

Development and validation of salivary analysis for forensic evidence (SAFE) scale

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Fida Mansha Ottapurakkal Thazath¹, Ram Sabarish², Akila Ganesh³

¹ Sri Ramachandra Dental College and Hospital, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, India. ² Department of Periodontology, Sri Ramachandra Dental College and Hospital, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, India.

³ Department of Public Health Dentistry, Sri Ramachandra Dental College and Hospital, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, India

Corresponding author:
akilaganesh@sriramachandra.edu.in

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ABSTRACT

Background: Despite the surging crime rates and diverse operating methods, identifying the perpetrators and suspects is challenging. Incorporating forensic odontology has expanded the scope of forensics. Over the years, saliva has become the hallmark of forensics. This study aimed to develop a scale for assessing the knowledge and awareness about the role of saliva in forensic odontology.

The scale was developed sequentially according to the qualitative research methodology: formation of a conceptual framework, systematic development of an item pool and refinement of an item pool (focus group discussion, cognitive interviewing and pretesting). Then, the validity and reliability of the scale were tested.

Results: A conceptual framework was developed based on five constructs, which included forensic sciences, forensic odontology, crime investigations, salivary analysis and forensic genetics. A pool of 28 items was designed based on existing literature and refined through a focus group discussion involving the target audience and expert panellists. After validation by cognitive interview and pretesting, the scale was condensed into a pool of 23 items. Statistical analysis revealed a Cronbach's alpha of 0.8 (good reliability) and kappa value of 0.79, indicating a strong level of agreement.

Conclusion: The Salivary Analysis for Forensic Evidence scale is a valid and reliable tool that consolidates all possible constructs involved in assessing the role of saliva in forensic odontology.

INTRODUCTION

With the surging crime rate and diverse operating methods, identifying the perpetrators and suspects has become increasingly challenging. Thus, the field has expanded into forensic odontology – a branch of forensic medicine that deals with proper handling, examination and presentation of dental evidence for appropriate justice. Forensic odontologists assist the legal authorities through dental evidence in various situations.¹ This includes management, examination, evaluation and presentation of dental evidence in civil or criminal proceedings along with background research.² In addition, saliva has become the mainstay in forensics and is now used as a biological sample like blood or urine.³ Currently, it is an indispensable fluid for various genomic analysis needed for

forensic applications. Identifying age and gender narrows down the profiles and restricts the suspects.⁴ Importantly, several advancements have been made globally in the field of forensic and crime investigations.²

Despite the efforts to raise awareness and apply forensic odontology, the potential of dental surgeons is yet underutilized. According to Milcah Roy et al.,⁵ the scope of forensic odontology is well-established among dentists; however, its practical application is limited. The end effects of abuse, assault or any other crime-related injuries that cause trauma to teeth, jaws or oral tissues should be appropriately evaluated, examined, recorded and documented at the Primary Health Centre for forensics.

Hitherto, the indispensable role of salivary analysis in crime scenes has been neglected, thereby necessitating the need to spread knowledge about the implications of forensic odontology. This lacunae led to the development and validation of a Salivary Analysis for Forensic Evidence (SAFE) scale to assess saliva's role in forensic odontology.

MATERIALS AND METHODS

The SAFE scale was developed using a valid and recognized methodology proposed by Vaughn et al.⁶ as follows: (i) Formation of a conceptual framework, (ii) Systematic development of the item pool, (iii) Refinement of the item pool, (iv) Validity testing and (v) Reliability testing (Figure 1).

The ethics approval was obtained from the Institutional Ethics Committee [Reference Number: CSP/22/MAR /106/74]. Informed consent and non-disclosure consent were obtained from all participants involved in the various stages of the scale development.

Formation of a conceptual framework

PubMed, Google Scholar and Scopus were used to retrieve complete, thorough and comprehensive literature on the concepts of forensic odontology⁷⁻⁹. A conceptual framework was designed to include the key components that defined the constructs intended to evaluate the role of saliva in forensic odontology.¹⁰ Each of the constructs was multidimensional and viewed as an individual component⁷ (Figure 1).

Systematic development of an item pool

An initial pool of items was generated for each construct extracted from literature and available instructional resources, such that they represented the whole attribute.¹¹ This perspective should be consistent across items,¹² and each item layout should be short, close-ended questions in simple language avoiding double-barrelled items, leading questions, double negatives, slangs and abbreviations, and based on knowledge and awareness (Figure 1).^{7,8,9,10} The items drafted should represent the construct of interest by ensuring the theoretical concept of the construct.⁸

Refinement of the item pool

The initial pool of items was refined via semi-structured and structured discussions through a sequence of processes: (a) Focus group discussion (FGD), (b) Cognitive interviewing and (c) Pretesting. The item pool was further refined based on the participants' feedback to ensure construct's conceptualization (Figure 1).⁸

Focus group discussion (FGD)

During the COVID outbreak, the FGD was conducted via video conferencing through Google Meet. It was moderated by the Principal Investigator. Two expert panellists from the Indian Academy for Clinical and Dental Genetics were part of the FGD, and the participants included undergraduate clinical dental students (Table 2). The ultimate goal of the FGD was to initiate interactions and structured discussions between researchers, expert panellists and the participants, as well as among the participants.¹⁰ The participants were informed of the FGD's objectives before volunteering, and consent was obtained to record the whole conversation. Each item was displayed and questions like 'Do you find this item misleading? Do you want to modify this item in any simpler form? Do you want to retain this item or exclude it?' were put forth, and participants were urged to voice their opinions. Also, new items generated from the participants were included. The elicited responses were analysed, and the items' language and phrasing were amended. The participants were awarded an incentive for their time and effort (Figure 1).

Cognitive interviewing

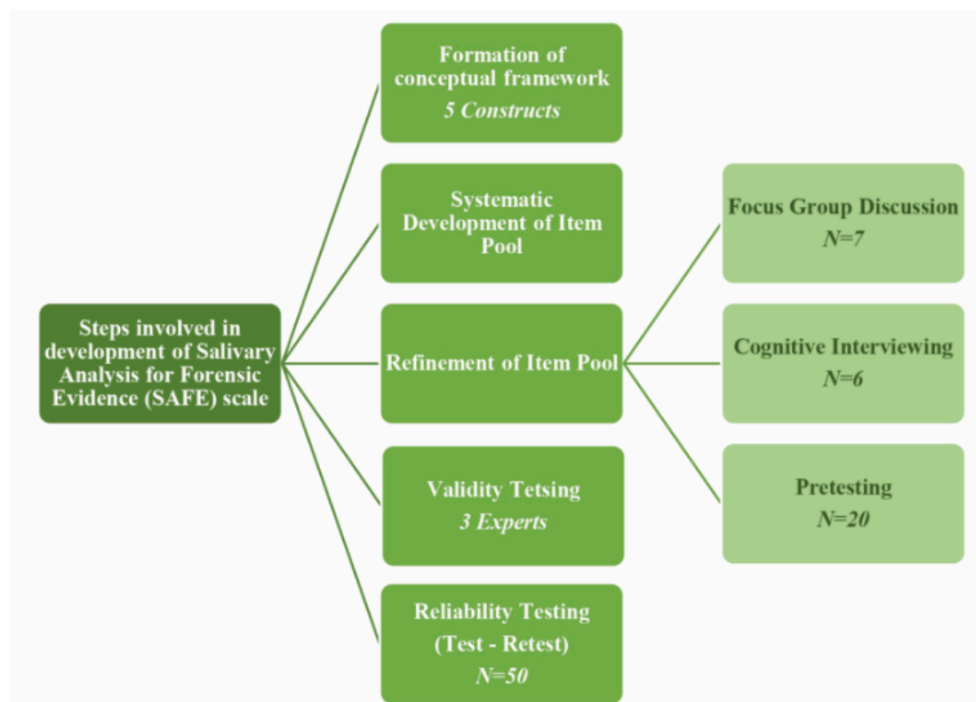
Cognitive interviewing was carried out as a face-to-face interview with six clinical undergraduate

dental students using the *'think aloud protocol'* (Table 2)¹⁰ in order to test the participants' understanding of terms, clarity of words, items relevance and response option of the developed scale and to address the related.⁸ The respondent's cognition of questions and response options were evaluated.¹⁰ Each item was read aloud to the participants with verbal probing questions: *'Was the question easy/hard to answer? Does this question make sense to you? Will this question make sense to people like you? Is there a better way to ask this?'* The answers were tabulated. Confusing, reductant and overlooking items were identified and modified accordingly following several discussions (Figure 1).¹⁰

Pretesting

Cognitive interviewing was followed by a qualitative evaluation.⁸ The revised items were pretested among volunteers to ensure accurate rendering of their vernacular usage¹⁰ and identify difficulties related to data collection and find solutions. The questionnaire was pretested on 25 clinical undergraduate dental students through face-to-face interviews (Table 2). The items were tested in terms of understanding, and adequate responses were documented with skipped patterns.¹⁰ The primary rationale was to eliminate items that participants perceived as relatively unimportant (Figure 1).¹³

Figure 1. Steps involved in the development of Salivary Analysis for Forensic Evidence (SAFE) scale. This figure depicts the five major steps involved in the development of a scale and the number of participants involved at each stage.



Validity Testing

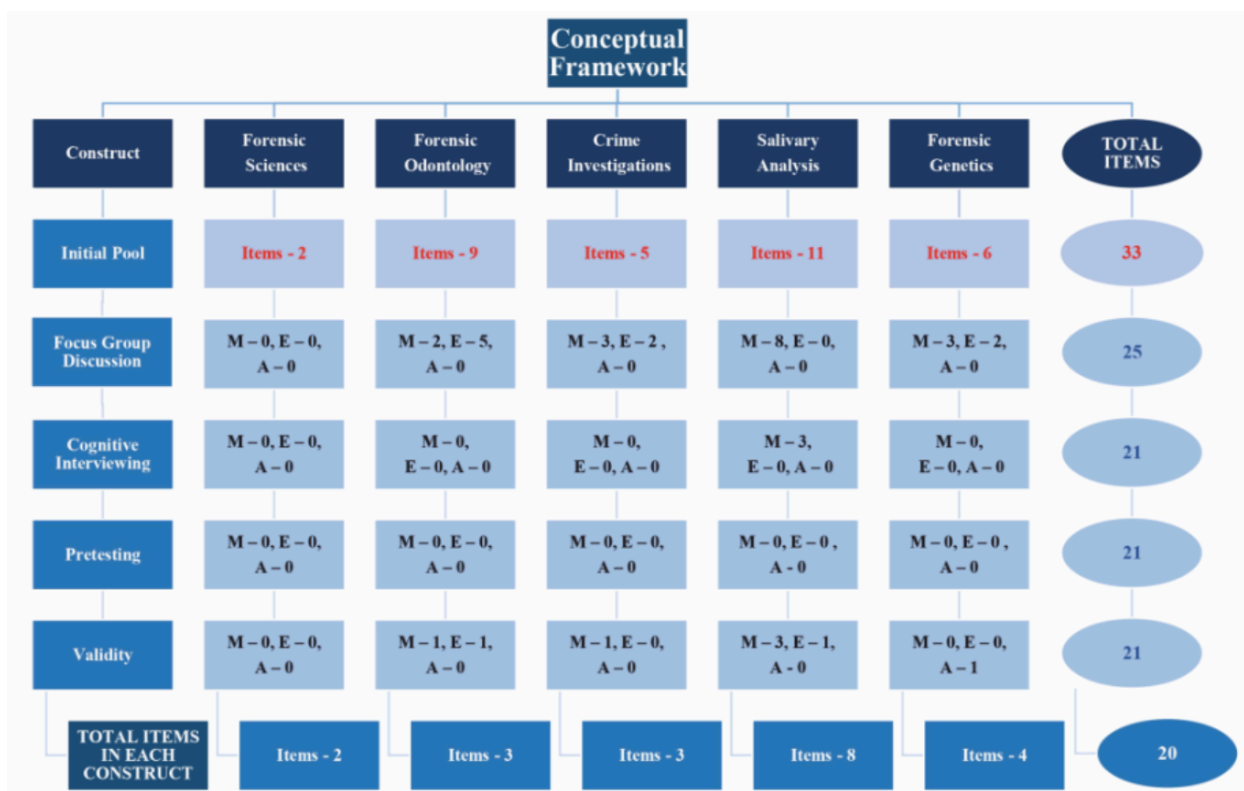
The subject experts reviewed the scale to assess the relevance, clarity and comprehension of the item.¹¹ A three-membered expert team, including an oral and maxillofacial pathologist, an oral medicine and radiology specialist and a senior forensic expert, validated the items' accuracy and representativeness. Each item was scrutinised, accepted and validated as excluded or to be modified, respectively. The recommendations for each item were recorded eventually and modified accordingly (Figure 1).^{8,10}

Reliability Testing

The consistency over time was assessed through internal consistency (Cronbach's alpha) and test-retest reliability (kappa). The reliability of the questionnaire was evaluated through face-to-face interviews conducted with 50 undergraduate clinical dental students (Table 2) who were unaware of the planned second test beforehand. About 10–14 days interval was planned between the baseline and the experts' second test (Figure 1).¹⁰

Figure 2. Conceptual Framework and items developed

This figure illustrates the number of items developed at each stage followed by the number of items modified (M), retained (R), added (A) and eliminated (E) at each stage.



RESULTS

Phase 1: Formation of a conceptual framework

Five main constructs, forensic sciences, forensic odontology, crime investigations, salivary analysis and forensic genetics, comprised the conceptual framework (Figure 2).

Phase 2: Systematic development of an item pool

The initial pool consisted of 33 items under the five broad constructs from the strongest to the weakest. The forensic sciences construct had two items related to basic knowledge of forensic science. The second construct had nine items on the basic knowledge of forensic odontology. The third construct, crime investigation, comprised five items related to the investigation of the crime scene. A maximum of 11 items were a part of the salivary analysis construct, which assessed the knowledge of the role of saliva in forensic odontology. Finally, the forensic genetics construct consisted of six items on the role of genetics in forensics (Figure 2).

Phase 3: Refinement of the item pool

Focus Group Discussion

Questions were discussed for each of the 33 items, and options were framed under the five constructs. Some items were eliminated, whereas some others were modified. At the end of FGD, 25 items were retained (Figure 2, Table 1).

Cognitive Interviewing

Undergraduate clinical dental students from various colleges were involved in cognitive interviewing. Each item was refined using the ‘Think Aloud Protocol’ and verbal probing. Although none were removed, the items were slightly modified by rephrasing the options for close-ended questions. For example, an option of ‘don’t know’ was added to some of the ‘yes/no questions’. Three items were modified, and the total number decreased to 21 items (Figure 2, Table 1).

Pretesting

A sample of 20 clinical undergraduates who volunteered to participate in the study were pretested, and the items remained unchanged (Figure 2, Table 1).

Table 1. Items involved in Salivary Analysis in Forensic Evidence (SAFE) scale
 This table shows the additions, modifications and eliminations of items involved during the development of this scale

	Added	Modified	Eliminated
Focus Group Discussion		<ul style="list-style-type: none"> - Dentists have role in person/victim identification in the event of mass disaster - Forensic odontology helps in identifying victims - Forensic odontology can help in person/victim identification (options) - Saliva can be used as an evidence (and its options) - Salivary analysis is a method to narrow down the suspects - In a crime scene where all could saliva usually be deposited (options) - Where all saliva could be identified as evidence (and its options) <ul style="list-style-type: none"> - Saliva can be recovered from skin - Saliva to detect recent drug abuse - Sex determination in identification using saliva - Types of DNA that can be isolated from Saliva - Salivary biomarkers play role in forensic genetics 	<ul style="list-style-type: none"> - Any postgraduate program in India offering Forensic Odontology - Dentists eligible to appear in court to present as evidence following the principle of forensic odontology - IPC section under which this fall - Maintaining dental record in your clinic - Duration of the records to be maintained - Maintain dental records at institutional level - Maintain dental records as a private practitioner - RNA markers in identified in saliva
Cognitive Interviewing	-	<ul style="list-style-type: none"> - Salivary analysis is a method to narrow down the suspects (Options – Crime detection, Drug abuse, Sexual Assault, Genocide, Suicide) - Where all saliva could be identified as evidence (Options – On the victim, crime scene evidence, sewer) - In a crime scene where all could saliva usually be deposited (options – Lip prints, Bite Marks, Chewing Gum/left over food, Clothes and surfaces.) 	-
Pretesting	-	-	-
Validity Testing	<ul style="list-style-type: none"> - It is possible to identify RNA markers from Saliva 	<ul style="list-style-type: none"> - Do you think forensic odontology has any role in dentistry? <li style="padding-left: 20px;">If yes, are you aware of a sub speciality as forensic odontology? -Do you think dentists can help in analysis of crime (in place of victim identification)? - In your opinions can individuals (in place of victims) be identified with forensic odontology? <li style="padding-left: 20px;">- Salivary analysis is a method to narrow down the suspects (Option - Child abuse in place of genocide) <li style="padding-left: 40px;">- Can saliva be collected from skin (Option - PHADEBAS test)? - Do you know the mechanism by which drugs end up in saliva? 	<ul style="list-style-type: none"> - Duration of records to be maintained - Where all saliva could be identified as evidence (Options – On the victim, crime scene evidence, sewer)

Phase 4: Validity

The scale was validated by three experts, whose comments were consolidated, discussed and duly incorporated into the scale. None of the

items were stated as ‘inappropriate’, and an item was added (Figure 2, Table 1).

Phase 5: Reliability

The test-retest reliability analysis showed a substantial level of agreement ($\kappa = 0.79$) and a statistical significance at $p \leq 0.05$. The internal consistency, measured with Cronbach's alpha, showed an agreement value of 0.816, indicating a good internal consistency (Figure 2).

DISCUSSION

Nowadays, the rising occurrence of both man-made and natural disasters highlights the critical need for accurate identification of individuals, particularly when bodies are severely decomposed or deliberately dismembered. Thus, comparing post-mortem and ante-mortem records is essential to determining a person's identity.¹⁴ A strong knowledge of this field is important for dental professionals.¹⁵ Recently, saliva analysis has emerged as a valuable diagnostic tool, offering an alternative to blood and urine and plays a significant role in victim identification during mass disasters.^{3,16} Saliva consists of 99% water and 1% solids, which include inorganic substances such as chloride, calcium and potassium, as well as organic compounds such as proteins, vitamins, hormones and amino acids. Saliva is common at crime scenes on various surfaces, including skin, food, clothing and other objects and is essential for linking individuals to criminal activities such as homicide, assault, abuse and substance misuse.¹⁶

SAFE is a new scale developed and validated to evaluate the knowledge and awareness of saliva's role in forensic odontology. The steps recommended by Vaughn et al.⁶ are crucial in directing the development process. Hitherto, studies have assessed knowledge and awareness using local inventory questionnaires. The major focus of the present study is on dental surgeons and undergraduate students' knowledge.

Two expert panellists from the Indian Academy for Clinical and Dental Genetics participated in the FGD. These experts were selected based on their extensive experience of a decade, which equipped them with in-depth knowledge of the role of saliva in forensic odontology.

The FGD, cognitive interviewing and pretesting was conducted on undergraduate clinical dental students selected for their limited knowledge of the role of saliva in forensic odontology, thereby deeming them an ideal group to gain valuable insights about the study.

A Senior Forensic Assistant from the Crime Branch with over six years of experience in forensic odontology was involved during the validity testing phase. Additionally, the expert served as a former senior resident in the Department of Forensic Odontology. The other two experts, each with over 15 years of experience, were from the Department of Oral and Maxillofacial Pathology and Microbiology and the Department of Oral Medicine and Radiology, respectively (Table 2).

The concept validity of the role of saliva in forensic odontology has been created and assessed in this study. The existing literature was beneficial during the development of an item pool that was further improved via FGDs. Although time-consuming, engaging diverse viewpoints from different groups is essential during the development process. This increases the scale's content validity by ensuring the consistent features of the trait. A panel of specialists evaluated each item according to its significance and implications, thereby improving the scale's comprehensiveness and removing any potential bias.

The test-retest reproducibility was used to gauge reliability, and most of the items' showed a kappa value of 0.8, indicating a substantial level of agreement. The internal consistency measured using Cronbach's alpha showed an agreement value of 0.816, indicating good internal consistency.

Importantly, the scale can be used for large-scale data collection. Experts from various fields participated at each stage of development, which strengthened the scale. The verbal probing in cognitive interviewing helped the participants to clarify the intent of the item without being presumptuous. The varied FGDs provided an in-depth perspective of the target audience.

Nevertheless, the scale has some limitations. It might be unable to capture nonverbal cues. The lack of context could lead to assumptions and misinterpretations. Also, due to the COVID pandemic, eliciting clarifications from online FGD participants would have been challenging.

To the best of our knowledge, this is the first scale developed to assess the knowledge and awareness about the application of saliva in forensic odontology. This scale can be further modified for medical and allied health science students and forensic experts, emphasizing the role of saliva in various forensic applications.

However, the potential role of saliva in forensic application is yet to be considered. Thus, further

studies are required to assess the awareness and knowledge of dental undergraduate students.

Table 2. Data of participants involved in Salivary Analysis in Forensic Evidence (SAFE) scale
This table displays information about the participants involved in the development of this scale, including their designations and the number of individuals in each group.

S. no	Participant	Designation	No of Participants
FOCUS GROUP DISCUSSION			
1	Expert Panelist	Founder, Indian Academy for Clinical and Dental Genetics and Founder and Chairman, International Saliva Summit of India (SALSI)	1
2	Expert Panelist	Assistant Editor, Genomeden e-magazine, Indian Academy for Clinical and Dental Genetics and Founder	1
3	Target Audience	Undergraduate Clinical Dental Students	4
COGNITIVE INTERVIEWING			
4	Target Audience	Undergraduate Clinical Dental Students	6
PRETESTING			
5	Target Audience	Undergraduate Clinical Dental Students	25
VALIDITY TESTING			
6	Expert Panelist	Ex. Senior Resident, Dept of Forensic Odontology, Current Designation: Senior Forensic Assistant, Crime Branch	1
7	Expert Panelist	Associate Professor, Department of Oral and Maxillofacial Pathology and Microbiology	1
8	Expert Panellist	Associate Professor, Department of Oral Medicine and Radiology	1
RELIABILITY TESTING			
9	Target Audience	Undergraduate Clinical Dental Students	50

CONCLUSION

The SAFE scale is a valid and reliable scale that involves all the possible constructs to assess the knowledge and awareness of the role of saliva in forensic odontology.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval for the survey was obtained from the Institutional Ethics Committee (Ref. No. CSP/22/MAR/106/74).

Participants was informed about the purpose and benefits of the survey and were invited to participate voluntarily. Informed consent was

obtained for each participant who was assured of data confidentiality.

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AUTHORS' CONTRIBUTIONS

All authors have made substantial contribution in the conception of the scale. FM was involved in conceptualisation, methodology, data collection, analysis and drafting of the manuscript. RS was

involved in conceptualisation, review of the study, revising of the manuscript critically and given final approval of the version to be published. AG was involved in conceptualisation, methodology, revising of the manuscript critically and given final approval.

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