

Efficacy of Natural Dye (Indigo blue), Lysochrome Dye (Sudan black) and Fluorescent Dye (Nile blue A) in Cheiloscopy as a Lip Print Enhancer in Forensic Odontology: A Cross-sectional Study

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ABSTRACT

Introduction: The study of lip prints is known as cheiloscopy. Lip prints are unique for each individual, which plays a key role in suspect identification. These imprints obtained on any surface may be in visible or latent form. Latent forms of lip prints are difficult to identify; hence various dyes are used to enhance the latent lip prints obtained from different surfaces. The present study has included the both porous and non porous surfaces. It is essential to determine how the surface of an object effects the enhancement efficiency of latent lip prints with different dyes.

Aim: To compare the enhancement efficacy among of Indigo dye (Natural dye), Sudan black (Lysochrome dye) and Nile blue A (Fluorescent dye) on porous and non porous surfaces, assessing their applicability in cheiloscopy.

Materials and Methods: The present cross-sectional study was carried out on 120 individuals. All the subjects were students from Vishnu Dental College, Bhimavaram, Andhra Pradesh, India. The Lip print collection completed within six months which was started in July 2022 and ended in January 2023. Individuals with age group of 18-25 years were considered. After obtaining

informed consent, subjects were asked to make an imprint on the given surfaces like porous and non porous (white and red colour surfaces). They were enhanced immediately by applying Indigo blue, Sudan black and Fluorescent dye for evaluating the clarity of lip grooves. Chi-square test was used to compare the enhancement efficiency among three dyes. The p-value<0.05 was considered as statistically significant.

Results: Fluorescent dye has better enhancement than the indigo dye and lysochrome dye with the significant p-value 0.001. When lysochrome dye and Fluorescent dye compared the results were not statistically significant (p-value=0.012). When compared between non porous and porous surfaces better enhancement was observed on porous surfaces predominantly by Fluorescent dye.

Conclusion: The present study revealed the usefulness of latent lip print in individual identification in forensic dentistry. Fluorescent dye gives a better positive result than Indigo blue, but when compared with lysochrome dye there was no obvious significance during enhancement.

Keywords: Chemical methods, Criminal investigation, Latent lip prints, Person identification, Porous and non porous surface

INTRODUCTION

According to Keiser-Nielsen, forensic dentistry is defined as the "proper handling and examination of the dental evidence, in the interests of justice, so that the dental finding may be properly presented and evaluated" [1]. Forensic odontologists play an important role not only in determining age and gender but also in identifying an unknown deceased person in a mass disaster; identification of the dead bodies or remains of the dead body is very important. The conventional methods for identifying a person include anthropometry, fingerprints, sex analysis, age estimation, height measurement, blood group, Deoxyribonucleic Acid (DNA) analysis, and odontology. To demonstrate the uniqueness of stomatological structures like teeth imprints, rugae, and lip prints as biometric tools, forensic odontologists have recently become technologically sophisticated and active in digital bite marks and lip print analysis [2]. The study and evaluation of lip prints based on the pattern of grooves of labial mucosa are termed cheiloscopy. It is a Greek word; cheilos means lip, and skopein means to observe [3].

Analysis of lip prints is helpful in estimating gender, race, and identification of suspects in the crime. Searching for imprints at a crime scene is one of the essential features in the criminal investigation [4]. They were commonly found in cases of sexual assaults, robberies, murders etc., and prints could be left on various objects, e.g., drinking glasses, bottles, cups, cigarette butts, tissues or napkins etc., [5]. They were available in two forms, visible and invisible (latent) forms. Visible lip prints are usually formed by lipsticks that contain oil, waxes, and colour pigments. Traces of these lipsticks are typically left behind on glass surfaces and cloth materials, which do not require any further development for their visualisation. Latent lip prints occur due to the oils released by the sebaceous glands mixed with saliva, which can be transferred to several surfaces which are difficult to identify and undetected until they are enhanced with physical or chemical methods [6]. They could be present on different surfaces, like porous and non porous surfaces with varied colours. They can be enhanced by using the materials that are most commonly used for fingerprint identification, such as aluminium powder, silver metallic

powder, silver nitrate powder, lead carbonate, lysochrome dyes and Fluorescent dyes etc., [7,8].

In the literature, no comparison has been observed among these three dyes; hence this study aimed to compare the efficacy of indigo dye, lysochrome dye and Fluorescent dye on different surfaces, which includes porous, non porous surfaces with varied colours. Null hypothesis of the study was there is no difference in efficacy of Natural dye (Indigo blue), Lysochrome dye (Sudan black) and Fluorescent dye (Nile blue A) on porous and non porous surfaces, whereas alternate hypothesis was there is significant difference in efficacy among these three dyes on porous and non porous surfaces.

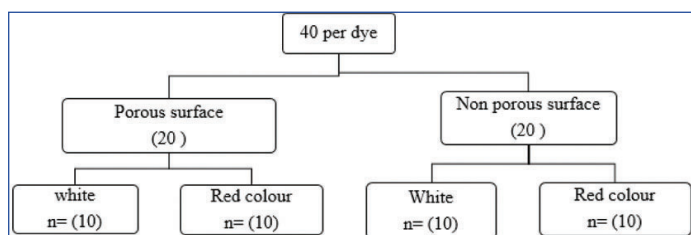
The aim of the study was to compare the enhancement efficacy among natural dye, lysochrome dye and Fluorescent dye on different surfaces. Primary objective of the study is to identify the most suitable dye for detecting the latent lip prints on porous and non-porous surfaces and also to evaluate the clarity of the lip prints.

MATERIALS AND METHODS

The present cross-sectional study was carried out on 120 individuals. All the subjects were students who were selected by random sampling method from Vishnu Dental College, Bhimavaram, West Godavari district, Andhra Pradesh, India. An institutional ethical committee approved the present study bearing project number IECVDC/2021/PG01/OP/IVV/04. The Lip print collection completed within six months which was started in July 2022 and ended in January 2023. The informed consent was obtained from all the participants.

Inclusion and Exclusion criteria: Individuals belonging to the age group of 18-25 years, visible and latent prints to be taken from same individual. Subjects with a history of severe trauma to lips and those who are associated with any lip pathologies were not included in the study.

Sample size calculation: Sample size was calculated using g power version 3.1.9.2, based on efficacy of different dyes as lip print enhancer as a primary outcome, with an alpha level of 0.05, power of the study 80%. The total estimated sample size was 120 participants, with 40 individuals in each of the three main groups. Each main group was then divided into two subgroups of 20 participants, based on nature of surface type (porous and non porous). Finally, each of these subgroups was further divided into two groups of 10 participants, based on surface colour (white and red coloured surface) [Table/Fig-1].



[Table/Fig-1]: Sample size presentation.

Materials used:

1. Natural dye (powder) - Indigo blue (Robin blue - Fabric whitener)
2. Lysochrome dye (powder) - Sudan black B (Fisher Scientific, Mumbai)
3. Fluorescent dye (powder) - Nile blue A (Avra Pvt., Ltd., Hyd)
4. Camilin hair brush
5. Porous surface: Paper cups -white and red coloured surface.
6. Non porous surface: Ceramic cups-white and red-coloured surface.

Here red surface was considered because it provides a high contrast between the lip print and the background, making it easier to visualise and analyse the print.

Study Procedure

Collection of patent lip prints: Asked the individuals to clean their lips with saline-soaked gauze, dried with sterile cotton, and then covered with coloured lipstick; advised them to gently press their lips onto a given surface to record the print, which was later compared to latent lip prints obtained by using Fluorescent, lysochrome and indigo dye for clarity of lip grooves and outline.

Collection of Latent lip prints: The lips prints were taken on ceramic and paper cups as well as on respective white and red coloured surfaces. After the collection of all the samples, a camel hair brush was used for the application of all three chemicals individually by powder and dusting method [9]. The application of dye was continued until the print became clearly visible. Two observers examined the latent lip print separately and compared it with the patent lip print for grooves and lip outline using a parameter of good (++), fair (+), and poor (-) to avoid inter-observer bias. Grading of lip prints based on clarity of the grooves/fissures given by Singh NN et al; detailed in the [Table/Fig-2] [10].

Grading	Features
Good (++)	Both the outlines of the lip as well as lip grooves are clearly visible.
Fair (+)	Clearly visible lip outline with less clarity of lip grooves.
Poor (-)	The outline can be noticed, but lip grooves cannot be appreciated.

[Table/Fig-2]: Grading of lip prints based on clarity of lip grooves.

STATISTICAL ANALYSIS

The data collected will be subjected to statistical analysis using IBM Statistical Package for Social Sciences (SPSS) version 24.0. Chi-square test was used to compare the proportions between three different dyes. The p-value<0.05 was considered statistically significant for all the comparisons.

RESULTS

When results are tabulated and analysed with the Chi-square test for the efficacy among all the three dyes, statistical significance has been obtained with the p-value of <0.001 [Table/Fig-3].

Name of the dye	Good	Fair	Poor	Total
Indigo dye	5	20	15	40
Lysochrome dye	16	19	5	40
Flourescent dye	25	13	2	40
p-value	<0.001*			
Chi-square value (χ^2)	27.37			

[Table/Fig-3]: Comparing enhancement efficiency among three dyes. Statistical test applied using chi-square test. *Denotes p-value significance

The results showed better latent lip print enhancement in Fluorescent dye predominantly, followed by lysochrome and indigo dye. When indigo dye compared with lysochrome and Fluorescent dye statistical significance was observed with p-value of 0.005 and 0.001, respectively [Table/Fig-4,5]. When lysochrome dye and Fluorescent dye compared the results were not statistically significant (p-value=0.012) was observed [Table/Fig-6]. When compared between non porous and porous surfaces better enhancement was observed on porous surfaces predominantly by Fluorescent dye

Name of the dye	Latent lip prints			
	Fair	Good	Poor	Total
Indigo dye	20	5	15	40
Lysochrome dye	19	16	5	40
p-value	0.005*			
Chi-square value	10.78			

[Table/Fig-4]: Comparing enhancement efficiency among indigo dye and lysochrome dye. Statistical test applied using Chi-square test. *Denotes p-value significance

Name of the dye	Latent lip prints			
	Fair	Good	poor	Total
Indigo dye	20	5	15	40
Fluorescent dye	13	25	2	40
p-value	0.001*			
Chi-square value	21.987			
[Table/Fig-5]: Comparing enhancement efficiency among indigo dye and Fluorescent dye. Statistical test applied using Chi-square test. *Denotes p-value significance.				

Name of the dye	Latent lip prints			
	Fair	Good	poor	Total
Lysochrome dye	19	16	5	40
Fluorescent dye	13	25	2	40
p-value	0.112			
Chi-square value	4.765			
[Table/Fig-6]: Comparing enhancement efficiency among Lysochrome dye and Fluorescent dye. Statistical test applied using Chi-square test. *Denotes p-value significance				

followed by lysochrome and indigo dye with statistically significance with p-value<0.001 using Chi-square test [Table/Fig-7,8].

Name of the dye	Good	Fair	Poor	Total
Indigo dye	3	8	9	20
Lysochrome dye	8	10	2	20
Flourescent dye	12	7	1	20
p-value	<0.001*			
Chi square value (χ^2)	98.2			

[Table/Fig-7]: Comparing the enhancement efficiency among three dyes on porous surface.

Statistical test applied using Chi-square test; *Denotes p-value significance

Name of the dye	Good	Fair	Poor	Total
Indigo dye	2	12	6	20
Lysochrome dye	8	9	3	20
Flourescent dye	13	6	1	20
p-value	<0.001*			
Chi-square value (χ^2)	100.48			

[Table/Fig-8]: Comparing the enhancement efficiency among three dyes on non porous surface.

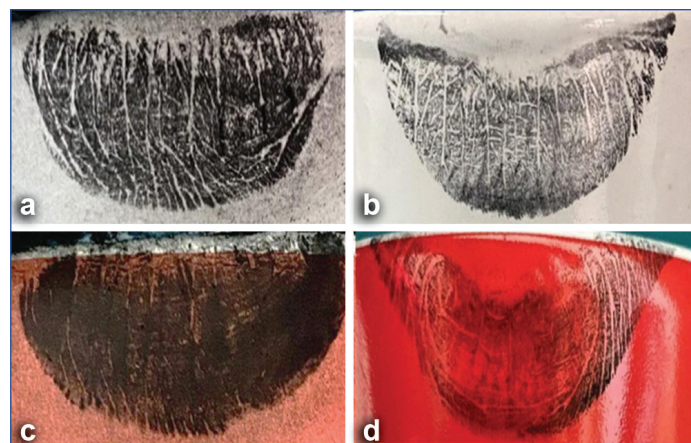
Statistical test applied using Chi-square test; *Denotes p-value significance

DISCUSSION

Building an individual's biological profile involves personal identification, age estimation, and sex determination. Personal identification includes DNA profiling, fingerprint examination, dental identification, and lip prints. Although DNA profiling is one of the most reliable methods, this may be time-consuming and expensive. Lip prints, however, are unique for individuals and easier to identify. The lines and fissures that form wrinkles and grooves on the lips between the inner labial mucosa and outer skin are known as lip prints. It has already been proven by a group of Spanish investigators in their study that lip prints are as reliable as fingerprints in a criminal investigation, especially during the absence of other means of identification [11,12]. The first investigation where lip prints were used for identification was observed on an envelope [13]. Foreign Beaurau of Investigation (FBI) first used lip prints during a robbery investigation where they collected from a glass door and matched it to a suspected robber [14]. Collecting both visible and latent prints is vital for positive forensic evidence [15]. The quality of lip prints is based on the lip outline and grooves imprinted on the surfaces, which can quickly evaluate by the examiner. When the efficacies of dyes are compared, most studies classify and evaluate them based on their appearance as good, fair, and poor [8,10,16]. Similarly, the

current study has also used this as a method of evaluation of the dyes for developing latent lip prints. Latent lip print enhancement depends on the biochemical reaction between lipid residues and the dyes used. They are not visible and require some enhancement for their development and evaluation. Additionally, with the development of persistent lipsticks which do not stain the surface of contact, no colour prints are left behind. Several physical and chemical methods, including dyes have been used to enhance and identify these latent lip prints. Over the years, the most commonly used materials have been fingerprint powder, lysochrome dyes, fluorescence dyes, and natural dyes. The conventional fingerprint powder, including silver metallic powder, red, white and black metallic powder, can be effective when the lip prints are dry [17]. It is crucial to determine how the surface of an object effects the efficiency of lip prints with various chemicals. To determine how each dye develops latent prints on several surfaces, the present study has included both porous and non porous surfaces. Porous surfaces are absorbent in nature, permit the flow of water, fluid, or vapour, whereas non porous surfaces do not absorb moisture and often appear polished. Indigo dye is a natural fabric whitener that contains Indigotin. Natural dyes are less expensive than other chemicals like lysochrome and Fluorescent dyes. Some of these include vermilion and indigo dye. Indigo dye is a fabric whitener that acts as a natural dye and is readily available. Vermilion is an opaque orangish red pigment. But particles are coarse in nature which leads to uneven dispersion; hence didn't trace minor details of the latent lip prints [16]. In comparison among indigo dye, vermilion, and Sudan black, Singh NN et al., observed that vermilion and indigo were better than Sudan black [10]. They also observed that these dyes were free-flowing and did not stick to the object where the lipstick was not evident. In the present study, indigo dye in comparison to lysochrome and Fluorescent dyes, the results showed that better lip enhancement was seen predominantly by Fluorescent dye, followed by lysochrome and indigo dye. In contrast to these results, a study conducted in 2016 comparing the efficacy of Indigo, Sudan III, and Aluminium powder concluded that Indigo dye showed significant results for visible and latent lip prints [8]. In addition, a study by Singh NN et al., proved that the indigo dye exhibits the same efficacy as lysochrome dyes on different surfaces, including cups and fabrics [10]. It is also essential to know that of all the samples, most of them are under the category of fair. Lysochrome dye is a dye that can dye fatty acids. These dyes act via dissolving upon contact with fat (lyso part), and the chrome part produces colour to show the print patterns. Sudan III, Oil Red O, and Sudan black are the most common [18]. As all lip prints contain lipids, thereby can be used to enhance latent prints. However, sometimes these prints may not be visible due to the lack of contrast between the dye and the surface. Of the three most commonly used dyes, Sudan black has known to produce better enhancement than Oil Red O and Sudan III. Based on this, Sudan black has been included in the present study. Castello A et al., documented that these compounds efficiently locate and develop both recent and older prints [18]. Navarro E et al., studied the effectiveness of several lysochrome dyes on human skin and have shown a positive development concerning shape and lip outline, along with grooves and wrinkles [19]. Fluorescent dyes adhere and chemically react with lipids on the lip prints. When the Fluorescent reagents usually used for fingerprint development were tested on latent lip prints, it was observed that they showed a medium-quality print for up to two months. The main advantage of Fluorescent dyes is that these dyes are more suitable on multicoloured surfaces. It has been studied that Nile red is a very efficient enhancer of latent lip prints. Kumar P et al., conducted a comparative study between Fluorescent and lysochrome dyes and found that Fluorescent dyes showed better results, with around 200 samples showing positive development with both the dyes but with a better appreciation of the patterns using Fluorescent dyes [20]. The overall results comparing the efficacy of indigo, lysochrome, and Fluorescent dyes

for lip print enhancement have shown that the best enhancement is by the Fluorescent dye followed by lysochrome dye and lastly, indigo dye. It is also crucial to note that when the indigo dye was compared with lysochrome and Fluorescent dyes, a statistical significance of 0.005 and 0.001 was observed, respectively. When the porous and non porous surfaces were compared, porous surfaces showed better enhancement, especially by Fluorescent dye, followed by lysochrome and indigo dye, with statistically significant results using Chi-square test. Through this study, it has been observed that different surfaces with different colours require specific dye for enhancement, hence null hypothesis was rejected. To understand this better, these three dyes including Indigo, Sudan III and Nile blue, have been used in both porous and non porous surfaces, which were in both white and dark colours. This has helped analyse the appropriate dye for each sample. It has been observed that on white porous surfaces, predominant grading was good, followed by fair and poor. Although this did not show statistical significance. Fluorescent dye displayed the best enhancement, followed by lysochrome and indigo dye. However, the coloured porous surface has shown very little difference between Fluorescent and lysochrome dye lip print enhancement. Indigo dye has shown poor results in the enhancement of coloured porous surfaces [Table/Fig-9]. These results show that the predominant grading was fair, followed by good and poor, with a statistical significance. The latent lip print enhancement on the coloured non porous surface has shown predominant fair grading, followed by good and poor with no statistical significance. Like other results, the Fluorescent dye has shown better enhancement overall, followed by lysochrome and indigo dye. Even on non porous white surfaces, the Fluorescent dye has shown better lip print enhancement in comparison to lysochrome and indigo dye [Table/Fig-10,11]. In addition, the grading on these



[Table/Fig-11]: a) Latent lip print enhanced by Fluorescent dye on white porous surface (Paper cup); b) Latent lip print enhanced by Fluorescent dye on white non porous surface (Ceramic cup); c) Latent lip print enhanced by Fluorescent dye on red porous surface (Paper cup); d) Latent lip print enhanced by Fluorescent dye on red non porous surface (Ceramic cup).

surfaces was also similar to non porous coloured surfaces with no statistical significance. Although the current study has compared the efficacy of several dyes on different surfaces, it is crucial to note that this study has to be expanded to more types of surfaces. This enables us to understand the efficacy of different dyes on different surfaces for better practical application.

Limitation(s)

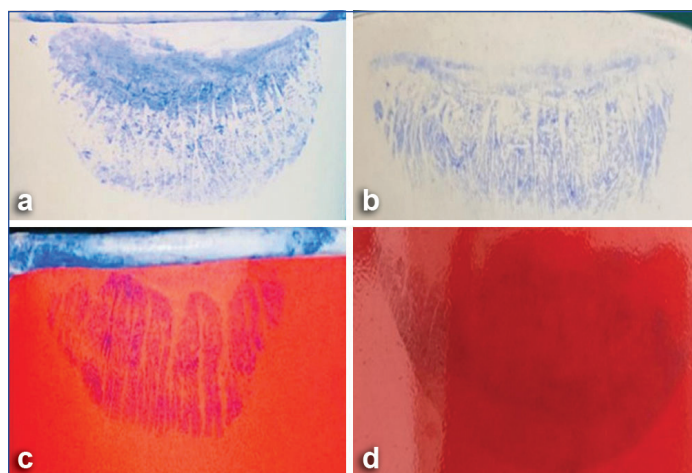
Obtaining lip prints is highly technique sensitive which depends on factors like the application of pressure, methods used for making prints. Apart from this, the quality of the latent prints is influenced by various environmental conditions, including temperature and humidity. As there is no standard technique for latent print enhancement, future research should address these limitations to improve the validation and quality assurance.

CONCLUSION(S)

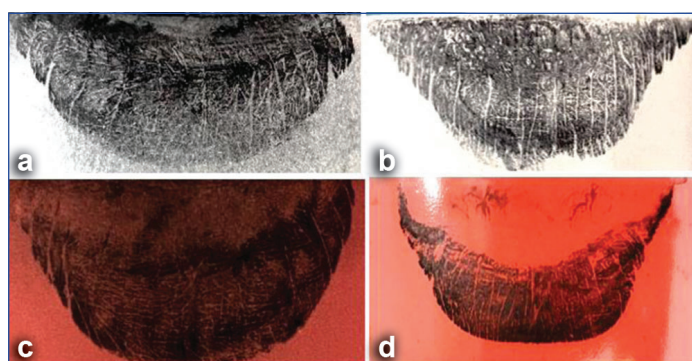
In this study, we have observed that both lysochrome and Fluorescent dye exhibit better enhancement even though the print is older than ten days. To validate the results, additional research on dark and multi-coloured surfaces with a larger sample size is required. Cheiloscopes protocols could be established with more relevant contributions, and this identification approach could be successfully and frequently used in practice.

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[Table/Fig-9]: a) Latent lip print enhanced by Indigo dye on white porous surface (Paper cup); b) Latent lip print enhanced by Indigo dye on white non porous surface (Ceramic cup); c) Latent lip print enhanced by Indigo dye on red porous surface (Paper cup); d) Latent lip print enhanced by Indigo dye on red non porous surface (Ceramic cup).



[Table/Fig-10]: a) Latent lip print enhanced by Lysochrome dye on white porous surface (Paper cup); b) Latent lip print enhanced by Lysochrome dye on white non porous surface (Ceramic cup); c) Latent lip print enhanced by Lysochrome dye on red porous surface (Paper cup); d) Latent lip print enhanced by Lysochrome dye on red non porous surface (Ceramic cup).

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