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REVIEW ON EXTRACTION OF DNA FROM SALIVA; STORAGE AND FURTHER MEDICAL STUDIES

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Abstract : A less painful method of DNA extraction and its further studies is explained . DNA is extracted from saliva with the help of several methods . Some methods are easy and they don't require any specific chemicals for the extraction process while some methods require specific DNA extraction kits . After the extraction, it is stored in specific conditions so that no changes take place in the original DNA. This stored DNA can be used for further studies regarding cancer detection and analysis of carcinoma cells.

IndexTerms **– DNA,Extraction,Saliva,Cancer**

I. INTRODUCTION

Mostly blood is used as a source of DNA in genetic testing today. However, taking blood samples are invasive, painful, and infectious[1].Large-scale epidemiological studies of DNA biobanks are increasingly using less invasive methods to obtain DNA samples, such as saliva collection. Epidemiologic DNA biobanking studies are increasingly using less invasive methods to extract genetic material, such as harvesting buccal epithelial cells from saliva.[2] DNA extraction is a critical step in forensic genetic analysis. DNA must be of sufficient quality with respect to fragment length, total amount and presence of inhibitor .There are various methods used in the process of extraction of DNA although some are quite expensive .The simplified manual protocol therefore serves as a cost-effective alternative to advanced automated solutions[3]. Currently, the optimal DNA input to PCR for STR profiling is ~500 pg, which corresponds to ~80 diploid cells (~6 pg/cell). Each item for which 80 cells are collected should have a complete DNA profile if the possibility of DNA loss is not taken into account by the workflow processing .[4]We are going to study DNA extraction from human saliva.

1] METHODOLOGY

1.1 By Buccal Swabs:-

DNA extraction from saliva involves diverse steps:
1) saliva collection and storage,
2) Initial preparations and cell lysis,
3) RNase treatment,
4) protein precipitation,
5) Isolation and purification of gDNA by ethanol precipitation,
6) gDNA rehydration.
The DNA Stabilization Buffer solution , functions adequately without alteration. [5]

1.2 By vortex method:-

After vigorous vortexing , the saliva sample was divided into six subsections. Three 100 mL sample aliquots were mixed with the same volume of Buffer 26 (20 mM Tris, 2 mM EDTA pH8, 1% Tween, and 400 mg/mL Proteinase K; Fermentation) and then incubated at 55 µC for 2-5 hr, followed by heating at 95 uC for 10 min to inactivate proteinase K. Three aliquots of 200 mL saliva samples were mixed with 700 mL of SL1 lysis buffer (containing SDS) and 120 mL of Enhancer SX from NucleoSpin Soil kit (Macherey-Nagel). The mixture was shaken in a NucleoSpin bead tube for 2 min at full speed on a Vortex-Genie 2 machine with a horizontal tube rack (Scientific Industries). From there, we followed the NucleoSpin Soil Kit (MachereyNagel) protocol. DNA was eluted in 100 ml of SE elution buffer. DNA extracts from both protocols were stored at 220 uC. [6]

1.3 By phenol-chloroform extraction process:-

The oral test was conducted strictly according to inclusion criteria, and participants were required to gargle with water for 30 minutes prior to sample collection. Unstimulated whole saliva samples were obtained, all spontaneously secreted from the mouth. Each person exhaled 2 ml of saliva and all samples were divided into two portions (1 ml each).
Modified phenol-chloroform extraction process. This DNA extraction protocol is the optimal condition for saliva samples according to Protocol 7 of Zoetenal et al. saliva samples at room temperature.

1.4 By QIAamp DNA Micro Kit-extraction process:-

All participants exhaled 2 ml of saliva,These 14 samples were assigned to group A(labeled A1eA14). Randomly two participants were selected .After three times, the participant was selected to provide a 2 mL saliva sample again.These two samples were assigned to Group B.(These two randomly selected participants No. 2 and 3, corresponding to samples A2 and A2A3, ie these two samples were collected at different times. Group B points were labeled B2 and B3). all the patterns out thereThe second stage was sent to the QIAamp DNA Micro.Kit extraction method.[7]

2] STORAGE OF DNA

The quality of DNA used for subsequent molecular analysis is a critical factor and depends on sample collection and storage prior to DNA extraction. Ideally, DNA samples are frozen soon after collection, but when samples are collected on-site, unavailability of resources and remote locations may limit appropriate storage methods.[8]
Methods of storing DNA depend on various factors. the type of DNA to be stored, the storage time, the storage temperature and conditions, and the downstream applications for which the DNA will be used. The procedure for isolating DNA and its purity at the time of storage
also determines the stability of the stored sample .
There are mainly four temperatures for long term storage of DNA
(a)Dry , room temperature
(b) –196° C
(c) – 80° C
(d) – 20° C [9]
In the glassy state, molecules cannot diffuse. That is, proton transport is limited to about one atomic diameter every 200 years, effectively suppressing chemical and nuclear decomposition. Raising the temperature restors and it can expose dna to potential damage. DNA stored in a dry state undergoes a hydrolysis reaction to remove active water and degrade the DNA. DNA stored dry should be kept at low humidity, as moisture can restore proton movement . [10]

3] DETECTION OF CANCER

The DNA methylation profile of ctDNA is thought to characterize DNA methylation in primary cancer tissues. In addition, cancer cells of various tissue types as well as normal cells are known to have characteristic DNA methylation profiles, and these methylation signatures can be used to identify the cell type or tissue type of origin.[11] Ongoing advances in saliva studies have allowed to the medical network that coined the term “salivaomics” . Salivary based processes were evolved for the invention of ability biomarkers into the six salivaomics which englobe the genome, the microbiome, the epigenome, the transcriptome, the proteome and the metabolome . Changes in salivary concentrations of those molecules may be implemented for the prognosis, person risk assessment, analysis and tracking of disorder. To this regard a huge number of research provided distinctive salivary biomarkers in head and neck cancers and also in cancers remote to the oral hollow space. No matter, the outstanding interest of saliva, to the fine of our knowledge, this is the primary meta-analysis that evaluates the diagnostic fee of salivary biomarkers for malignant non-oral tumours detection. We summarized the medical evidence and carried out a systematic review and meta-evaluation comparing the effect of salivary biomarkers for the diagnostic of malignant non-oral tumours.[12] No matter the susceptibility of human body’s to the most cancers cells proliferation, destiny techniques can be advanced to differentiate low biomarker concentrations in saliva to counter the proliferation .Currently, numerous techniques based totally on the proper detection of most cancers biomarkers in saliva had been advanced, including enzyme-related immunosorbent assay (ELISA), northern blot, western blot, Radioimmunoassay (RIA), polymerase chain reaction (PCR), microarrays, chromatography, and mass spectrometry ,these strategies no matter the reality that touchy, accurate and specific.[13]

4] RESULTS AND DISCUSSION

The extracted DNA obtained from the mentioned methods or process has a good yield. The tests performed by these processes are 99.9% accurate. If the extracted DNA yield from saliva is compared to that of extraction of DNA from blood , then blood gives more yield as compared to saliva . But extraction of DNA from saliva is less painful and easy as compared to extraction of DNA from blood. The quality of DNA extracted from saliva is sufficiently good to perform necessary tests.

A total mean DNA yield of 64.1 µg (range 3.9–176.0 µg) was obtained from 3.3 ml of saliva, giving a mean yield per ml of 18.5 µg/ml (range 1.2–44.0 µg).[14]

After extraction of DNA it is stored by the most common method known as freezing. This method of storage helps in the long term storage of extracted genomic DNA.

4.1 Results of Descriptive Statics

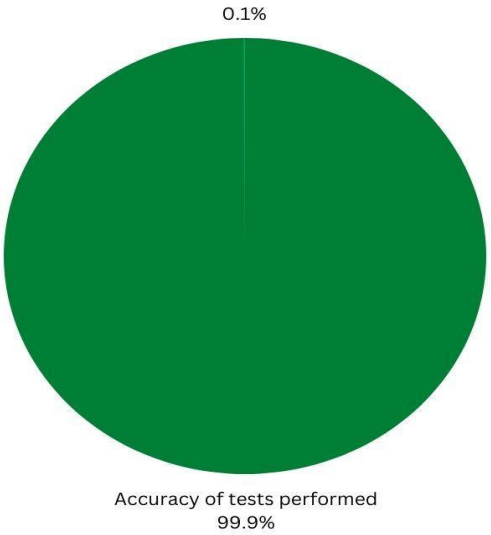

Figure 1
Accuracy of tests performed on the DNA extracted from saliva

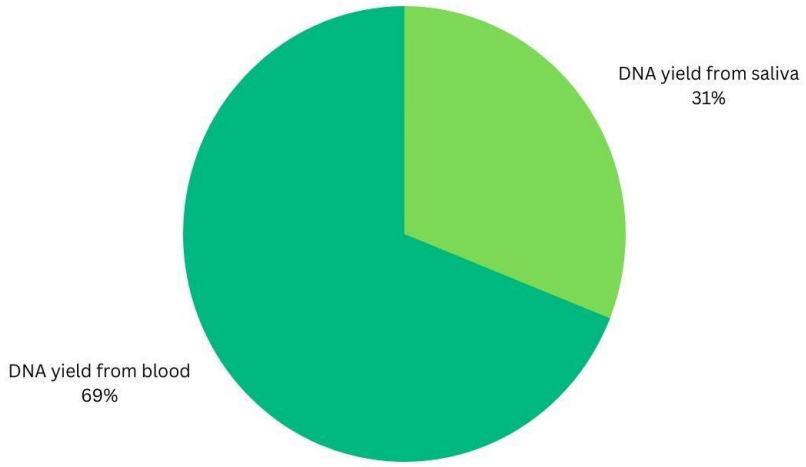

Figure 2
Percentage yield of DNA

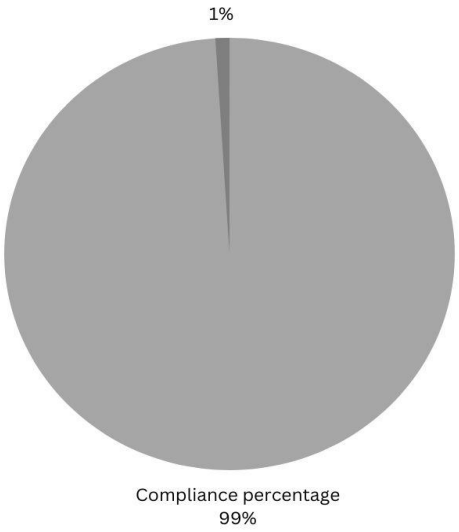

Figure 3
Patient compliance of sampling

4.2 Applications and future prospects

1. The patient compliance of saliva sampling is great as compared to other sampling methods.
2. Pediatric sampling can be done easily without any harm and DNA extraction can be done.
3. Extraction of DNA from saliva is a painless , easy and effective process.
4. Rapid detection and testing kits require saliva samples , this process is also very effective.
5. Rapid detection kits , RT PCR tests are necessary for detection of infections from various microbes and viruses.
6. These kits provide quick results as compared to other methods .
7. In Future these kits or methods have huge applications at the time of a pandemic.
8. As this provides quick results it has a vast application in studies and research.
9. Extraction of DNA from saliva would be necessary in future for gene analysis and medicinal purposes.

4.3 Acronyms

DNA - Deoxyribonucleic acid
PCR - polymerase chain reaction
STR - short tandem repeat
pg - picograms
RNA - Ribonucleic acid
gDNA - Genomic deoxyribonucleic acid
ml - milliliter
mM - millimolar
EDTA - Ethylenediaminetetraacetic acid
mg - micrograms
pH - power of hydrogen
µ C - micro celsius
hr - Hour
SDS - sodium dodecyl sulfate

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