology-Research and Practice 189:66-72.

Lin J, Kennedy SH, Svarovsky T, Rogers J, Kemnitz JW, Xu A, Krina T (2009). High-quality genomic DNA extraction from formalin-fixed and paraffin-embedded samples deparaffinized using mineral oil. Analytical biochemistry 395(2):265-267.

Naing L, Winn T, Russil BN (2006) Practical issues in calculating the sample size for prevalence study. Archives of Orofacial Sciences 1:9-14.

Olert J, Wiedorn KH, Goldmann T, Kuhl H, Mehraein Y, Scherthan H, Niketeghad F, Vollmer E, Muller AM, Muller-Navia J (2001). HOPE fixation: a novel fixing method and paraffin-embedding technique for human soft tissues. Pathology-Research and Practice 197:823-82652.

Ortiz-Pallardo ME, KO Y, Sachinidis A, Vetter H, Fischer HP, Zhou H (2000). Detection of alpha-1 antitrypsin PiZ individuals by SSCP and DNA sequencing in formalin-fixed and paraffin-embedded tissue: a comparison with immunohistochemical analysis. Journal of hepatology 32:406-411.

Schander C, Halanych KM (2003). DNA, PCR and formalinized animal tissue – a short review and protocols. Diversity and Evolution 3:195-200.

Serth J, Kuczyk MA, Paeslack U, Lichtinghagen R, Jonas U (2000) Quantitation of DNA extracted after micropreparation of cells from frozen and formalin-fixed tissue sections. The American journal of pathology 156:1189-1196.

Srinivasan M, Sedmak D, Jewell S (2002). Effect of fixatives and tissue processing on the content and integrity of nucleic acids. The American journal of pathology 161:1961-197

Sung HW, Chang Y, Liang IL, Chang WH, Chen YC(2000): Fixation of biological tissues with a naturally occurring crosslinking agent: fixation rate and effects of pH, temperature, and initial fixative concentration. Journal of Biomedical Materials Research 52:77-87.

Shibutani M, Uneyama C, Miyazaki K, Toyoda K, Hirose M (2000). Methacarn fixation: a novel tool for analysis of gene expressions in paraffin-embedded tissue specimens. Laboratory investigation 80:199-208

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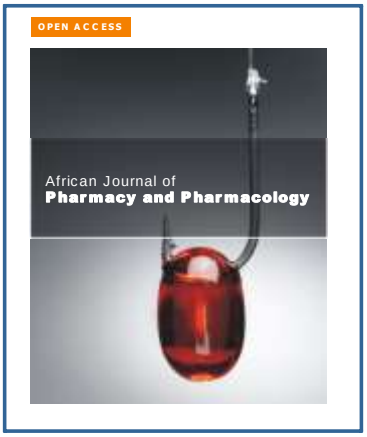

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