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Review Article

Determination of reliability and practicality of saliva as a genetic source in forensic investigation by analyzing DNA yield and success rates: A systematic review

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ABSTRACT

Background: Genetic analysis has become the mainstay of forensic identification protocols. Oral fluids including saliva are invariably present in crime and disaster scenarios; their availability probably exceeding that of conventional sources like blood. Due to the intrinsic nature of fluids, the substrates may be degraded due to time delay and environmental exposure. Therefore, a review of the reliability and practicality of saliva in forensic analysis is warranted.

Objectives: This review is designed and planned to present and critically analyze the most relevant clinical trials on the application of saliva in forensic settings in order to assess the reliability of saliva for DNA analysis.

Data sources and eligibility criteria: Thorough literature search was performed via Pubmed/Medline database, the Cochrane Trial register and Clinical Trial registry of India. Only the clinical trials that specifically mentioned the use of saliva for DNA analysis were eligible and included. Eight trials were included for the analysis.

Study appraisal: Customized data collection were generated and compiled by two independent evaluators. Risk assessment and qualitative synthesis was performed.

Results and conclusions: Using qualitative thematic synthesis, it was observed that saliva, especially whole saliva, collected in specific kits under stabilization, was a sufficient source of DNA, especially for PCR and genotyping methods, irrespective of environmental conditions and time delay. The observations indicate that saliva is a reliable and practical source of DNA for forensic settings.

1. Introduction

Crime scenes present genetic evidence mostly from the body remains, blood and other body fluids, and from inanimate objects in the vicinity. Analysis of body fluids is the most reliable; however, they are susceptible to degradation due to environmental conditions and the time delays that ensue. Blood samples may not be available all the time; and may involve issues of law, especially in criminal investigation. Body fluids like saliva, seminal and vaginal fluids are present in many of the crime scenes and are often indispensable for identification [1]. Saliva may be available from a variety of sources, like bitemarks, drinking glasses, tissues etc. The main source of DNA from saliva is the desquamated epithelial cell component of the oral mucosa. There are various methods for collection of saliva, including swabs, oral rinses, cytobrush, and whole saliva in vials, especially by the Oragene™ method [2]. Genetic analysis of saliva and other sources can be done by numerous protocols. The older methods of hybridization have given way to PCR-based techniques, SNP genotyping, sequencing based and DNA micro-array. There are now a range of new generation techniques that are faster, simpler and inexpensive [3,4]. However, specific protocols for discovery, collection and preparation of saliva for genetic analysis are yet to be established. There are currently very few Cochrane-registered clinical trials of the use of saliva in forensic settings [5–12]. Thus, a thorough review of the reliability and practicality of saliva in forensic science is warranted. The objective of this review is to assess saliva as a DNA source in forensic and other settings. It endeavors to discuss and present the most accepted and validated methodology for the collection, storage, preparation and analysis of saliva to achieve the

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highest success rates.

2. Methods

2.1. Protocol

This systematic review protocol is available at PROSPERO (International prospective register of systematic reviews) database. Registration number is CRD42018097300.

2.2. Research question (in PICOS format)

In cases of unknown individuals in forensic settings, is genetic analysis of saliva reliable and practical for forensic procedures?

2.3. Sources, search and study selection

Electronic literature search of Pubmed/Medline database, the Cochrane Trial register, and Clinical Trial registry of India, were undertaken. Only the clinical trials registered by the above bodies that specifically mentioned the use of saliva in DNA analysis were eligible and included. The main search terms that were entered were “saliva”, forensic dentistry and saliva”, “saliva and DNA”; an illustrative screenshot is included (Fig. 1). Original research in journals with adequate impact and citation were preferred. Fully published English language articles in scientific journals were selected with year range of 2000 to present (18 years). Table 1 gives the criteria for selection. Table 2 is the flowchart of the screening process.

2.4. Data collection

A customized data extraction form was generated for this review (Table 3 A to 3 H). Basic information, participant details, outcomes, and other measures were included. Two independent observers collated the data and filled the forms. In case of disagreement a consensus was reached.

2.5. Data items

The variables for data collection are tabulated. The included variables were study design, number of participants, age group, gender (when specified), setting/country, collection methodology, DNA quantification procedures, statistical analysis, self SWOT analysis (when specified), results and funding sources. (Table 3A to H)

Table 4A and B include the assessment of risk of bias in individual studies, derived from Cochrane risk of bias tool (Cochrane Handbook v 5.1.0, March 2011). Risk assessment across studies has also been done by the above mechanism (Table 5) [13].

2.6. Qualitative synthesis

A qualitative synthesis was performed, specifically a thematic analysis, as outlined by Thomas J and Harden A (BMC Med Res Methodol 2008) and Bearman M and Dawson P (Med Ed 2013) [14,15]. The parameters (themes) include age group, collection methods, time delay, DNA quantification techniques, and other measures that may be relevant to the review. The focus of the synthesis is to determine the effect of these parameters on the quantification of DNA from saliva, and to therefore discuss and conclude on the reliability and practicality of

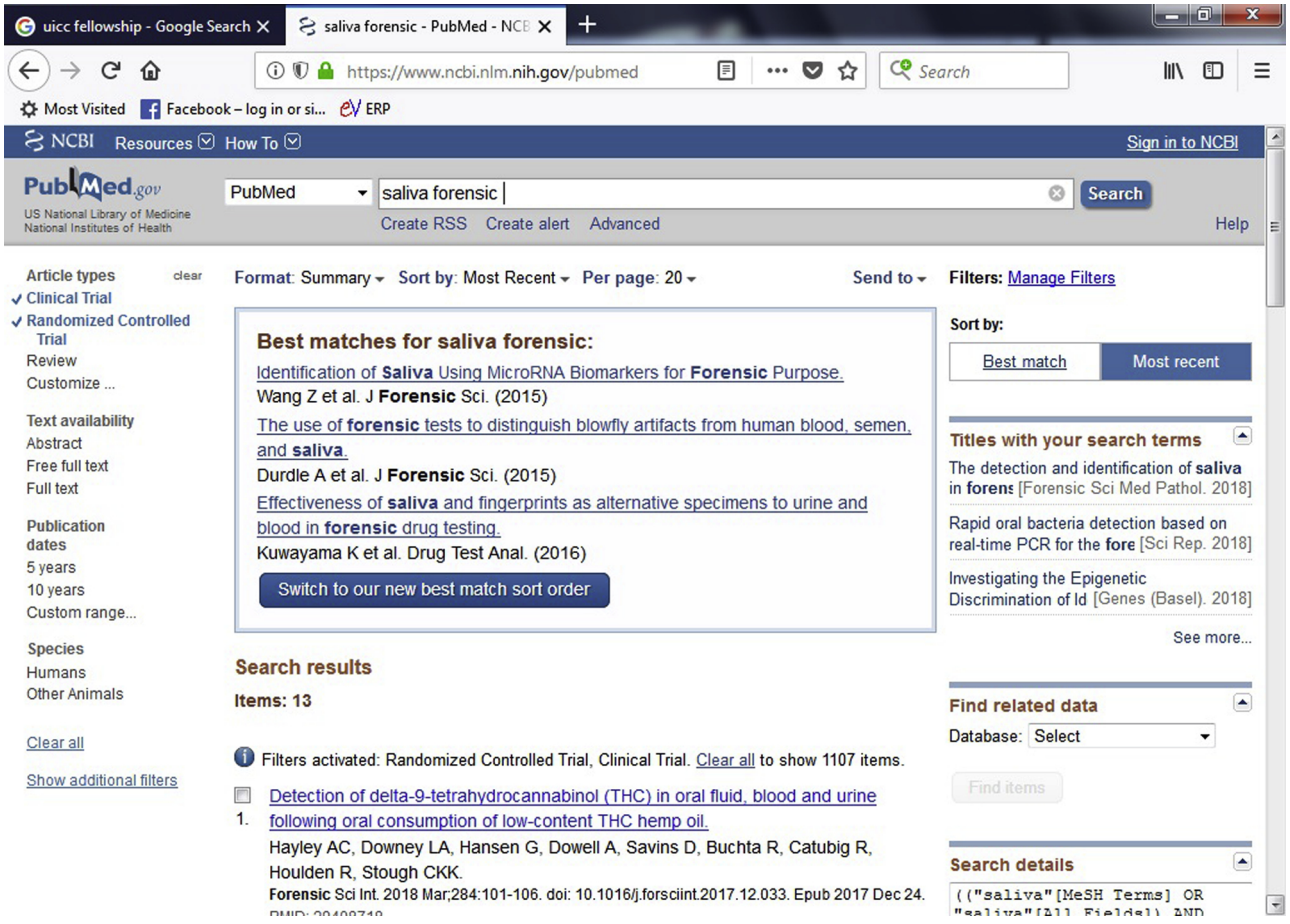

Fig. 1. Figure showing the screenshot of an example web search.

Table 1
Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Clinical trials registered in Cochrane Library and Clinical Trial Registry of India	Trials deemed to be unsuitable for our analysis due to lack of adequate information and major deficits in study design, methods, etc
Published Trials not registered but assessed to be otherwise competent for our analysis	
Studies that specifically mention the genetic analysis of saliva in forensic settings	Trials that mention saliva in non-genetic analysis including proteomics, drug testing, metabolite analysis etc
Studies that mention the genetic analysis of saliva in settings other than forensics like academic research, quality analysis, comparative studies, genotyping	Trials that have included saliva in genetic analysis but have not adequately presented relevant data required in data collection
Publications ranging from year 2000 to 2018	Studies earlier than 2000 (initial search results did not fulfill basic criteria)
English language articles (including translations)	Other language articles
Studies in humans	Studies in animals (initial search results did not fulfill basic criteria)

saliva in DNA analysis.

3. Results

Table 3A to H record the main data collected from the clinical trials. Emphasis was given to the type of trial, the setting, number of participants, collection methods, DNA quantification techniques and results, with SWOT performed by the authors themselves, wherever specified, as reported by the authors. Funding sources were also included. Many of the trials had not explicitly stated the type of trial (random, fixed, control etc.). Some of the trials had low sample size; one of the studies did not mention the sample size in few groups.

In collection techniques, the protocol generally recognized as optimal, i.e. whole saliva collection in a stabilization kit, was followed in

Table 2
Screening process adapted from PRISMA flowchart (2009) [16].

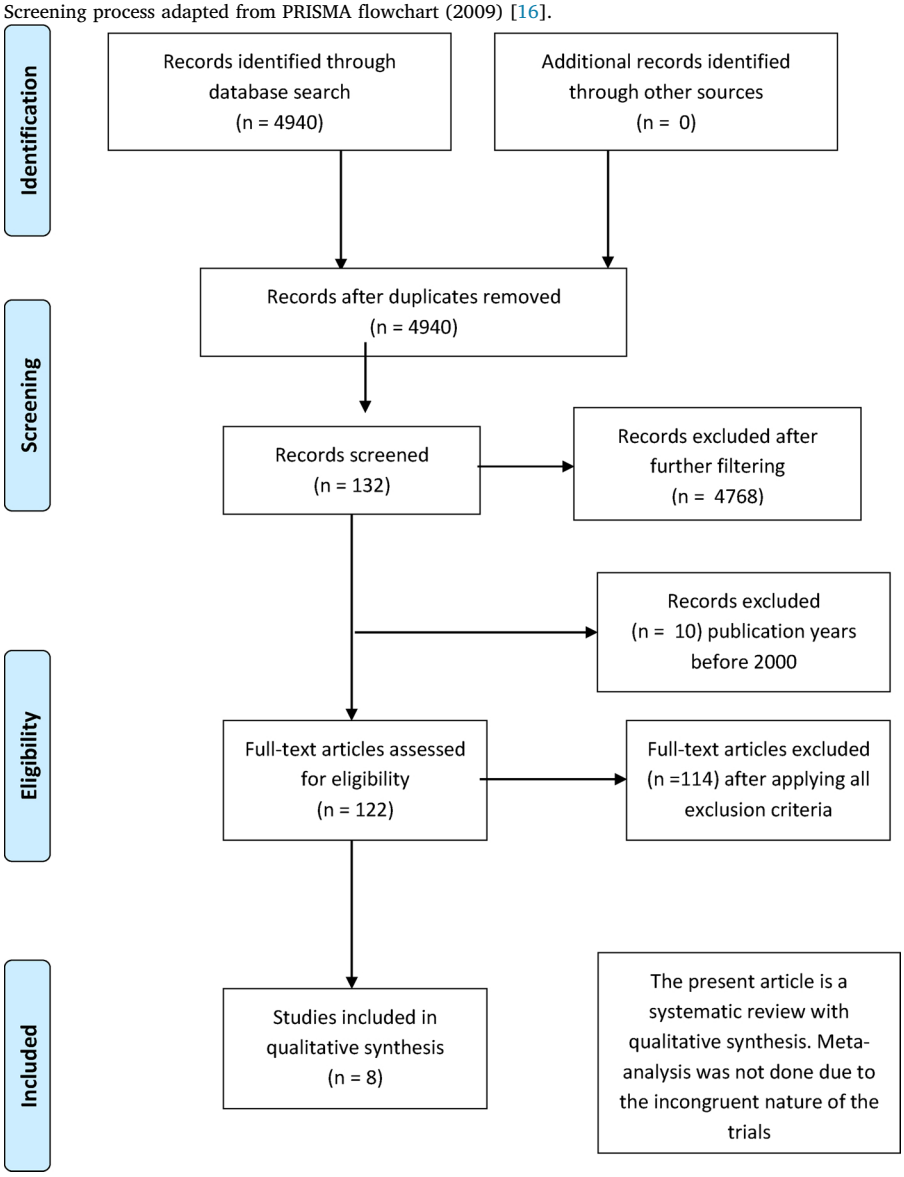

Table 3
Comprehensive data form for the clinical trials (A to H).

A

Trial 1	Design	Setting/ Country	No of participants	Age range	Collection methodology	
Imabayashi et al 2005	Not specified	Japan	142 in which 6 involved saliva sampling	NS	Cotton swabs, saliva samples were used either immediately or stored 5-10 Y at room temperature	
Quantification methodology	**Statistical analysis**	**Results**	**Strengths**	**Weaknesses/ limitations**	**Conclusions**	**Funding source**
QIAamp DNA minikit (Qiagen) Gene Quant II (US) HLA DrB1 genotyping with SNP analysis	Not specified	Correct typing of all saliva samples in both cases HLA-DR3, DR9, DR1, DR2	Useful for mixed source samples Simpler, cost effective compared to PCR (RFLP, SSP, SSOP) and comparable to STR	Not specified	Methodology is accurate for mixed samples	Not specified

B

Trial 2	Design	Setting/ Country	No of participants	Age range	Collection methodology	
Kanto et al 2005	Randomized trial	Brazil	20, non-related	24-47y	Saliva stored in Axygen cryotubes at -20C Sweet's double swab technique used for 5 random samples deposited on skin	
Quantification methodology	**Statistical analysis**	**Results**	**Strengths**	**Weaknesses/ limitations**	**Conclusions**	**Funding source**
Beckman DU 640 spectrophotometer PCR with ALF express sizer STR single loci amplification	Not specified but likelihood ratios calculated	DNA from saliva deposited in skin was 14-10 times lower than the actual samples	Care taken to avoid contamination	Multiplex loci amplification not done DNA in 4 / 5 skin samples	Methodology can be upgraded to include more samples and multi-loci analysis	Not specified

C

Trial 3	Design	Setting/ Country	No of participants	Age range	Collection methodology	
Rogers et al 2007	Randomized crossover	Health center faculty, Ohio, US	17	24-56	Multiple collection techniques including cotton swab, oral rinse, cytobrush, Oragene™ are compared	
Quantification methodology	**Statistical analysis**	**Results**	**Strengths**	**Weaknesses/ limitations**	**Conclusions**	**Funding source**

(*continued on next page*)

Table 3 (*continued*)

C

Trial 3	Design	Setting/ Country	No of participants	Age range	Collection methodology	
Whole genome scan with 400 microsatellite markers PCR: ABI – Prism linkage mapping set	SAS 9.1 ANOVA with Bonferroni adj. Wilcoxon signed rank	Whole saliva and oral rinse provided high DNA yield with 98-99% PCR success. Cytobrush and swabs were lower in yield and PCR percentage, (even with Oragene™)	Crossover study	Age and ethnic group evaluation not done Differential participation, spectrophotometry	2 ml saliva or 10 ml oral rinse superior sources of DNA than cytobrush and swabs	NIH: NICHD, NIDDK, NHLBI; Grant numbers: HD12252, DK64870, DK64391, HL69995; Grant sponsor: National Center for Research Resources, NIH; Grant number: RFIP Grant 1 C06 RR13556-01.

D

Trial 4	Design	Setting/ Country	No of participants	Age range	Collection methodology	
Nishita et al 2009 (Reg no: NCT00301145 at clinicaltrials.gov)	Randomized	COMPASS participants in US	565 67% female, 88.5% Non Hispanic Caucasians	19-76y	Saliva collected by Oragene™ kit via correspondence and transport. Avg of 63.8 days taken for DNA extraction. DNA samples were stored at 4C for at least 3 days	
Quantification methodology	**Statistical analysis**	**Results**	**Strengths**	**Weaknesses/ limitations**	**Conclusions**	**Funding source**
UV spectrophotometer Picogreen (PG), qPCR, SDS v1.2.3 software Genotyping, SNP, VNTR	SAS 9.1	Saliva classified as cloudy and clear. Cloudy samples had high yield probably due to non-human DNA. PCR 77% Genotyping rates were 98% in SNP, and 97.5% to 98.5% in VNTR	Higher genotyping rates achieved	Limited participation (25% sample collection) No second observer Collection protocols and accuracy not monitored	Whole saliva even under contamination was able to generate high genotyping rates	National Cancer Institute, R01 CA071358 and National Institute on Drug Abuse U01 DA020830

E

Trial 5	Design	Setting/ Country	No of participants	Age range	Collection methodology	
Nunes et al 2012	Cohort study	1993 Birth Cohort, Brazil	4110/822 50.7% male	14.69 y on avg	Oragene™ kit to collect whole saliva stored in 2 modes: 3 days, and 8 months. Pts advised to use mouthwash 15 min prior to sampling	
Quantification methodology	**Statistical analysis**	**Results**	**Strengths**	**Weakness/ limitations**	**Conclusions**	**Funding source**
Eppendorf Biophotometer PCR,SNP and HRM, (Tagman)	STATA 10.0 Paired T test	3 days: 2-160 ng/μL 8 months: 1-251 ng/μL. not significant. 98-99% rates on PCR, SNP, HRM	Better human DNA yields probably due to mouthwash rinse Sample size	Not randomized Age restricted	2.2 ml saliva helpful for DNA analysis, higher volumes not additive. Time delay not a factor	PRODOC grant from the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES-Brazil)

F

Trial 6	Design	Setting/ Country	No of participants	Age range	Collection methodology	
Khare et al 2014	Not specified	Lucknow, India	20	Not specified	Blood and saliva were collected in vials and stored at -20C	
Quantification methodology	**Statistical analysis**	**Results**	**Strengths**	**Weakness/ limitations**	**Conclusions**	**Funding source**

(*continued on next page*)

Table 3 (*continued*)

F

Trial 6	Design	Setting/ Country	No of participants	Age range	Collection methodology	
Hitachi spectrophotometer STR-F13 (PTC-100 (MJ Research Inc)	Not specified	9 of 20 samples had Optical density 1.6-2.0 DNA yield was 48.4 μg/ml with 75% rate STR was 100% positive	Not specified	Small sample size Possible contamination by proteins, phenol Purification steps not performed Only one STR gene was assessed	Apart from blood, saliva is a good source of DNA in forensic applications	Not specified

G

Trial 7	Design	Setting/ Country	No of participants	Age range	Collection methodology	
Kakuda et al 2016	Control trial	Yamanashi U, Japan	8 voluntary saliva samples and unspecified number of ancient samples from remains from regional university archives	Not specified	Forensic swab (Sarstedt, Germany)	
Quantification methodology	**Statistical analysis**	**Results**	**Strengths**	**Weakness/ limitations**	**Conclusions**	**Funding source**
Multiplex Inosine flapped APLP for haplogrouping mtDNA (Qiagen) PCR and electrophoresis, Nanodrop 1000, Phylotree	Not specified	DNA identified in all samples at level of 100 fg (1×10^{-13} g)	Identified femtogram level DNA. Most studies require DNA atleast in picogram levels	Did not compare with SNaP shot analysis or HTS	Developed method for ancient and degraded samples	Grants-in-aid for Scientific Research from the Japan Society for the Promotion of Science (Nos. 26440257, 25251043, 16H01954), and JSPS Core to Core Program (Advanced Research Networks)

H

Trial 8	Design	Setting/ Country	No of participants	Age range	Collection methodology	
Forat et al 2016	Not specified	U of Bonn, Germany	100 blood, 100 saliva, and various samples of menstrual blood, vaginal and seminal fluids	35-39 y	75 samples of cotton swabs in 3 forms: (Dry, Wet and Outside in Ground) sampling and analysis done for Immediate, 1,2,3 and 6 months of storage	
Quantification methodology	**Statistical analysis**	**Results**	**Strengths**	**Weakness/ limitations**	**Conclusions**	**Funding source**
QIamp (Qiagen), Spectrometer, Bisulfite conversion (Epitect) PCR, Next Gen Sequencing with SNuPE method of methylation analysis	CLC Genomics Workbench	Saliva sampling was as good as other sources in a mixture of body fluid, specific markers able to analyze. Methylation did not change in any forensic condition	Considers exogenic and endogenic parameters that interfere with true results Analyze fluid mixture with wide array	Age dependent DNA methylation and other causes not taken into account	Analysis of complex fluid mixture possible and successful using specific marker panel	SF was supported in part by ZIM project Nr. KU2117901AJ8.

Table 4
Risk assessment in individual studies (A and B).

A – Trials 1 to 4

	Trial 1 Imabayashi et al 2005		Trial 2 Kanto et al 2005		Trial 3 Rogers et al 2007		Trial 4 Nishita et al 2009	
Domain	Risk	Review authors' judgment	Risk	Review authors' judgment	Risk	Review authors' judgment	Risk	Review authors' judgment
Selection bias	High	No comment on randomization in the study	Unclear	Stated like "Five samples from the 20 donors were randomly chosen.." which is not specific	Low	Explicit statement of a randomized crossover trial, with group reversal	Low	Explicit statement and system of a randomized trial
Randomization	Unclear		Unclear		Unclear		Unclear	
Allocation concealment		No mention of allocation concealment		No mention of allocation concealment		No mention of allocation concealment		No mention of allocation concealment
Performance bias	High	No statement on blinding. However it is unlikely to affect outcome	High	No statement on blinding.	High	No statement on blinding.	Low	No statement on blinding, however, the nature of data collection renders low risk of bias
Blinding of personnel and participants								
Detection bias	Low	No mention of blinding assessment, however being a laboratory assessment, unlikely to affect outcome	Low	No mention of blinding assessment, however being a laboratory assessment, unlikely to affect outcome	Low	No mention of blinding assessment, however being a laboratory assessment, unlikely to affect outcome	Low	No mention of blinding assessment, however being a laboratory assessment, unlikely to affect outcome
Blinding of assessment								
Attrition bias	Low	No missing data	Low	No missing data	Low	No missing data	Low	All received samples assessed and reported
Incomplete outcome data								
Reporting bias	Unclear	No information provided on selective or actual reporting, though no cause to suggest bias	Unclear	No information provided on selective or actual reporting, though no cause to suggest bias	Unclear	No information provided on selective or actual reporting, though no cause to suggest bias	Unclear	Study states "Having genetic material from asubset of all participants in a study could be a limitation and could potentially bias genetic association results"
Selective reporting								
Other bias	Unclear	Insufficient information	Unclear	Use of higher per-sample volume of saliva than recommended, may influence the success of tests	Unclear	Study states "we cannot measure possible differential participation and return rates for the different specimens"	Unclear	Reported to have received more samples from older participants than expected

B – Trials 5 to 8

	Trial 5 Nunes et al 2012		Trial 6 Khare et al 2014		Trial 7 Kakuda et al 2016		Trial 8 Forat et al 2016	
Domain	Risk	Review authors' judgment	Risk	Review authors' judgment	Risk	Review authors' judgment	Risk	Review authors' judgment
Selection bias	High	No statement of a randomized trial	High	No statement of a randomized trial	High	No statement of a randomized trial	Unclear	No statement of a randomized trial,
Randomization	Unclear	Sourced from birth cohort thus very restricted age group.	Unclear	Sample size is low	Unclear	Sample size is low	Unclear	however the authors perceive from scrutiny it was probably randomized
Allocation concealment		No mention of allocation concealment		No mention of allocation concealment		No mention of allocation concealment		No mention of allocation concealment
Performance bias	Low	No statement on blinding, however, the nature of data collection renders low risk of bias	Low	No statement on blinding, however, the nature of data collection renders low risk of bias	Low	No statement on blinding, however, the nature of data collection renders low risk of bias	Low	No statement on blinding, however, the nature of data collection renders low risk of bias
Blinding of personnel and participants								
Detection bias	Low	No mention of blinding assessment, however being a laboratory assessment, unlikely to affect outcome	Low	No mention of blinding assessment, however being a laboratory assessment, unlikely to affect outcome	Low	No mention of blinding assessment, however being a laboratory assessment, unlikely to affect outcome	Low	No mention of blinding assessment, however being a laboratory assessment, unlikely to affect outcome
Blinding of assessment								
Attrition bias	High	All received samples were not assessed and reported. Selection of 822 from 4110 samples for testing was not explained	Low	All received samples were assessed and reported.	Low	All received samples were assessed and reported.	Low	All of received samples were assessed and reported.
Incomplete outcome data								
Reporting bias	Unclear	Insufficient data on this since only 20% of samples were analyzed and reported	Unclear	No clear indication of protocol involved in reporting	Low	No cause to suggest bias	Low	No cause to suggest bias
Selective reporting								
Other bias	Low	No other bias	Low	No other bias	Low	No other bias	Low	No cause to suggest bias

Table 5
Risk assessment across studies.

Domain	Risk	Review authors judgement
Selection bias	High	Most trials demonstrated a lack of
Randomization	High	explicit randomization
Allocation concealment		None of the studies have concealed allocation
Performance bias	High	Blinding was not reported in any of
Blinding of personnel and participants		the studies, though influence in outcome is unlikely
Detection bias	Unclear	Blinding was not reported in any of
Blinding of assessment		the studies, though influence in outcome is unlikely
Attrition bias	Low	Most of the trials reported complete
Incomplete outcome data		analysis and reporting of data
Reporting bias	Low	No explicit data on selective reporting
Selective reporting		
Other bias	Low	No significant risk noted

3 trials out of 8. Other trials used swabs, without DNA stabilization. DNA quantification methods included spectrophotometry, Polymerase chain reaction (PCR), genotyping, whole-genome scan etc. Thus, there was no single quantifying method that was common in all trials.

Five of the trials had self assessment of strength and weaknesses. Most of the trials mentioned the sample size or the laboratory procedure as superior or comparable to the previous studies. The main weaknesses reported were limited samples, lack of age and ethnic categorization, and the sensitivity/limitations of laboratory procedures.

Risk analysis of individual studies is presented in Table 4A and B. Considering the enormous impact of bias on the validity of the trials, a comprehensive analysis based on the Cochrane Handbook was performed. Most of the trials did not use the term randomized in their methodology. Allocation concealment and blinding was not mentioned in any clinical trial. However, most trials have analyzed and reported all the analyzed samples. There was no data regarding other possible sources of bias. Table 5 shows the risk analysis across studies. Lack of randomization and blinding are the most significant sources of possible bias. Analysis and reporting of outcomes did not present a significant risk of bias.

Qualitative synthesis of the trials is summarized in Table 6. Overall, the age group of the participants did not affect the DNA success rates. Sample source and Collection methodology was critical to the success of DNA quantification. Studies that employed whole saliva and DNA stabilization kits reported higher percentage of DNA rates compared to those that employed swabs, cytobrush, oral rinses etc., especially with the use of DNA stabilization solutions. The time delay between sample collection, storage and DNA analysis was not a significant factor, even in room temperatures. DNA quantification using PCR and genotyping had more success rates than spectrophotometry. There was no major information to flag conflict of interest regarding funding in these trials.

4. Discussion

Forensic identification relies on proper collection and analysis of evidential specimens. The most conclusive identification requires genetic analysis. DNA, RNA, cellular proteins, and other molecules may be used for the analysis. The most common sources of the aforementioned components include blood, soft tissue, secretory fluids like saliva, vaginal and seminal secretions, teeth, bones etc. Saliva is one of the most common and effective sources of DNA. However, guidelines for the use of saliva in DNA analysis are lacking. This reflected in the wide variation in the collection, storage and analysis protocols [1–4].

This systematic review aimed to establish the reliability and practicality of saliva for DNA analysis in forensic settings. Using strict inclusion and exclusion criteria, eight studies were selected. English language articles with proper study design and relevance to the research question were preferred. Studies that were published since the year 2000 were included due to high likelihood that up-to-date analytic techniques would be employed. Comprehensive data collection was performed and tabulated. Risk of bias assessment with and across studies was analyzed. Qualitative thematic synthesis was done since the studies presented with wide variations in study design and methodology. Therefore quantitative syntheses and meta-analysis was not deemed appropriate for this review.

Analysis of the collected data revealed that many of the studies had not specified a particular study design. Explicit statements regarding randomization, allocation concealment, blinding etc were lacking in most trials. The methodology varied significantly between the trials. However, the main DNA quantification techniques were similar, though no single technique was observed to be common to all the studies. The results and conclusions converged among the studies, reporting that saliva is an acceptable and reliable source of DNA, when standard guidelines of collection, stabilization, storage and analysis were followed [5–12].

The settings involved in the trials varied and were spread across countries like the US, Germany, Japan, India and Brazil; thus reflecting varied ethnic and racial populations. The age range of the participants in the studies included adolescents, young adults, middle aged and elderly. Wherever specified, the studies appear to have employed

Table 6
Summary of qualitative (thematic) synthesis.

Theme / Parameter	Nature of variability	Effect on DNA analysis	Report
Age group	Studies had analyzed wide variation in age group from adolescents to elderly	All age group samples were satisfactory for analysis	Age is not a factor in saliva samples for DNA analysis
Source of samples	Whole saliva, swabs, cytobrush, oral rinses	Whole saliva followed by oral rinses reported higher DNA yield.	Irrespective of technique, whole saliva tends to be the best source of DNA
Sample Amount	Trace level to 10 ml	Affects DNA yield in all techniques except PCR	Spectrophotometry and whole genome scan require larger samples to be successful. One study implies more than 2.2 ml of saliva is redundant for PCR based methods.
Contamination of samples	Pure whole saliva, saliva with food and other debris, cloudy saliva in smokers, saliva in mouthwash rinse, cotton swab etc.	Unreliable results in spectrophotometer, due to non-human DNA. PCR, genotyping not affected	Spectrophotometer is largely unreliable compared to PCR and genotyping, STR, etc which employ specific human primers and gene loci.
Sampling technique	Storage in swabs, vials, cryotubes, Oragene™ kit	Oragene™ kit and cryotubes use reported higher DNA yield	Use of DNA stabilizing solution dramatically improves DNA success rates and reduces false positives from non-human DNA
Temperature	Room temperature, 4C and -20C	No significant differences in DNA yield	Temperature is not a significant factor, esp with PCR
Quantification techniques	Spectrophotometry, PCR, genotyping, whole genome scan	PCR was more successful in all employed scenarios compared to spectrophotometry	PCR is the preferred method of DNA analysis, with specific claims of higher success made by individual studies e.g. Kakuda et al 2016
Funding sources	Many not specified. All reported funds are research and education grants		No basis for influence on outcome in the trials that specified the funding

balanced gender participation. The number of samples ranged from 8 to more than 800. As mentioned earlier, sample collection methodology was not made explicit in some trials, rendering the validity of such samples in some doubt [5,9,10].

One of the most accepted and reliable methods for sampling saliva for DNA analysis is by collecting whole saliva in a stabilization medium, and storage in either room temperature or cryostorage. Oragene™ seems to be the preferred method of collection, as seen in many articles, and based on author experience, the method of choice in many government forensic laboratories. In this method, whole saliva is placed in a kit containing a medium that destroys microbes and stabilizes human DNA, thus significantly increasing success rates. Three out of eight trials have employed this technique. Even in trials that compared the above method to other methods like swabs, bitemark sources, cytobrush and oral rinses; Oragene™ sampling produced high and reliable results. Therefore, saliva sampling is best done by whole saliva in a stabilization medium. Results of the trials indicate that temperature and time duration do not affect the DNA success rates especially in this method [7–9].

Laboratory methods for DNA analysis differed widely between the studies. Every trial seems to use a particular quantification apparatus, though the reason for selection of the particular method was not explicitly stated. The major analysis techniques included spectrophotometry, genotyping with different loci and techniques including Polymerase chain reaction (PCR), Single nucleotide polymorphism (SNP), Short Tandem Repeat (STR), Variable Number Tandem Repeat (VNTR), microsatellite markers, High resolution melting (HRM), Amplified Fragment Length Polymorphism (APLP), gene sequencing etc., with each trial elaborating on the relative superiority of its method. More studies are required to analyze and recommend a particular technique. However, PCR and other genotyping methods seem to be sensitive and specific, with high success rate compared to spectrophotometry. Since spectrophotometry relies on Optical Density to analyze DNA, and cannot accurately distinguish human and non-human DNA, its results are not comparable to PCR. PCR employs primers of human DNA and is therefore significantly more accurate. Genotyping methods like SNP, VNTR, and STR were reported to have higher success rates compared to PCR. Use of multiplex analysis, i.e., panels of multiple gene loci, are reported to present higher success rates compared to single locus analysis. This seems to be the common interpretation among the trials [5–12].

A critical review mechanism should address risk of bias. Based on the Cochrane Handbook for systematic reviews, risk assessment was performed within and across studies [13]. As mentioned above, some trials may suffer from high risk of bias regarding randomization, allocation concealment and blinding. Detection and reporting bias is low. In our case, Kanto et al, Rogers et al and Nishita et al provided explicit statements of randomization [6–8]. Nunes et al 2012 did not specify under what mechanism 822 samples were selected among 4110 participants [9]. A major factor in most of the trials was the absence of proper criteria for selection of samples for analysis. Most studies have noted that all analyzed samples were reported. Two of the studies have flagged the differential participation and return rates of the participant pool, and observed that such factors may affect the final outcome to an extent. On the whole, across the trials, the methodology and conclusions reported may be considered as reasonably free from major bias risk.

Considering the number of trials selected for this review and the wide variations in the methodology, including sample size, age and gender profile, geographic and ethnic variations, laboratory methods etc.; the authors relied on general guidelines of Cochrane reviews, Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement of 2009, the observations of Thomas J and Harden A (2008) and Bearman M and Dawson P (2013) [13–16]. A qualitative (thematic) synthesis was considered to be the analysis of choice. The themes selected were age group, source of samples, sample amount, contamination of samples, sampling technique, temperature, quantification techniques and funding sources.

As mentioned earlier in the discussion, there was a wide variation in the age range, geographic and ethic characteristics of the participants. The synthesis found these factors to not affect the success of DNA analysis. Findings regarding the significant parameters are presented in Table 6. The findings of Terada et al (2014) and Mee BC et al (2011) indicate that temperature of storage (room temperature, 4C-8C or freezing) did not significantly affect quality of DNA. This coincides with our synthesis. The above articles reported a significant difference in DNA quality when time was delayed from 30 days to 180 days [17,18]. However, this difference did not affect the gene mapping via STR analysis. Our analysis revealed that though time delay might affect the quantity and quantity of DNA, the DNA-based identification techniques like STR analysis still present with high success rates, if adequate sample volume is available. Conversely, in a study by Utsuno H and Minaguchi K (2004), samples stored for longer time (> 7 to 9 years and more) did present with allelic imbalance and false allele amplification, evidences of degradation and contamination of DNA. Therefore it is safe to postulate that beyond 180 days the possibility of DNA degradation resulting in reduced success rates in PCR methods must be considered. [19]

The problem of degradation of samples and inadequate sample volume for techniques like STR and SNP does affect the success of forensic identification. Some of the trials had addressed this issue and recommended techniques like Next generation sequencing (NGS), HLA genotyping, Multiplex APLP and mtDNA haplotyping, stating that these techniques are superior in results compared to the conventional methods. Gettings KB et al (2015) and Borsting C et al (2015) elaborate on the high identification potential of NGS for highly degraded samples. There has been an explosion in the use of NGS and single molecule sequencing for clinical and forensic applications due to high success rates, increased speed and reduced costs. [20,21]

5. Conclusion

In summary, the use of body fluids in DNA-based forensics has been reported for many years. The preferred source was blood, due to the high cell content. However, saliva has emerged as a simpler and inexpensive source. The present article seems to be the first to systematically review the published clinical trials that reported the use of saliva as a DNA source in forensic and other settings. Extensive search of databases revealed no such attempt in the literature. Our review affirms that saliva is a reliable DNA source, especially when few guidelines are established for collection, stabilization and storage. Limitations of this study include the low number of clinical trials that fulfilled the inclusion criteria. Quantitative synthesis and meta-analysis was not performed. In general, the amount of research on saliva as a DNA source seems to be limited, compared to the wealth of data on forensic studies involving anthropomorphic analysis, use of blood and tissue etc. Future work should include more such research in order to analyze this crucial aspect of DNA-based forensic science.

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Conflict of interest

None declared.

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