



**ORIGINAL RESEARCH PAPER**

**Oral Pathology**

**DRIED SALIVARY SPOT AS A DIAGNOSTIC AID- A REVIEW**

**KEY WORDS:** Salivary Diagnosis, Forensic Odontology, Dried Salivary Spot, Fluorescence.

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**ABSTRACT**  
 Saliva is a colourless, watery and complex fluid secreted by the salivary glands into the oral cavity. It plays an important role in maintaining the moisture and well being of the oral cavity. In recent years, Saliva has been used by the researches, as it is non invasive technique, limited training required, potentially valuable for children and elderly patients, cost effective, eliminates the risk of infection and screening in large population. Collecting dried salivary sample is still being a greater challenge in the forensic. The process of identifying a disease, condition, or injury from its signs and symptoms is referred as diagnosis and diagnostic tool helps in simplifying the clinical findings to obtain a reasonable and relevant differential diagnosis. Recently, Dried salivary spot has been most commonly used among Forensic Odontology which can be used as an efficient diagnostic aid. This article aims to review the updates and significance of dried saliva spot and sampling in forensic odontology.

**INTRODUCTION**

Oral cavity being the moistest environment which consists of Saliva is constantly coats the inner surface of the oral cavity, occupies the space between the lining of mucosa and teeth. Human saliva comprises of organic and inorganic substances like proteins, hormones, antibodies and other molecular compounds which acts as a promising diagnostic fluid, helps in diagnosis of disease state. Saliva is an important source of DNA, hence salivary spot encountered in forensics is an important objective for forensic serologists. Dried saliva spot (DSS) which has seen growing interest in the past few years is which helps in the bio-detection of numerous medications and metabolomics analysis, thereby forms the widespread usage. The dried salivary spot has made an advancing progress among therapeutic drug monitoring, disease diagnosis and also it plays a major role in forensic Odontology in victim identification.

**Dried Salivary Spot (DSS)**

Dried salivary spot is defined as a technique for collecting dried saliva samples in the forensic odontology. In forensics, identification of salivary stains is important evidence where the salivary act as an important source of DNA. It is difficult to collect salivary stains from skin, clothing, paper or other inanimate objects since it remains invisible. Hence, a proper method of collection is required for identifying the invisible salivary stain on human skin and which can further be preceded with the methods of DNA extracting. (1,2)

Saliva collection is painless and non-invasive technique. Therefore, saliva is more effective as a diagnostic fluid than blood and other non-blood materials. Approximately, 3-100 mL of Saliva is used as sampling volume in the dried salivary spot method. (3)

**Methods Of Collection Of Dried Saliva Spot**

There are various methods used in the collection of dried salivary spots, which includes;

1. Chemicals
2. Lasers and UV lights
3. Fluorescent spectroscopy

**Chemical Method:**

- Enzymes: Alkaline phosphatase, starch and amylase

- Salts: Nitrate, thiocyanate has been used to detect dried saliva spot.
- Procedure: Dried salivary spot is collected through a single cotton swab or filter paper and the chemicals acts as a reducing sugar and forms a red insoluble precipitate.

**Advantages:**

- Collection of sample is easy
- Easy transportation and storage

**Disadvantages:**

- Depending on the age and quantity of the salivary sample are various disadvantages present;
- Alkaline phosphatase is not very specific because it fetches a false positive result.
  - Starch or iodine test for Amylase has been used in which excess starch leading to false positive result.
  - Salts like nitrate has a limitation because it can be used only on the fresh samples
  - Salts like thiocyanate may not be always present in the saliva. (3)

**Lasers and UV light:**

Quartz arch tube and Argon ion laser can be used

**Procedure:**

- The dried salivary spot is excited at a wavelength between 200 and 320 nm. The emission spectrum of the peak excitation Wavelength is used for the collection of dried salivary spot sample.

**Advantages:**

- Simple screening technique for identifying DSS
- Lasers and UV light were detected in 21 & 13% where as Argon and quartz arch tube is about 30 & 48%. (5)

**Fluorescent Spectroscopy**

Structure, dynamics, functional interactions of proteins can be assessed using fluorescent material at specified wavelength

**Procedure:**

Aromatic amino acid, tryptophan, α-salivary amylase present in saliva can emit a characteristic spectrum on fluorescent spectroscopy which helps in detection of the DSS on the skin.

The fluorescent material is excited by a particular wavelength; it emits radiation at a longer wavelength. The bands of dried salivary spot were analysed under a Fluorescent spectroscopy, confirmed with the bands of pure tryptophan.

Tryptophan is the most widespread probes in dried saliva stains for Fluorescent analysis.

**Advantages:**

- Diagnostic tool for larger sample
- Rapidity and specificity is higher, since this technique is considered to be 100-1000 times more sensitive than other techniques.

**Disadvantages:**

- Strong dependence on light scatter
- Environmental factors such as temperature, pH, and viscosity can alter the analysis (1, 4)

**Salivary Biomarkers using DSS**

A biomarker is defined as a biological molecule found in blood and other body fluids, or tissues that is a sign of a normal or abnormal process or of a condition or disease. Salivary biomarker is a non invasive method of diagnosing the disease and monitoring the general health. (2)

Some important biomarkers include;

- 1) Locally produced proteins of host and bacterial origin which includes enzymes and immunoglobulin.
- 2) Genetic /genomic biomarkers such as host-derived DNA and mRNA
- 3) Bacteria and bacterial products, steroid hormones and volatile compounds
- 4) Salivary proteomic, genomic and metabolite biomarkers for periodontal diagnosis. (6-9)

**Brindle et al 2010** States the comparison between stability of C - reactive protein (CRP) in dried blood spot and dried salivary spot. Sample of dried blood spot is stable at room temperature for about 1 week after collection and sample of dried salivary spot is stable for about 8 hours at room temperature before freezing. (10)

**Goodson et al 2014** States that CRP in dried salivary spot is 6 times higher in obese children than those of healthy normal weight children. Salivary insulin and leptin were 3 times higher in obese children. (11)

**Stefan MM et al 2020** States that C - reactive protein (CRP) levels obtained from dried blood samples and CRP levels from dried salivary spot correlated with each other. But salivary CRP shows increased result for stress reactivity. (12)

**Plank Anne Christine et al 2021** States that the comparison of C - reactive protein (CRP) in dried blood spot and dried salivary spot and its ability to diagnose systemic inflammation precisely. It states that dried blood spot can reflect systemic inflammation more precisely than saliva. (13)

**Sodnom-Ish et al 2022** States that the addition of methyl group to the DNA sample from dried salivary spot is one of the most effective method for early diagnosis of oral squamous cell carcinoma. (14)

**Masha babaei et al 2022** States the comparison of CRP of dried salivary spot and plasma. CRP of dried salivary spot has proven to be more definitive in diagnosis of medical conditions. (15)

**CONCLUSION**

In recent years, Saliva has become a useful tool and a detective aid in diagnosis of various diseases both in clinical and forensics. In a forensic crime scene, it is of great difficulty

in collection of evidence. Dried salivary spot is important evidence which helps in the identification of the victim. This article describes how the dried salivary spot can be used as a diagnostic tool, their method of collection and use as a biomarker. But its knowledge and importance is not well aware among many. Hence more researches are needed for its implications in various fields in the future.

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