

MICROSCOPIC COMPARISON OF HAIR IN THE HUMAN BODY

By

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A thesis submitted in fulfilment of the requirements for the degree of
Master of Forensic Science (Professional Practice and Research)

in

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Murdoch University

Academic Supervisor: Associate Professor James Speers

Semester 2, 2019 and Semester 1, 2020



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Declaration

I declare that this thesis does not contain any material submitted previously for the award of any other degree or diploma at any university or other tertiary institution. Furthermore, to the best of my knowledge, it does not contain any material previously published or written by another individual, except where due reference has been made in the text. Finally, I declare that all reported experimentations performed in this research were carried out by myself, except that any contribution by others, with whom I have worked is explicitly acknowledged.

Signed: Jaipratavi Bahadur Jung Chettri

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Part One

Literature Review

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Abstract

Hair is one of the characteristics present in humans and it possesses several functions such as protection against external factors, producing pheromones and apocrine sweat and also thermoregulation ¹. It is also found in crime scenes as physical evidence. Hair recovered at a crime scene often denotes that there has been some physical contact between the victim and the perpetrator which could have possibly occurred as a result of a serious or violent offence ⁵. After the hair is recovered with the appropriate procedures and submitted to the laboratory it can help provide compelling evidence placing the suspect at the scene of crime ⁵. The examination procedure explored in this study will include macroscopic and microscopic. This type of analysis has its own drawbacks which will be explored in detail as well as ways to prevent and eradicate issues such as bias and carelessness of the examiner. One of the main topics focused will be regarding the detailed comparison of the characteristics of the cuticle, cortex and medulla. Lastly, samples will be selected without the examiner's knowledge of their source and identification of the source will be made based on the characteristics of the samples.

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List of Abbreviations

RCMPFL: Royal Canadian Mounted Police Forensic Laboratory

NAS: National Academy of Sciences

DNA: Deoxyribonucleic acid

mtDNA: Mitochondrial DNA

FBI: Federal Bureau of Investigation

PCAST: President's Council of Advisors on Science and Technology

DOJ: Department of Justice

ENFSI: European Network of Forensic Science Institutes

SWGMAT: Scientific Working Group on Material Analysis

1. INTRODUCTION

There are two essential parts of human hair, the hair follicle and hair shaft. The hair follicle is responsible for the generation of hair and the hair shaft is the hair fiber that is seen above the skin which consists of the cuticle, cortex and medulla ¹. The hair follicle has a growth cycle where it grows, stops to grow occasionally and starts again. The growth cycle has three stages; Anagen, Catagen and Telogen phase ¹. During the Anagen phase, the hair follicle generates the entire hair shaft from root to tip whereas in the Catagen and Telogen phase the follicle resets and the stem cells prepare to receive signals in order to start the next cycle ².

1.1 Growth cycle of the hair follicle

Anagen Phase

This phase is also known as the growth phase as the complete growth of the hair shaft occurs in this phase ³. It begins by the hair follicle growing and extending deeper into the skin ³. Additionally, a hair bulb grows at the base of the hair follicle which then grows dermal papilla cells that initiate the growth of a new hair shaft ³. If the hair shaft from the previous growth cycle has not already been shed, then the growth of hair shaft in the current cycle helps push out the previous hair out of the follicle ³. This phase has a rapid rate of cellular growth compared to other tissues in the body ³. The rate of growth regarding scalp hairs in this phase is approximately half an inch per month which lasts for four to six years ³.

Catagen Phase

This phase is also known as the regression phase where the hair follicles start to shrink, and the growth of hair stops ³. The follicle bulb degenerates and moves towards the dermis and

away from the capillaries which were surrounding the bulb whereas the blood supply to the upper area of the follicle and sebaceous glands remain unharmed ⁴. The hair shaft now becomes susceptible to being shed even from normal grooming activities and bathing ³. This phase lasts approximately two to three weeks ³.

Telogen Phase

This phase is also known as the resting phase where the hair follicles stop shrinking, the follicle becomes inactive and the hair shaft continue to shed ³. This stage generates the most naturally shed hair which can usually be found in clothing, bedding and comb/brush ^{3,4}. This phase is complete when mitotic activity begins in the basal cells of the lower follicle which creates cells that surround the dermal papilla and the new Anagen phase starts ⁴.

1.2 Different layers of the hair follicle

Hair grows out of the organ known as the hair follicle and it extends from the root or bulb ⁵. The part that we can see growing out of the skin is known as the hair shaft. The hair shaft is composed of three different layers which are the cuticle, cortex and medulla ⁵.

Cuticle

The cuticle is the outermost part of the hair and it is well known for its resistance to chemical decomposition and its ability to retain its structural features for an extended period ⁵. It contains overlapping scales which are formed due to the keratinised cells progressing from the follicle ⁵. A fully developed cuticle surface is hard and protects the cortex from wear and tear but eventually, the cuticle is also damaged over time due to weathering, combing, washing and abrasion with other hair shafts ⁴. The cuticle is formed from a single layer of cells in the

follicle and the cuticle cells form a pattern called scale pattern which can be visualized under a microscope ⁴. The damage caused to the cuticle causes the scale edges to become irregular and develops the irregular pattern seen under a microscope ⁴. The scale patterns on the hair shaft can be analysed by first making a cast of the surface which can be done by embedding the hair in a soft medium such as clear nail polish or softened vinyl ⁵. After the medium has hardened, it can be viewed under a microscope to examine the scale patterns ⁵.

Cortex

The cortex is the part of the hair that is embedded with pigment granules which are responsible for hair colour ⁵. The shape, colour and distribution of the granules are of great importance as they are used as points of comparison when comparing hair of different individuals ⁵. Examination of the structure of the cortex can be microscopically undertaken using a mounting medium which has a refractive index similar to the hair which helps in reduction of reflected light from the surface of the hair and increase in the amount of light penetrating the hair ⁵.

Medulla

The medulla is created as a column of cells that produce a protein which contains amino acid citrulline ⁴. During its formation, the cells collapse, and they appear as a network of cells with spaces and gaps ⁴. The medulla is not always present in human hair, especially in very fine hair ⁴. Moreover, the medulla can be either continuous throughout the hair shaft (excluding the root and tip) or discontinuous ⁴. In cases where the medulla is discontinuous, it may be broken at irregular intervals by the cortical materials which are also known as interrupted medulla or it may be present in small amounts irregularly in the cortex which is also known as fragmented

medulla⁴. When it comes to the visualisation of the medulla, light microscopy can prove not to be useful especially if the mounting agent has taken the place of the air present in the intercellular and intracellular gaps⁴. In cases where the mounting agent does not fill the gaps, the medulla can appear dark which leads to very little detail being observed and it can also lead to the medulla being mistaken for pigmentation⁴. Polarised light can prove to be more useful and more structural details can be seen when using a mounting medium⁴. The function of the medulla still seems to be unclear in human hair but Kassenbeck has speculated that its function is to maintain the diameter of the hair without producing excess weight by using minimal resources⁴.

1.3 Human hair comparison methodology

The techniques of comparing human hair have not undergone any drastic changes in the past few decades. In order to perform a comparison, a questioned sample or unknown sample of hair is compared side by side with a known sample of hair using a comparison microscope⁶. There are various features compared which include color, structure, cuticular traits, and acquired characteristics⁶.

1. Color: This includes the hue of the hairs and also the pigment density, distribution, aggregation, size, shape and color^{6,7}.
2. Structure: This includes shaft characteristics such as its diameter, cross-sectional shape, medullation, cortical structure, and the presence or absence of aberrations in the shaft^{6,7}.

3. Cuticular traits: This includes the thickness, colour, clarity and also the appearance of the inner and outer cuticular margin ⁷.
4. Acquired characteristics: This includes any chemical treatments done on the hair such as dyes, bleach and also any abnormalities and cleanliness of the hair shaft ^{6,7}.

The best-case scenario outcome of this microscopic examination would be one or more characteristics being similar which would lead to an association being identified between the questioned and known sample ⁶. In general, the forensic hair examiner would come to one of three conclusions:

1. The hair samples match: This would be the case where there is evidence of similarities between the questioned and known samples which suggests that a possible association exists ⁶.
2. The hair samples do not match: This would be the case where there is evidence of dissimilar characteristics between the questioned and known samples. Additionally, any significant features that are not common between the two samples would also strongly suggest that the source of the hairs being examined are not the same ⁶.
3. No conclusion can be drawn: This would be the case where there is not enough visual evidence provided by the hair shaft, and it is not possible to make a judgment on the correspondence of the hair samples.

1.4 Process of hair examination by microscopy

As stated by Sir Edmund Locard in 1930, whenever there is contact between two objects, a transfer of materials will occur between those two objects ⁴. Those materials can be

categorized as trace evidence which has the potential to identify the objects and location ¹¹. Due to the nature of such trace evidence, there is a high risk of contamination and cross-transfer while processing the evidence which is why maximum care should be taken in order to prevent this ¹¹. For the recovery of hair samples from a crime scene, various techniques can be applied such as scraping, shaking, taping and picking from clothing or bedding items ¹¹. To recover samples from a large carpeted surface, it could be more efficient to use a vacuum cleaner that contains a filtered system to filter the hair separately for the ease of obtaining the hair samples ¹¹. An appropriate method should be chosen with regards to the type of surface from where the samples are being obtained for minimal contamination ¹¹.

1.4.1 Creating a Cast

In some cases, it might prove useful to make a cast of the hair follicle in order to visualize the scale pattern under a microscope ¹¹. In order to create a cast, a polaroid film-print coater can be used. This method was discovered by Ogle and Mitosinka in 1973 ¹¹. Next, a thin layer of the fluid is applied to a microscopic slide, and the hair sample is lightly pressed in the fluid until it is dry ¹¹. The hair can then be pulled out which leaves a cast of the scale pattern on the fluid ¹¹.

Another method was created by Crocker in 1998, which utilises a clear tape instead of a mounting medium which is then placed on a microscopic slide ¹¹. This process also helps visualise the scale patterns under a microscope ¹¹. Lastly, one more mounting medium that can be used to create a cast is nail polish. The hair simply needs to be embedded on a glass

slide containing a thin layer of nail polish upon which a scale pattern is left once the fluid dries and the hair is pulled out ⁵.

1.4.2 Sampling method

After the collection of hair evidence is complete it is vital to select the appropriate number and types of hair to use for the analysis ¹¹. In cases where the hair is collected in a crime scene with the use of a special vacuum cleaner, the filter should be inspected, and only representative samples should be collected ¹¹. This would mean that hair of different length, colour, racial group, and body area should be collected or in cases where the questioned or known samples are present, hair samples similar in appearance to those should be obtained ¹¹. The selection of hair samples can start at the crime scene as well or in the laboratory during the processing of obtained samples ¹¹. The characteristics of the hair are viewed under a microscope and selected with the intention of providing the examiner with a good range of the hair types present ¹¹. Among the different regions of the body, head and pubic hair exhibit the most characteristics useful in an examination ¹¹. Nevertheless, the collection of standard samples from an offender can take a few months or even years after the hair samples are collected from the scene of crime ¹¹. This would mean that the standard samples could have undergone changes and might not have as many similar characteristics to the samples collected when the crime first took place ¹¹. Some experts have identified that two hair samples from the same person might not be possible to identify as the same if they are collected after a one-year gap ¹¹. Moreover, various environmental factors or cosmetic changes could make the time gap even shorter ¹¹. Although, it has been found that pubic hair retains their characteristics longer than any other hair of the body ¹¹.

1.4.3 Slide preparation

Hair shafts can be prepared for microscopy by mounting them in a mounting medium which has a similar refractive index to that of the hair ^{5,11}. Having similar refractive index is crucial for the optimum visualization of the hair shaft ^{5,11}. In order to position the hair on the glass slide, a small amount of mounting medium needs to be applied on the slide after which the hair can be placed ¹¹. For long hairs, a figure-eight can be made with the hair so that the examiner can analyse the full length of the hair ¹¹. More than one hair can be analysed in one glass slide at the same time as long as the hair shafts are not overlapping with each other and obscuring the visualisation under microscope ¹¹. Any excess mounting medium can be removed using a piece of blotter paper ¹¹. Finally, after applying a few drops of the mounting medium on top of the hair, another glass slide is used to cover it ensuring that no air bubbles are formed ¹¹. It is also vital to ensure that the hair is adequately cleaned to visualise maximum detail; nonetheless, if the hair is covered in blood or any other liquid, the examiner should keep in mind that it might be of evidentiary value before cleaning it ¹¹.

1.4.4 Microscope

The quality and reliability of the examination can be directly proportional to the quality and reliability of the microscope in use ¹¹. Proper care should be taken for maintenance and cleanliness of the microscope such as preventing dirt/dust, fingerprints and other contaminants from affecting its performance ¹¹.

1.5 Past studies conducted on hair analysis

The entire basis of hair analysis is formed on the fact that humans shed their hairs daily which can easily be transferred from one individual to another or from an individual to the crime scene and vice versa ⁸. Humans normally lose about 75-100 scalp hairs in one day and this number is even greater if the person is involved in a struggle or if the hair has been pulled on during the occurrence of a crime ⁴. The hair evidence recovered from such incidents can be analyzed and can provide useful insights into the activities that occurred at the scene of a crime. There have been various studies conducted in the field of hair analysis to understand the evidential value of hair and to what extent it can be used to prove or disprove different theories and hypotheses.

1.5.1 Gaudette and Keeping (1974)

In one of the earliest studies regarding the accuracy of microscopic analysis of head hair for identification purposes, Gaudette and Keeping proposed in the year 1974 that the chance of finding a difference between two hairs from the same person is 1 in 4500 ⁸. They stated that the false positive rate in hair analysis is 1 in 4500. In this study, the authors used one examiner to do over 350,000 comparisons which resulted in only nine pairs of hair that could not be identified ⁹. This study had three major components:

- 1) Obtaining 6 to 11 head hairs each from 100 different individuals
- 2) Comparing the hair samples to identify the pairs of hair with less than four differences
- 3) Comparing the pairs of hair obtained from step 2 to analyse if the hair samples can be differentiated

However, their study has been heavily criticised by Barnett and Ogle who said that the probabilities shown in the study were unreliable ⁸. Additionally, the two authors also mentioned that the study did not include the chance of asserting a match between two dissimilar hairs which is their true negative rate⁸. Moreover, Barnett and Ogle raise a few concerns about the study such as the absence of any objective basis of selecting the hair samples. They base this claim on a statement given by the authors of the study who said that the hair samples were not chosen randomly from the population but were selected so that the probability of two hairs being similar would be much greater ⁸. The criticism also went on to talk about statistical analysis which were questionable and there was a possibility of examiner bias ⁸. The scientific community strongly criticized the study by Gaudette and Keeping (1974) and the strongest criticism was that they only examined hair from different individuals but not hairs from the same individual ⁹. Regarding the examiner bias mentioned by Barnett and Ogle, the 1990 study published by the Royal Canadian Mounted Police Forensic Lab also mention that the low false-positive rate portrayed in the 1974 study could have been due to examiner bias ⁹. The examiners performing the study were already familiar with the fact that all of the hair samples being examined had come from different individuals which could have caused them to be more inclined towards searching for differences even if two samples looked very much alike ⁹. The examiners were fully aware whether the hair samples came from the same person or not which could have led to an unconscious bias of the results ⁶.

1.5.2 Gaudette (1978)

In the follow-up study conducted by Gaudette, he made a revelation regarding their 1974 study and its inaccuracy. In the 1978 study, three trainee examiners were hired who had completed

a full year of training in this area⁹. The false-positive rate for identification regarding pubic hair was estimated to be 1 in 800¹⁰. The examiners were given one hair sample (pubic) which were different from each other and also a set of 100 reference samples⁹. The examiners were not told that the reference samples were from different people and the one hair sample given to each of them should ideally come up with only one match among the 100 samples⁹. As a result, the first two examiners came up with one match, but the third examiner got four matches which in total would be three correct and three incorrect matches⁹. This showed that the false positive rate in this study proved to be 1 percent which is 400 times higher than the rate predicted by the authors in their 1978 study⁹. The author responded to the criticism by eliminating examiner bias in this study which gave very different results. Although, the hair samples used in both the 1974 and 1978 studies were Caucasian which would mean that the statistics calculated in these studies would only apply to Caucasians and not to the population from different ancestries⁶. The authors Wickenheiser and Hepworth from the 1990 paper still believed that Gaudette did not address the problem of examiner bias appropriately in his second study¹². Gaudette used the same elimination criteria used in everyday casework but the examiner in that study still knew that any indistinguishable hair would not be associated correctly¹². In order to fix the problem of examiner bias, an unknown number of potentially similar hairs should have been added¹². Moreover, more than one examiner should be involved in the study which would provide a wider perspective as the two or more examiners would have found characteristics at different levels would also contribute to the validity of the findings in study¹².

1.5.3 Royal Canadian Mounted Police Forensic Laboratory (1990)

Two authors from the hair and fibre unit of the RCMPFL used a similar study design to that of Gaudette and Keeping 1974 study. The only difference was that they used two examiners to examine all the hairs ⁹. As a result, the authors found 'non-repeatability' and 'non-reproducibility' among examiners ⁹. Non-repeatability meant that among the two examiners, there was a certain amount of variation in the way they classified the features of the hair sample ⁹. Similarly, non-reproducibility was when the two examiners classified the same hair differently ⁹. The most notable finding from this study was that even though the examiners did not find any similarities between hair samples from different individuals, they also did not find any consistent similarities between hair samples from the same individual in most cases ⁹. Ideally, this study should have resulted in the examiners finding a match for 15 hair samples, but they ended up finding only two ⁹.

1.5.4 Wickenheiser and Hepworth (1990)

Wickenheiser and Hepworth also conducted a study that was solely focused on addressing examiner bias. The results from the study showed that the examiners did not make any incorrect associations ⁸. However, heavy criticism was received by these authors because they only used two examiners for a study associated with examiner bias ⁸. A study that has used only two examiners cannot accurately assess the amount of bias for a large group of examiners ⁸. The authors attempted to replicate the work of Gaudette and Keeping (1984) in this study while making improvements such as trying to eliminate examiner bias ⁶. A sorting procedure was also developed where obviously dissimilar hairs were eliminated to avoid a microscopic comparison ¹². The sorting procedure had two difficulties in which if it was too strict, two potentially similar hairs could have been disregarded and if the procedure were too general

then a large number of hairs would pass this step and the study would not be completed in a reasonable amount of time ¹². It was crucial to creating a procedure that would be strict enough to disregard dissimilar hairs and general enough to allow any similar hair to proceed to the microscopy stage ¹². In the end, the examiners selected 13 categories for the characterisation of the hair samples ⁶.

HAIR CLASSIFICATION									
Colour:		White	Light Brown	Dark Brown	Red				
		Yellow	Medium Brown	Red Brown	Black				
MAJOR CHARACTERISTICS									
Treatment		Texture		Pigment Distribution		Medulla			
None	0	Smooth	1	Absent	0	Absent	0		
Dyed	1	Streaky	2	Uniform	1	Continuous/Opaque	1		
Bleached	2	Very Streaky	3	Peripheral	2	Continuous/Translucent	2		
Curled/Permed	3	Granular	4	About Medulla	3	Continuous/Opaque/Translucent	3		
Other	4			Unilateral	4	Interrupted/Opaque	4		
				Clusters	5	Interrupted/Translucent	5		
						Interrupted/Opaque/Translucent	6		
						Fragmentary/Opaque	7		
						Fragmentary/Translucent	8		
						Fragmentary/Opaque/Translucent	9		
Medulary Index		Maximum Diameter		Cortical Fusi					
Non Applicable	0	Fine <0.04mm	1	Absent		0			
<0.250	1	Med 0.04-0.08mm	2	Present - Root		1			
>0.250	2	Coarse >0.08mm	3	Present - Shaft		2			
SECONDARY CHARACTERISTICS									
Length		Cuticular Margin		Tip					
<2.5 cm	1	Smooth	1	Natural Taper		1			
2.5 - 7.5 cm	2	Slightly Serrated	2	Recent Cut		2			
7.5 - 15 cm	3	Serrated	3	Rounded		3			
15 - 30 cm	4			Rounded Frayed		4			
>30 cm	5			Split		5			
				Crushed		6			
Pigment Density		Pigment Size		Shaft					
Absent	0	Absent	0	Constant		1			
Sparse	1	Fine	1	Slight/Smooth/Variation		2			
Light	2	Medium	2	Wide/Smooth/Variation		3			
Medium	3	Large	3	Slight/Abrupt/Variation		4			
Heavy	4			Wide/Abrupt/Variation		5			

Figure 1: Primary and Secondary characteristics used for analysis by the examiners in Wickenheiser and Hepworth study ¹².

Among the 13, there were seven primary characteristics and six secondary characteristics ⁶. The primary characteristics were treatment, texture, pigment distribution, medulla, medullary index, maximum diameter and the presence of cortical fusi whereas the secondary characteristics were length, cuticular margin, tip characteristics, pigment density, pigment size, and shaft diameter variation ⁶. Two hair samples were concluded as a match if all of these characteristics were in common between the two samples ⁶. As a result, none of the hair samples was incorrectly identified as a match with another different hair sample ⁶. In conclusion to this study, there was not a proper representation of the range of microscopic features available in hair ⁶.

1.6 National Academy of Sciences report on hair analysis

The National Academy of Sciences (NAS) is a private, non-profit organisation consisting of scholars who are involved in scientific and engineering research ⁸. The main aim of this report was to make an agenda for progressing forensic science and its disciplines ⁸. The NAS report is considered to be an essential document because it elaborates on the flaws and drawbacks of scientific disciplines used in forensic science. Additionally, it also creates an opportunity for various research to be done and paths that can be followed to improve on the flaws that exist in the disciplines of forensic science. This report also includes a section that discusses the current status as of 2009 regarding hair analysis and its drawbacks. Additionally, one of the

essential recommendations provided by the NAS report is that more research and validation studies need to be conducted in various areas of forensic science, including hair analysis¹³.

There are various physical characteristics in the recovered hair evidence that can prove useful in identifying similarities with the questioned hair sample⁸. However, these physical characteristics can only be used to the extent of eliminating the hair sample from a group of samples that also have a likelihood of being similar to the questioned sample⁸. These are also known as class characteristics where several features are similar but are not enough to definitively prove two samples alike. This report emphasizes on the theory that identification of physical characteristics by itself cannot identify a person but can only narrow the possibilities by excluding some samples⁸. In comparison to all current forensic methods available, nuclear DNA has surpassed all those methods as it is the only method available that can provide, with a great level certainty, a connection between a questioned sample and a specific source¹³.

One of the methods used by examiners to narrow the possibilities of a sample includes identifying the part of the body where the hair originated⁸. The NAS report states that suspects can be eliminated by using microscopic methods to identify the origin of the hair on the human body. This can prove to be efficient when dealing with a large number of samples, but it can also lead to corroborative evidence being discarded. Without the use of DNA analysis, the sample can only be proved to be dissimilar with the questioned sample, but even though the hair originated from a different part of the body, there is still a chance of it originating from the same human being. Nevertheless, an examiner should be able to identify the class characteristics which would raise suspicion on the hair as it might be from a different part of

the body, but it could have originated from the same individual. Another characteristic that can prove to be important is hair colour that has been applied artificially ⁸. An artificially coloured hair can provide more robust information than natural hair as the added artificial colour brings into account additional variables such as the time since treatment, chemicals used and also the natural hair colour of the individual ⁸. Such information can provide additional assistance in a case and aid in the elimination of any false positive hair samples.

During the process of analysis, a control sample or a group of control samples need to be retrieved from a known hair source ⁸. When it involves head hair, samples should be collected from the top, front, sides and the back, including the nape area ⁸. The collection method should be a combination of combing and pulling and a total of 50 hairs should be obtained. In regard to a pubic or any other sample from a somatic region, a total of 25 hairs is highly recommended by this report ⁸. Even though it is still possible to conduct the analysis without achieving the target number of samples, a less number would increase the probability of an incorrect exclusion of a sample ⁸.

One of the current problems with human hair analysis is the lack of characteristics that help in individualisation ⁸. The microscopic characteristics analysed are generally accepted but other characteristics such as physical which include refractive index and density have been questioned and deemed unreliable ⁸. The NAS report also states that microscopic techniques alone are not enough and the findings from those analyses should be confirmed by mtDNA analysis ⁸. In a study conducted by the Federal Bureau of Investigation (FBI) showed that out of 80 hair comparisons, 12.5% of the results yielded false positive ⁸. This shows the high error

rate and indicates that it is not enough to make a conviction based on the microscopic technique alone.

The process of using DNA analysis on hair samples has significantly reduced the need for microscopic hair analysis⁸. A great majority of cases involving hair samples have known to contain useable DNA material years after the commitment of the crime⁸. Even though the hair sample may only contain mitochondrial DNA, they are still more likely to provide robust evidence compared to a microscopic examination⁸. The NAS committee consisted of prominent scientists who were provided with 1.5 million U.S. Dollars for the project which took over two years and generated a 300-page report¹³. This committee, when talking about microscopic hair analysis, mentioned that there is no evidence to support those microscopic methods alone are enough for individualisation⁸. The researchers involved in the NAS report have also estimated that due to the high accuracy and quality of DNA evidence, microscopically analysed hair evidence can become less utilised in the future as a source of evidence⁸. However, microscopic methods can still be used to narrow the possibilities which can proceed on to the DNA method of analysis which can provide individualization of the sample⁸.

1.7 President's council of advisors on science and technology report on hair analysis

The President's council of advisors on science and technology (PCAST) is an advisory group of scientists and engineers appointed by the president of the United States that gives advice regarding science and technology. The president also consults with them while making policy changes in regard to science and technology⁹. The research scientists involved in the group of advisors reviewed over 2000 publications which were submitted by the forensic community¹⁵.

Additionally, the group also consulted with nine current and former federal judges, a former U.S. Solicitor General, a former state Supreme Court justice, two deans of law schools, and two statisticians with forensic science expertise ¹⁵. This report explores any additional steps that can be taken to ensure the validity of evidence used by the legal departments in the United States ⁹. This report was published in 2015, which is six years after the NAS report was published. Hence, the PCAST report tries to explore the flaws that still have not been improved after the NAS report exposed them. The PCAST committee has outlined two essential areas that need to be worked on which are:

- 1) Clarity on the scientific standards for the reliability and validity of forensic methods.
- 2) Evaluation of various methods and protocols in forensic science to verify if they are scientifically valid and reliable.

Before the publication of the PCAST report, the Department of Justice (DOJ) had released a document concerning the validity and reliability of forensic hair analysis ⁹. The document by the DOJ stated that hair comparison by microscopy is a reliable method, but it should not be used for individualisation purposes ^{9,14}. The characteristics of a hair cannot be used as a basis for personal identification ^{9,14}. The DOJ has also made a conclusion that if a questioned hair sample is found to be similar to a known sample which can be verified as not being the source, then the microscopic examination cannot be at fault ⁹. This demonstrates the limitations of the science that there is an unknown group of people present from where the hair could have originated ⁹. However, the PCAST report has identified this statement as being disingenuous ⁹.

Additionally, when an expert witness informs the jury that the questioned hair sample is microscopically indistinguishable from the known sample, the expert and the prosecution intend for this statement to carry weight⁹. Nonetheless, the DOJ document continues to say that no information is available about individuals with similar hair characteristics⁹. Moreover, another concern raised about the DOJ document is that there are implications made that there is no empirical evidence about the accuracy of hair analysis⁹.

A 2002 study conducted by the FBI is considered to be a landmark in forensic science because it comprehensively analysed a large group of cases to measure the rate of false-positive associations⁹. Mitochondrial DNA was analysed to re-examine 170 samples on which the FBI had conducted hair examinations⁹. The findings showed that 11 percent of samples which were found to be microscopically indistinguishable had actually originated from different individuals as found by the mt-DNA tests⁹. The conclusion drawn by the FBI were of great significance to forensic science, police, courts and juries because it was found that when hair examiners state in their report that a hair sample is microscopically indistinguishable, 1 in 9 times the hair actually come from a different source⁹. The DOJ report, on the other hand, did not include this finding but instead used it to support their claim on using microscopic hair comparison alongside with DNA testing⁹. They made a misleading statement involving the FBI study and mentioned that out of 80 associations made by microscopy, 88 percent of those were included by additional DNA tests⁹. The document did not mention that the rest of the samples were found to be false associations which could sway the jury about the origin of those hair samples⁹.

In the reports submitted to the PCAST, there were flaws in almost all of them regarding their designs and out of those reports only one related to the work conducted by a forensic examiner in preparation of a trial ¹⁵. The results obtained from that report was quite unpleasing as it showed that there is an 11% false identification rate ¹⁵. Moreover, it was uncovered that invalid expert testimony was given in 95% of the cases that were reviewed ¹⁵.

Following the publishing of the PCAST, there was a particular concern raised regarding the conclusions provided by them ¹⁵. Prosecutors accused that the conclusions drawn by the group were invalid as there was significant research that had been left out from their report ¹⁵. Various forensic science disciplines including bite marks, firearms, complex DNA mixtures and shoe prints lacked scientific foundational support and it would be incorrect to be admissible in a criminal courtroom ¹⁵. Following the allegation that PCAST had left out numerous research and scientific evidence, they issued a notice requesting the submission of any research studies that were allegedly left out ¹⁵. As a result, they did not receive any submissions following the notice that they put out ¹⁵.

Additionally, the Department of Justice also stated that they did not have such studies to submit ¹⁵. Nonetheless, the conclusions reached by the PCAST group are significant and it represents an essential aspect in the field of science ¹⁵. Similarly, courtrooms should also take this report's claims with seriousness ¹⁵. Lastly, it was concluded by the report that evaluations of feature-comparison methods are foundationally valid only in regard to latent fingerprint comparison, single-source DNA and simple mixed DNA analysis ¹⁵.

2. Issues and possible research gaps regarding hair analysis

The hair comparison studies submitted to the PCAST in 2015 had major flaws in their design ¹⁵. These flaws have the potential to affect the interpretation of the results received from the analysis. One of the studies submitted had shocking results that showed an 11% false identification rate which was the number of times hair examiners had wrongly associated the hairs which belonged to different people ¹⁵. Moreover, in a study conducted by the DOJ, it was also found that hair examiners had given scientifically invalid testimony in 95% of the cases reviewed ¹⁵. Moreover, one of the biggest research gaps exists in comparison of hair between different parts of the body, as shown in section 3.1. There is also a lack of studies and research in the field of hair analysis as there is data available regarding the characteristics of hair but no studies comparing the structure of the hair from different parts of the body of the same person using microscopic analysis ²². These are examples of some of the many issues regarding hair analysis. Following are such similar issues and possible research gaps that are still faced by hair analysis which critically affects the interpretation of the results.

2.1 Lack of foundational Validity

In order to consider a method or discipline scientifically valid, it needs to be considered foundationally valid, and this is essential in hair analysis because the methods used during the examination of hair must be valid in order to be admissible in a court of law ¹⁵. A method or discipline can be foundationally valid if it has undergone empirical testing by multiple groups under conditions where it is intended to be used ¹⁵. Additionally, the method should be repeatable and reproducible. A method is considered to be repeatable if the examiner can get the same results while examining the same samples, whereas the method is considered reproducible if different examiners have similar results for the same samples ¹⁵. Moreover,

there should also be valid estimates of accuracy for the techniques used which would demonstrate the frequency in which an examiner comes to an incorrect conclusion ¹⁵. The authors of the PCAST report have two different ways in which an examiner can establish foundational validity. In cases of single-source DNA analysis, the foundational validity can be established using published research done on the different steps of the DNA analysis procedure that demonstrates its accuracy and reproducibility, but this is not the case for the analysis of mixed DNA profiles ¹⁵. Since mixed DNA profiles require human judgement, the only way to establish scientific validity is by using black-box error rate studies ¹⁵. The black box error rate analyses the validity of a method by measuring how often an examiner gets correct results by applying the appropriate techniques ¹⁵. There is a great importance of foundational validity because neither experience nor good judgement and professional work, such as certifications and accreditations, standardized protocols, proficiency testing and code of ethics, can act as a substitute for foundational validity ¹⁵.

2.2 Cognitive Bias

Bias is one of the major concerns in hair examination because it has the ability to change the outcome of the analysis. It can mislead and guide the examiner in a different direction which would hinder the accuracy and reliability of the examination. There are various forms of bias that are found in this process.

2.2.1 Motivational Bias

Simply stated, this type of bias occurs when a person favours one side over the other. Fred Zain fits this category of bias due to his misconduct in the state crime laboratory of West

Virginia ¹⁸. An investigation into him revealed that he would almost always favour the prosecution and whenever in doubt he would state that the suspect is guilty ¹⁷. Moreover, even after his relocation from West Virginia laboratory to a different state, he would still receive evidence from prosecutors in West Virginia for retesting ¹⁷. This shows the effect of a bias driven outcome whereas a laboratory should not have any bias at all and most importantly cannot favour the prosecution over defence or vice-versa.

2.2.2 Role Effect Bias

This is a type of cognitive bias where the motivation in bias is subconscious ¹⁷. In other words, a person's perception of his/her role can affect their decisions and cause a biased outcome. There have been cases where the forensic examiners saw themselves as a part of the attorney's team and though they were the prosecution witnesses when in fact they were just scientists ¹⁷. Furthermore, in 1997, an Inspector General of the Department of Justice issued a report on the FBI laboratory's explosive unit stating that their report had various faults including inaccurate testimony, insufficient documentation of results, and testimony beyond the competence of the examiners ¹⁷. The Inspector-General concluded that the report by the FBI had relied on speculation and titled the document in a way that would support the incrimination of the defendant ¹⁷.

2.2.3 Contextual Bias

This is another type of cognitive bias where any extra information tends to influence the decision-making process ¹⁷. An example of this is when an FBI examiner identified a substance

as being related to explosives based on the fact that there were pieces of detonation cord found in the garbage outside the house ¹⁷.

2.2.4 Confirmation Bias

This is another type of cognitive bias, and it refers to the tendency of looking for instances that confirm the hypothesis rather than deny ¹⁷. This was the case in FBI's misidentification of fingerprints in the Madrid terrorist train bombing investigation. In a later review by the FBI, it was found that the unique automated fingerprint correlation system had an influence on the examiner's judgment and examination that followed ¹⁷. The misidentification was also confirmed by three other experts. Moreover, the review conducted on the FBI was not a blind review and the reviewer knew that a positive identification had already been made which also made him prone to confirmation bias ¹⁷.

2.2.5 Reconstructive effects

Lastly, this is another type of cognitive bias when the examiner relies on his/her memory which causes them to fill in gaps with what they believe should have happened when their recollection is vague ¹⁷. This issue was mentioned in the Inspector General's report on the FBI where he claimed that the examiner could be influenced by protocol requirements while relying on his/her memory that would cause the documentation of what they thought should have occurred when the memory of it is not intact ¹⁷.

2.3 Measures to help eliminate the issues regarding hair analysis

One of the significant issues related to hair analysis is the influence of bias in the examination. The use of appropriate methods and scientific practices such as blind testing, use of precise

measurements, standardised procedures, statistical analysis can assist in controlling the problem of bias in an examination ¹⁷. Additionally, more critical procedures that need to be followed are:

- i) Proper documentation of laboratory analysis in the case files. The lack of laboratory notes by the examiner had been a significant issue on the FBI explosives unit where there was lacking documentation in case files ¹⁷.
- ii) The laboratory notes should be recorded simultaneously with the examination; otherwise, there is a potential of reconstructive effects bias from the examiner ¹⁷.
- iii) The protocols given to the examiner should only contain relevant information and protect them from causing contextual bias ¹⁷.
- iv) A comprehensive report should be submitted detailing the methods and procedures used, as well as the results found in the examination. There have been cases where only a short statement had been provided regarding the results and no mention of the methods used and whether they were foundationally valid ¹⁷.
- v) The results of the examination should contain a description of the significance of any finding. There have been instances in DNA exoneration cases where the forensic experts gave misleading testimony by leaving out important information ¹⁹.
- vi) The forensic experts should also be restricted from providing testimony beyond the report submitted ¹⁷. This will prevent the prosecutors from using the expert to draw conclusions that were not intended by the expert. For example, if a suspect's hair was found at the crime scene and no one else's, the prosecutor should not use this information to reach to a conclusion that the crime has been committed by the

suspect as the hair could have gotten there for several other reasons. The expert can only confidently state that the hair evidence found at the crime scene is consistent with the hair samples from the suspect.

Furthermore, the proper imposition of these procedures can be achieved through accreditation ¹⁷. Currently, the American Society of Crime Lab Directors/Laboratory Accreditation board conducts quality assurance programs which include proficiency testing, technical reviews, corrective action procedures and audits which play an enormous role in the enforcement of such procedures mentioned above ¹⁷. Additionally, in support of this, the NAS report had also recommended accreditation of laboratories and the certification of the examiners ^{13,17}.

2.4 Procedural bias experiment

The process of examining human hair starts from obtaining the hair samples from the suspect and submitting them to the hair examiners. The samples submitted are a known sample and a questioned sample for comparison purposes along with a synopsis of facts regarding the investigation ²⁰. The synopsis usually contains information regarding other evidence collected, eyewitness information, any confessions made by the suspect which induces a perception to the police personnel involved in the case ²⁰. They generally have very little or no doubt with respect to the guilt of the suspect as they obtain ample evidence for a conviction ²⁰. This notion can also be passed on to the hair examiner, which causes a potential for bias regarding the guilt of the suspect which can influence the results of the hair examination ²⁰.

2.4.1 Experiment hypothesis

It was speculated by Miller that forensic examiners are prone to be influenced by socialisation between themselves, the police or attorney requesting the examination and the situation they are requested to conduct the examination ²⁰. Therefore, it was hypothesised that the submission of more than one known hair sample for comparison purposes might increase the accuracy of the results and help eradicate the bias received by the examiner ²⁰.

2.4.2 Methods and results achieved

Fourteen students were used in this experiment. All 14 students who were enrolled in advanced crime laboratory courses were trained in human hair identification techniques which included 60 hours of lectures and 60 hours of laboratory practical experience under the supervision of qualified professionals in the field ²⁰. This qualified the students for providing expert testimony regarding human hair analysis in a court of law ²⁰. Next, 56 fictional cases were created for the experiment out of which the first 28 cases were prepared in the usual way that contained:

- i) Questioned hair evidence recovered from the scene of the crime
- ii) Known hair samples from one suspect
- iii) A brief synopsis of the case which only contained the fact that a certain crime had been committed and a suspect was brought in to custody.

The rest of the 28 cases included:

- i) Known hair samples of 5 different suspects
- ii) Questioned hair evidence recovered
- iii) A brief synopsis of the case

All of the hair samples from the 56 cases had similarities with the questioned samples which included characteristics such as pigmentation, scale patterns, colour, length and width.

Nevertheless, none of the questioned samples had the same source as the known samples. The 14 examiners were given 4 cases each where 2 of them were prepared in an initial way with questioned hair, known samples from one suspect and a synopsis whereas the only difference with the other 2 cases given to them was that it contained known hair samples from five different suspects instead of one. This was also referred to as the “line-up” procedure which required the examiner to analyse samples from five suspects rather than one ²⁰.

The results indicated a 16% of the conclusions given by the examiners were incorrect ²⁰. The incorrect examinations occurred 30.4% of the time when the examiners analysed the evidence using the primary method ²⁰. The incorrect examinations were 3.8% when the line-up procedure was utilised ²⁰. The correct examination rate was 83.7% and there were 7 cases where the results were given as inconclusive ²⁰. One of the most noteworthy outcomes of this experiment is that all of the seven inconclusive results were given with the use of the line-up procedure.

2.4.3 Critical analysis

In the process of human hair comparison an examiner can point out the similarities and differences of characteristics, but if the hair samples have a majority of similarities, the examiner is prone to overlook substantial differences which results in a subconscious bias ²⁰. Moreover, a preconceived notion of the questioned and known samples to have originated from the same source can impact the examiner’s judgment when the samples have few

similarities in their characteristics²⁰. The results from this experiment show that the procedure of submitting the hair evidence to the examiners has an impact on the conclusions provided from the analysis. There was definitely an increase in accuracy as there was an increased number of suspect known samples. Moreover, this also shows that the examiners were more indeterminate of the origin of the samples when there were more known suspect samples to compare. Even though an inconclusive result does not resemble accuracy, it is still a more relevant result than a false positive. This leaves room for more examination to occur and a more appropriate judgment to be made. Following the procedures of the line-up, method gives a more accurate decision as the initial method did not give any inconclusive results.

3. Examination of characteristics in hair

There is a wide range of microscopic characteristics that can be identified in hair. A complete hair sample will consist of the hair shaft and the root⁴. The initial examination can be done with low power magnification microscopy with the help of a stereomicroscope with magnifications up to $\times 40$ and $\times 50$ ^{4,21}. Following this, high powered magnification is used it can go up to $\times 60$ to $\times 100$ where a source of illumination is present such as in a transmitted light microscope^{4,21}. Optimal results for hair analysis can be achieved when examined using a ring light source as it will provide an even epi-illumination²¹. This will enable the examiner to identify whether or not the root is present, its shape and appearance, basic features in the hair shaft, the general appearance of the terminal end, any damage, disease and other features^{4,21}. It is also essential to create a checklist in order to encourage a detailed record of notes^{4,21}.

These are the characteristics used for analysis by the European Network of Forensic Science Institutes (ENFSI) in 2015.

FEATURE		Stereomicroscopy (Incident Light)	Transmitted Light Microscopy
General Characteristics	Colour - Described using colour chart, text, etc.	White, Blonde, Red, Brown, Black, etc.	Colourless (white), Blonde, Red, Brown, Black, etc.
	Cosmetic Treatment	Dye lines	Dyes, Styling products, Bleaches, Lighteners
	Shaft Form		Straight, Arced, Wavy, Curly, Twisted, Tightly coiled, Crimped
	Shaft Length (in mm / cm)		
	Shaft Thickness / Shaft Diameter	Fine, Medium, Coarse	Measured in μm
	Shaft Cross-section	Round, Oval, Triangular, Flattened	Round, Oval, Triangular, Flattened
	Shaft Form		Buckling, Convoluting, Shouldering, Undulating, Splitting, Regular
	Proximal End	Root presence / absence and Appearance	Root presence / absence and Appearance
	Distal End	Tip Appearance - Natural, Cut, Abraded	Tip appearance - Natural, Cut, Abraded, etc.
	Cuticle		Presence, Thickness, Margin features, Pigment
Cortex	Cortex Texture		Coarse, Medium, Fine
	Other Cortical Features		Ovoid bodies, Cortical fusi
	Pigmentation (in discrete granules)		Presence / Absence
	Pigment Size		Coarse, Medium, Fine
	Pigment Aggregation		Streaked, Clumped, Patchy
	Pigment Aggregate Size		Large, Medium, Small
	Pigment density		Absent, Light, Medium, Heavy, Opaque
	Pigment distribution		Uniform, Peripheral, One-sided, Random or variable, Central or medial, Pigment in cuticle, Banded
Medulla	Medulla Type	Presence & Appearance; Continuous, Discontinuous, Fragmented, etc.	Presence & Appearance; Continuous, Discontinuous, Fragmented, etc.
	Damage	Singeing, Crushing, Cutting, etc.	Singeing, Crushing, Cutting, etc.
Other	Special Characteristics	Post Mortem Banding, Lice, Mould, Fungal tunnels, Insect bite marks	Post Mortem Banding, Lice, Mould, Fungal tunnels, Insect bite marks
	Diseases		Pili annulati, Trichoschisis

Figure 2: Features used for the analysis of human hair by the ENFSI.

Whereas, the features recommended by the Scientific Working Group on Material Analysis (SWGMA) have provided a list of microscopic as well as macroscopic characteristics. This list was generated in April of 2005.

Macroscopic Characteristics

- Color
 - White, blonde, red, brown, black
- Structure
 - Shaft form
 - Straight, arced, wavy, curly, twisted, tightly coiled, crimped
 - Shaft length range in cm or in.
 - Overall shaft thickness
 - Fine, medium, coarse

Microscopic Characteristics

- Colour
 - Colourless (white), blonde, red, brown, black
- Natural pigmentation
 - Pigment size
 - Coarse, medium, fine
 - Pigment aggregation
 - Streaked, clumped, patchy
 - Pigment aggregate size
 - Large, medium, small

- Pigment density
 - Absent, light, medium, heavy, opaque
- Pigment distribution
 - Uniform, peripheral, one-sided, random or variable, central or medial, pigment in cuticle, banded
- Colour treatments
 - Dyes (permanent, semipermanent)
 - Temporary dyes (rinses, sprays, gels, mousses)
 - Bleaches or lighteners
- Structure
 - Shaft characteristics
 - Diameter range in micro meter
 - Cross-sectional shape
 - Round, oval, triangular, flattened
 - Shaft configurations
 - Buckling, convoluting, shouldering, undulating, splitting, regular
 - Medulla
 - Absent, continuous, discontinuous, fragmented, opaque, translucent, relative width, amorphous, other (doubled, tripled)
 - Cuticle
 - Cuticle
 - Present

- Absent
- Cuticle thickness
 - Thin, medium, thick
- Outer cuticle margin
 - Flattened, smooth, serrated, cracked, looped, irregular or other
- Inner cuticle margin
 - Distinct, indistinct
- Cuticle colour and clarity
 - Natural, pigment, dye
- Cortex
 - Cellular texture
 - Coarse, medium, fine
 - Ovoid bodies
 - Size, distribution, abundance
 - Cortical fusi
 - Size, shape, distribution, abundance
- Ends
 - Proximal ends
 - Root present
 - Telogen, catagen, anagen, sheathed, follicular tag, postmortem banding, putrid
 - Root absent

- Severed, decomposed, crushed
- Distal ends
 - Tapered tips (uncut), rounded or abraded, square cut, angular cut, frayed, split, crushed, broken, singed

3.1 Classification of hairs from different parts of the body

Most of the research done in this field have published data regarding the features of hair from different parts of the body, but a majority of the literature only concern the scalp, pubic and axillary region. This can also be considered as one of the significant research gaps in the field of microscopic hair examination since there is a minimal amount of research regarding hair characteristics and additionally there are no studies conducted regarding the microscopic comparison of the structure of the hair from different parts of the body ²². It is possible to classify hair from different body parts based on their size, structural variation in the follicles such as follicle density, size of follicular orifices, hair shaft diameter, volume and surface of the infundibula ²². The hair shaft diameter usually ranges from 16 to 42 μm depending on the area of the body with the highest diameter observed in the sural/calf area (42 μm) and the lowest in the forehead (16 μm) ²². The length of hair cycle of the different phases, anagen, catagen and telogen, also depends on the area of the body. For example, a complete cycle of the eyebrows takes an approximate of four months whereas the scalp hair takes three to four years to complete one cycle ²².

3.2 Classification of hair follicles

The human hair follicles can be further classified into two types: i) androgen-independent hair and ii) androgen-dependent hair/ hair on hormone-dependent areas of the body ²². The androgen-independent hair includes eyebrows and eyelashes whereas the androgen-dependent hair includes scalp, beard, chest, axilla and pubic regions ²². The hair shafts in androgen-dependent regions include terminal hair shafts that are long, more than 2cm, and thick, more than 60mm in diameter. These hairs are medullated except scalp hair where most of it does not contain a medulla ²². Apart from these regions, the rest of the body contains vellus hair which is also androgen-independent hair and they are usually short (less than 2cm), thin (less than 30mm diameter), unpigmented, and reaching only about 1mm into the dermis ²². The difference between vellus and terminal hairs is that vellus hairs are the non-pigmented and usually non-medullated short hairs that can barely be seen in the human body and it develops mostly during the childhood of the person ¹. Whereas, terminal hairs are the opposite, meaning that they are great and pigmented hairs that are found in abundance on the scalp, axillary, pubic regions and face (where the person is a male) ¹.

The information assisting the identification of hair from different body parts are given in detail below.

3.3 Scalp Hair

Every hair on the scalp grows about 1cm in a month for three to five years in the anagen phase ²². This is followed by a brief catagen phase and then the telogen phase, which lasts another two to four months which is when the shedding of hair is at its maximum ²². The hair is generally 60 to 80 μm in diameter and the outer layer of the cuticle consists of a flat layer of

overlapping scales which points outwards from the root to the tip of the shaft ²². Additionally, the total length of the follicle and infundibulum in terminal hair can be anywhere between 3259 μm to 4469 μm whereas in vellus hair 496 μm to 664 μm ²². Moreover, the diameter of the terminal hair follicle on the skin surface can be almost twice as large (31 μm to 59 μm) as compared to vellus (45 μm to 85 μm) ²².

3.4 Pubic and Axillary hair

The growth of pubic and axillary hair starts when the age of puberty is reached in both male and female ²². Any hair located in the frontal genital area, around sex organs, the crotch and inner upper thigh area of adolescent and adult humans are referred to as pubic hair and hair present underarms or the armpit is referred to as axillary hair ²². There is vellus hair present in these areas during childhood but during puberty when there is an increase in the levels of androgen, pubic hair starts to grow which are substantial, long and coarse hair compared to the vellus hair ^{22,23}. There are exceptions to the growth of pubic hair when there is an involvement of any hormonal disease and the growth also decreases with age, for example, after menopause in women ^{22,23}. Regarding medullation, there is a higher probability of its absence in scalp hair as compared to pubic or axillary hair ^{22,23}. Whereas, the diameter of the hair shaft and the medulla of pubic hair is significantly higher than that of the scalp or axillary and the diameter of the scalp hair generally has the second-highest value after pubic hair ^{22,23}.

4. Experimental methodology

One of the first task to complete when hair evidence is received at the laboratory is to identify whether it is human or animal hair. In order to identify the hair as human or non-human, the hair shaft should be analysed microscopically from the root end to the tip end ⁴.

4.1 Classification of human hair from non-human hair

As we know, the hair shaft is composed of 3 central regions which are the cuticle, cortex and medulla. The detailed analysis of these regions will lead to the identification of human or non-human hair. There are different types of hair present in the non-human fur or pelage and they are usually visually identifiable due to the coarseness ⁴. The most commonly found types of non-human hair are guard and under-hairs ⁴. The guard hairs are generally longer and coarser than the under-hairs that are finer ⁴. The guard hairs have a wide range of features that can be identified microscopically which makes them one of the most useful for identification purposes as it can also be used reliably for species identification compared to under-hairs ^{4,5}. Some of the major characteristics for comparison of the features between human and non-human hair are given below:

Table 2.1 Comparison of features of human and non-human hair

Feature	Human	Non-human
Colour	Relatively consistent along shaft	Often showing profound colour changes and banding
Cortex	Occupying most of width of shaft – greater than medulla	Usually less than width of medulla
Distribution of pigment	Even, slightly more towards cuticle	Central or denser towards medulla
Medulla	Less than one-third width of shaft. Amorphous, mostly not continuous when present	Greater than one-third width of shaft. Continuous, often varying in appearance along shaft, defined structure
Scales	Imbricate similar along shaft from root to tip	Often showing variation in structure along shaft from root to tip

Figure 3: Major features used for analysing human vs. non-human hair.

Additionally, in order to confirm the questioned hair as being animal hair, it can be compared with a known animal hair samples which can prove the hair as being non-human ⁴. Once the hair is identified as human hair, the experiment can move on to analysing the features of the human hair sample.

4.2 Examination of the hair shaft

The examination of the hair shaft will be done macroscopically first. In order to do this, the SWGMAT and the chart from Wickenheiser and Hepworth study (figure 1) will be used. Second, the hair shaft will be subject to microscopic examination with the help of a high-powered microscope. Any measurements and observations will be recorded as per the SWGMAT guide as well as the guide from the study, as shown in figure 1. This will help achieve the collection of data regarding the structure of the hair shaft and any potential correlations or similarities

between hair from the same area of the body and any differences between hair from different regions of the body.

The second part of this study will involve randomly choosing hair shafts without the knowledge of the examiner of its source. These samples of hair will then be subject to critical microscopic examination in order to identify the source/body part of the hair sample. The data collected regarding hair structure in the first part of the study will assist in the identification of the source.

4.3 Materials

The materials used for the analysis of the hair samples will be a high-powered microscope, hair samples collected from a random population and a suitable mounting medium for the hair such as Histomount.

5. Research Aims and Objectives

This literature review will be solely focused on microscopic comparison techniques regarding human hair. Various journals, articles and studies will be analysed to assess their reliability and to discover any flaws that exist in the process of microscopic examination of hair. Data will also be collected regarding the structure of the hair shaft from different regions of the body. Following this, the data gathered will be used to verify whether it is viable to identify the source of random samples of hair. The examiner, in this case, will not be informed about the source but the hair sample will be documented appropriately in order to verify whether the examiner's justifications are correct. The different sources of hair used in this project are the scalp, axillary, and pubic.

6. Conclusion

Various studies conducted in the past have shown flaws in the experimental design, false identification rate, invalid testimony and occurrences of examiner bias ^{15,17}. These flaws can affect the interpretation of the results achieved and the outcome of the result as well if appropriate procedures are not utilised. Moreover, proper documentation procedures should also be followed since they are one of the most critical aspects of the examination. A lack of appropriate documentation can result in misinterpretation of results ¹⁷.

The use of the SWGMAT guidelines and Wickenheiser and Hepworth study were used as a guide for the characteristics in a hair sample. A detailed list of characteristics of each hair sample should also help establish a specific correlation in the hair samples from one region of the body as they will have similar measurements in follicle size and diameter as well as additional characteristics ²². Moreover, these characteristics can then be used as a guide to differentiating hair from different regions of the body. The effectiveness of this method will be tested when the examiner will have to individually observe the features of each hair shaft and make a decision on which category it fits into. This will assist in identifying the source of the hair sample and can be double-checked to see if the acquired results are accurate and reliable.

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Part Two

Manuscript

MICROSCOPIC COMPARISON OF HAIR IN THE HUMAN BODY

By

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List of Abbreviations

NAS: National Academy of Sciences

DNA: Deoxyribonucleic acid

mtDNA: Mitochondrial DNA

FBI: Federal Bureau of Investigation

PCAST: President's Council of Advisors on Science and Technology

DOJ: Department of Justice

ENFSI: European Network of Forensic Science Institutes

SWGMAAT: Scientific Working Group on Material Analysis

POI: Person of Interest

Abstract

The forensic microscopic analysis of hair has been a standard procedure but there are also various limitations to it that have caused false convictions in the past. Some of the problems faced include the use of poor and outdated techniques, overinterpretation of results and the involvement of bias. This paper will explore these drawbacks and will outline various steps that can be taken to overcome these limitations. Techniques such as blind evaluations quality assurance have the capability of greatly reducing the involvement of bias. Additionally, the 3 part sequence of characteristics for the examination of a hair shaft can also aid in increasing efficiency of the examination and reducing bias. Furthermore, with careful evaluation of the standardised methods of the ENFSI (1) and SWGMAT (2) documents, recommendations have been made to advance the quality and efficiency of examination. Even though the use of DNA analysis has advanced the determination of the origin of hair samples, microscopic techniques still have the capability of comparing and screening samples suitable for DNA analysis, saving time and increasing efficiency. Lastly, the importance of microscopy is also explored in this document where, in some circumstances, DNA methods cannot yield any probative results whereas microscopy can provide a helpful insight. With careful examination of the pros and cons, it can be said that the joint use of both DNA and optimised microscopic techniques will greatly assist in providing accurate results in hair examination.

1. Introduction

Microscopic analysis is applied to the comparison of human hairs recovered from a scene or alleged victim and a person of interest (POI). Individualisation is not always possible solely with

the use of microscopic hair examination due to the lack of studies and research on the techniques used. As the NAS (National Academy of Sciences) report specified, there is crucial importance for more research and validation studies to be conducted not only for microscopic hair analysis but also for other techniques such as toolmark identification, questioned document comparisons and bite mark identification (3). The major limitation of forensic hair analysis is that the techniques have not gone through rigorous testing to prove that a questioned sample can be connected to the source with the use of microscopic techniques. Additionally, only nuclear DNA has proven to have the capability of individualisation with certainty and consistency (3). The aim of this project is to analyse the different types of limitations faced by microscopic analysis of hair and ways to overcome these limitations to advance the viability of microscopic methods.

1.1 Outline of issues faced in microscopic examination of hair

There are also a few specific concerns about microscopic hair examination mentioned by the NAS report (4):

- No scientifically accepted statistics regarding the frequency with which certain hair characteristics are distributed in the population (5).
- No uniform standards on the number of characteristics to examine before the examiner declares a match (5).
- Depending on the examiner's experience, the categorization of the hair characteristics will vary (5).

- The incorrect terminology used in the reporting of details can cause misunderstanding and also incorrectly imply individualization (5).

According to the follow-up PCAST (6) (President's Council of Advisors on Science and Technology) report, the essential areas that need to be focused on are:

- Clarity on the scientific standards for the reliability and validity of forensic methods.
- Evaluation of the methods and protocols to verify if they are scientifically valid and reliable.
- Quantitative information about the reliability of the method should also be clearly stated in an expert testimony, such as the frequency of false associations in the analysis.

Moreover, poor methods in the legal system such as influencing the jury, overstepping by the prosecutor, bias from police officials involved in the case and expert witnesses is also a major issue that can potentially led to miscarriages of justice. The case of Jimmy Ray Bromgard from 1987 shows these issues as he was convicted of rape based on a sketch of the perpetrator from the victim's recollection and hair evidence found at the crime scene which was compared to his hair (7). The failing of the legal system here was that even though the victim mentioned that she was only 60-65% positive of Bromgard being the perpetrator, one of the police officers had suspicion on Bromgard and said the sketch resembled his physical appearance (7). In regard to the hair evidence found at the crime scene, the prosecution's expert witness testified that there was a 1 in 10,000 chance that the hair came from a person other than Bromgard (7). The legal system failed here again as the only assurance provided by the expert witness was that the possibility of the hair sample not belonging to Bromgard was 1 in 10,000 which is not

a substantial amount. Moreover, there was also a lack of database that could possibly support this calculation. With the probability provided, the hair sample could have come from many different people whose hair have not been compared. Due to this Bromgard was convicted and 14 years later when he was exonerated after a DNA test showed he could not have been the perpetrator (7).

In a different case from the year 1992, Guy Paul Morin was convicted for the murder of Christine Jessop after 8 years from the occurrence of the crime (8). The primary evidence presented in this case was a hair sample found on the victim's necklace and the expert witness from the testified that Morin's hair could not be excluded as a possible source (8). Since the statement made by the expert witness is meant to carry a substantial amount of weight in court, Morin was convicted of murder.

It was later found that a hasty preliminary comparison of hairs was conducted in the presence of police officers. The examiner conveyed an opinion to them which was overstated and caused the police officers to believe that the hair comparison yielded critical information implicating Morin. In such cases, the trial judges need to undertake stringent critical analysis of the admissibility of the hair comparison evidence (8). Guilt cannot be assigned solely based on the fact that the suspect's hair sample cannot be excluded as a possible source for the unknown hair from the scene of crime (8). There can be a wide range of characteristics that are similar but since hair evidence cannot be used for individualisation, hair comparison alone cannot be used to convict a suspect for the crime. Even with a probable correlation established, confirmatory tests using DNA analysis need to be conducted before convicting anyone of a crime. The need to use DNA testing is a must, especially in cases involving murder because

microscopic tests alone cannot lead to identification of a person (6). All the major documents, NAS, PCAST, ENFSI and SWGMAT have mentioned the point that hair examination should not be used on its own and would be beneficial to accompany with DNA testing especially when the hair is one of the primary evidences.

Due to such cases, a consortium was formed in 2013 to review an approximate of 3000 cases where microscopic hair analysis was used (7) This group included members of the Innocence Project, the National Association for Criminal Defence Lawyers, law firm Winston and Strawn LLP and the Department of Justice (7). By 2015, they had reviewed over 269 cases and found that 96% of the transcripts had at least one testimonial error (7). The review done by this group caused the FBI to admit flawed testimony in numerous cases from the 1980 to 1996 which is when they began using mitochondrial DNA for hair comparisons (7).

Lastly, another major concern in human hair analysis is the involvement of cognitive bias which causes complications leading to the miscarriage of justice. It is an error that occurs in reasoning, evaluating, remembering or any other cognitive process that occurs when an individual stick to their own preference and beliefs regardless of any other information provided (9). It can also occur when the individual is overwhelmed by additional, unnecessary biased information which causes the judgement of the examiner to be biased. There are different types of cognitive bias and the remedy to minimise it will be explored later in this manuscript.

1.2 Addressing the issues mentioned by NAS and PCAST

One of the issues raised by the NAS report was regarding the examiner's experience and the way it would potentially affect the characterization of hair characteristics. This has been

addressed by the ENFSI (European Network of Forensic Science Institutes) as they have recommended on the standardisation of examination and further improvements through increased scientific education, training and continued professional development will enhance hair analysis (1). Since the process of hair comparison is a manual method, the examiners need to demonstrate competence by providing consistent, reproducible and valid results that are similar to results produced by other competent examiners (1).

The ENFSI has also covered the topic relating to the reporting of details and how it can cause misunderstanding. Words and phrases such as 'associated with' or 'could be from' can suggest individualization even when the samples have different origins (1). Suggestions have also been made regarding the proper reporting of results in a court of law by the expert witness.

In regard to the issue of number of characteristics to examine before declaring a match, However, more than 5 years after the publishing of the NAS report, the ENFSI report has simply restated the issues from the NAS report regarding statistics about the frequency with which hair characteristics occur in the population has also not been addressed. Nonetheless, suggestions have been made by ENFSI for the presentation of written reports and presentation of oral evidence in court. One of the most important suggestions being that the expert witnesses should not deviate from their field of expertise and answer unrelated questions unless directed by the court. Even though if the court directs the witness to deviate from their field of expertise, a declaration should be made by the witness regarding the limitations in their expertise (1). The PCAST report on the other hand have focused only on scientific foundational validity regarding various forensic disciplines including hair. They have not addressed any of the issues raised by the NAS report.

The SWGMAT document was published prior to the NAS report and has stated one of the issues mentioned in the NAS. As the NAS stated that there are no statistics regarding the distribution of hair characteristics in the population, the SWGMAT document had stated that statistics and probabilities should not be used in hair comparison techniques (2). There are no recommendations made by the SWGMAT and have only posted the standards and guidelines for hair examination which are also the same guidelines utilised by the FBI (Federal Bureau of Investigation).

Both the ENFSI and SWGMAT documents contain guidelines and procedures regarding microscopic hair examination making them very similar to each other. One of the differences is that ENFSI provides a separate table with the features to be analysed for animal hairs. Additionally, the ENFSI has provided details on the features that can be observed under a stereomicroscope versus a transmitted light microscope. Both these documents contain ample information regarding the procedures and guidelines for microscopic hair analysis and it would be beneficial to refer to both these documents in order to ensure no critical information is overlooked.

1.3 Analysis of macroscopic and microscopic characteristics

One of the initial and most obvious features of hair is the colour. The Fischer-Saller scale classifies hair colour into 8 categories: very light blond, light blond, blond, dark blond, brown, dark brown/black, red and red blond (10). The word 'blond' can be substituted by the colour yellow because if the shade of being light or dark blond is removed then the only colour remaining is yellow, brown and black (10). Both the ENFSI and SWGMAT document have used

the term blond as a category of colour but the Wickenheiser and Hepworth study have not (11). Even though the ENFSI and SWGMAT documents were published at a later date, they have not used the correct terms for colour classification because there are only two pigments that produce hair colour; Eumelanin produces black and brown colours whereas pheomelanin produces yellow and red colours (10). There is an appropriate scientific foundation that explains how hair colour is formed (10).

Robertson (year), on the other hand, has classified colours into five categories: colourless, yellow, brown, red and black with further classification by shade (light, medium, dark) (10). This creates an efficient way to categorize colours in order to reduce subjectivity and to increase the consistency of analysis for different examiners (10). Although, Robertson fails to mention the colour white which is mentioned in the other 3 documents. Since, white hair is caused due to lack of pigmentation, it can be assumed that the term 'colourless' mentioned by Robertson would also include the colour white as it is mentioned in the SWGMAT document that this colour falls under the colourless category. Hair colour is one of the most initial and basic characteristics that are analysed and there should be uniformity across all institutions and agencies in identifying them with the correct term. The classification categories used by Robertson is the most scientifically accurate and it is highly recommended that his classification technique be used.

Robertson also provided a list of features that can be assessed microscopically. These included pigment density, distribution, shape and size of pigment, granules, aggregates, presence/absence of ovoid bodies and cortical fusi, descriptions of medulla (presence/absence and if present distribution and appearance), and features associated with cortex and cuticle.

These features are presented in rigorous detail in his book on Forensic Examination of Hairs (12). Assessing all the features mentioned by Robertson, it can be seen that he has provided the most details on the features as compared to the other 3 documents. Although, the drawback for having a lengthy list of features is the amount of time taken to examine a single hair shaft. The SWGMAT document has efficiently provided maximum features for analysis in an organized manner. As suggested by Robertson, every hair sample should not be examined initially for all the features as there would be an elimination process where screening of hairs would be conducted based on the obvious macroscopic characteristics or with the use of a low powered microscope (10). The screening process would majorly contribute towards the efficiency of the analysis as a lot more time would be saved.

Additionally, comparison microscopy involves viewing the hair shaft from one angle at a time where only one part of the hair is in focus. This problem has not been mentioned by the 3 other documents, but Robertson has suggested the technique used by Brooks et al. (13) which utilises the automontage approach which produced a stacked image of different photographs of the hair shaft with all of them in focus (10). Their work also goes on to focus on the potential for digital image analysis to extract pigment pattern analysis for comparison purposes. This has been the only attempt at capturing pigment pattern in hair shafts as an operator-independent method (10). This method needs to progress further which would eventually help in creating databases for hair features and storing feature-related information for comparison purposes. In response to the various hair feature lists explored, there are various features mentioned in one document which is lacking in the other. A combination of all these features need to be created in order to ensure that an appropriate analysis has been done in an efficient manner.

1.4 Evaluation of methods involved in the examination of hair

The current methods of hair examination are similar to the methods that were used prior to the NAS report. There have not been any major discoveries in the field of microscopic hair examination besides the increase in use of nuclear and mitochondrial DNA introduced in the early 2000's. Most of the foundational work and research were done in the 20th century which include works from groups such as Gaudette and Keeping (14). The main focus of the work done was to analyse the hair sample in order to identify its origin. However, in the year 2002, a study conducted by the FBI used mt-DNA analysis and made a major revelation that exposed some of the flaws in microscopic hair examination (15). One of the flaws included a 12.5% error rate in stating the similarity of two hair samples and calling it a match (4). As the availability of DNA analysis increased, microscopic methods became less reliable. Additionally, DNA analysis was more likely to provide a reliable and specific result as compared to microscopic examination (4). This resulted in an increased number of researches done in the field of DNA analysis and very little done in microscopic examination as results provided by DNA analysis had the capability to lead directly to the origin with a miniscule error rate, whereas microscopy had a higher error rate in associating one hair sample to another.

1.5 Importance of microscopic methods

There are also a few instances where it is essential to the investigation for microscopic methods to be conducted first and it might also be the only way to provide any results. For example, regarding mt-DNA analysis, groups of people from the same maternal line may not have different mt-DNA types and will not be able to provide any discriminating factors to

differentiate the hair samples (2,16). Additionally, the main reason for using mt-DNA techniques is because most of the hair evidence encountered at a crime scene are naturally shed hairs which means that they are in the telogen phase of growth. There is a lack of nucleated cells in the follicular tissue of hairs in the telogen phase as compared to the anagen phase where there is an abundance of nuclear DNA which is why mt-DNA analysis is used (2,16). Moreover, the macroscopic and microscopic analysis of hair will also define the type of DNA analysis that can be used (2,16). Microscopically analysing the growth stage of the hair will aid in planning whether nuclear DNA or mt-DNA analysis methods should be used.

1.6 The evolution of methods and techniques over a decade

There have not been any significant changes in the methods and techniques involved in microscopic hair examination. The textbook on forensic hair analysis (James Robertson editor) which was first published in 1999, has been cited by the SWGMAT and ENFSI manual which were published in 2005 and 2015 respectively (1,2). Even though Robertson's book provided detail regarding hair and its analysis, significant improvements and findings have not been made in the field of comparison. There have been changes in our perception of microscopic techniques since the introduction of DNA analysis for hair examination. It has been responsible for turning over wrongful convictions that were caused by microscopic hair analysis in the past (6). The specificity of DNA was much higher than that of microscopic techniques and provided more accurate results as compared to microscopic techniques which can only provide a scientific opinion on the similarity between two hairs (4).

The joint use of microscopic and DNA analysis methods is highly recommended in current times and is also referred to as complementary comparison methods (1). DNA analysis of hair is a destructive method and, in most cases, cannot be used for microscopic analysis as a second step (2). Microscopic methods need to be utilised prior to DNA analysis because it is capable of providing an association between known and questioned hairs and can also be used as an eliminating factor for hairs that have no similarities (2). In such cases there is no need to conduct a DNA analysis because the microscopic methods have already proved the dissimilarities between the questioned and known samples. Hence, the combination of mt-DNA and microscopic methods will aid in excluding or providing an even stronger relation of two hair samples as compared to using either of the methods alone (2).

2. Minimising cognitive bias

Cognitive bias is a major concern when a method or technique involves an element of subjective judgement (9). Due to this, it is essential to follow calculated steps in order to minimise the influence of cognitive bias wherever possible.

2.1 Role effects bias

This is a subconscious bias which causes the person's perception of his/her role to affect the results (17). An instance shared by a former director of the Illinois crime laboratory shows that most forensic scientists at the state police laboratories saw themselves as members of the state attorney's team and identified themselves as prosecution witnesses (17). It is understood that the scientists overstepped their role and created their own illusion. There have also been reports of inaccurate testimonies provided by the laboratory scientists (17). Appropriate

training and workshops need to be provided as the scientists should clearly know their role and not overstep their duties. Their main duty is to examine evidence and provide the results in relation to the case and not to take sides.

2.2 Contextual bias

This type of bias occurs when extra information is provided that influences the decision of the examiner. An example of this is when the investigators would provide their personal views on the suspects and evidence before the examiner has a chance to perform an analysis on the evidence (17). Such extraneous information results in an alteration of expectations of the examiner which inevitably affects their perception and decision-making process (17). The most effective procedure to reduce such bias is to adopt blind testing procedures. This greatly impacts the examiner's ability to form an unbiased opinion. In other words, an examiner who does not have any irrelevant information and cannot be influenced by it (17). When the investigators or police officials do not tell the examiner their views on the case or the evidence, it will reduce the likelihood of a biased result.

2.3 Confirmation bias

This bias occurs when the examiner reaches the result that they were expecting to reach (1). This type of bias is also known as the "tendency to test a hypothesis by looking for instances that confirm it rather than searching for potentially falsifying instances" (17). One of the precautionary methods to avoid this is by including psychology training for forensic examiners that addresses experimental methods, perception, judgement, decision making and social

influence (13). Such steps at the very least would ensure that the examiners receive appropriate training in making judgements.

2.4 Reconstructive effects

This occurs when the examiner uses their memory to remember the case details (17). This can cause them to generate false information in their recollection and tend them to “fill in gaps” with what they believe should have occurred (17). In addition to bias, to reduce memory bias there needs to be contemporaneous documentation of every case (17). Detailed notes should be stored on every technique used on every piece of evidence gathered. This will ensure proper recollection about any case and ensure that no details are incorrect.

2.5 Blind and double blind-study techniques

Additional proposed methods to reducing the effect of bias is managing the flow of information in a forensic laboratory in order to minimize any possible exposure of the examiner to irrelevant contextual information. This information also includes eye-witness identifications and confessions. It is essential to document the finding of the evidence before performing feature comparison methods of a known and suspected sample. This is similar to blind testing which is one of the most effective techniques to tackle bias. It is unnecessary to provide unrelated information to the examiner such as contact with the investigating officer, the victims and their families, or whether the suspect has confessed or not (13).

An example of a blind study is the experiment conducted by Wickenheiser and Hepworth (18). They tried to replicate the work of Gaudette and Keeping and at the same time tried to eliminate examiner bias (8,4). This was considered a blind study as it was also an experiment

to eliminate examiner bias which was done by giving no information about the results of the hair samples to the examiners. Their task was to analyse the hair samples to find any similarities. No incorrect correlations were made by the examiners in their study, but heavy criticism was received by them for using only two examiners to analyse the bias for a large group of examiners (4).

Blind procedures such as the evidence line-up procedure (13) should be adopted by forensic laboratories if not already done. The evidence line-up procedure can be executed by comparing a questioned sample to the known sample along with a few other samples that are not related to the case. Instead of comparing a questioned sample with a sample obtained from a POI, various other samples should be added to the set of known samples that are valid hair samples but not related to the case at hand. This aids in reducing a false positive result that could possibly be caused due to confirmation bias.

Additionally, the use of double-blind procedures are also vital to the examination. This can be adopted in the verification process of the decisions and results obtained by the forensic examiner (13). In this procedure, the verifier should not know the examiner and vice-versa. If possible, the verifier should also not know about the conclusion reached by the examiner and cross-laboratory verification can also be conducted where the examination report is sent to a different laboratory for verification. It is highly recommended that such blind and double-blind study techniques be adopted by laboratories as it has a major impact in controlling any potential bias that may arise in the examination.

Another technique that can be applied is the ACE-V method that is more commonly used in fingerprint examination (19). This method can also be applied to hair examination which can aid in reducing the effect of potential bias. The ACE-V method is short for Analysis, Comparison, Evaluation and Verification (19). This method lays out the order in which examination should be done and it can also be applied to hair examination procedures where the hair is first analysed in detail before any comparison techniques are done which is then followed by the evaluation of the results achieved and a conclusion is formed. Finally, the verification of the conclusion is done by another qualified member of the laboratory where he/she performs the analysis and comparison of the hair shaft in order to provide their conclusion to check whether both examiners reach the same conclusion. Blind procedures can also be integrated in this where the two different examiners do not know each other, or the conclusion reached by them before performing the examination.

3. Improving interpretative evaluation and reporting of results

The proper evaluation and reporting of results in hair comparison is vital in conveying the correct message in a scientific report or testimony. It is essential to have knowledge regarding the transfer and persistence of hair, methods and techniques available for analysis, detection and collection of the hair sample (1). The success in making a positive identification is limited in various ways as valid and standardised techniques and methods need to be followed during the examination process. Moreover, the knowledge and experience of the examiner also play a major role in the assessment of the strength of the results (1).

There are no two hair samples that are completely identical in every detail, therefore all the characteristics of the hair sample must be identified. If the characteristics are similar to that of the questioned sample, it can be said that there is a possibility that the two hairs have a common origin (1). Additionally, the examiner must also identify how common the shared characteristics are. This is done in order to assess the likelihood of finding a hair sample with the same characteristics if it had not originated from the same source (1). Additionally, there are characteristics such as hair dyes or abnormalities which increase the likelihood of proving that two samples of hair originated from the same source as it is rarer to find the exact same dye or abnormality between two different hair samples (1). In contrast, the likelihood of proving the origin of two samples are greatly reduced as the number of usable characteristics are reduced in a hair comparison.

One of the major drawbacks for interpreting hair evidence has been the conflict about how to evaluate the evidential value of hair comparisons (1). Additionally, there are also no concrete number of characteristics that can be analysed before declaring that the two samples of hair share the same origin. This is due to the subjective nature of the analysis and its high dependence on examiner experience and proficiency.

The major improvement that needs to take place in terms of the reporting of results in hair comparison is the involvement of various types of bias which causes the examiner to present the evidence in a non-professional manner. The reporting of results should always include relevant information which is clear and concise and structured in an unambiguous manner. Additionally, there should also be peer review conducted of the report in order to make sure there is no bias involved and no unnecessary information included in the report (1).

In regard to the microscopic characteristics that need to be analysed, the SWGMAT (2) and ENFSI (1) documents have formed a list that includes all the necessary features that need to be analysed. Both documents have not specifically mentioned a sequence in which those characteristics should be analysed. A combination of both these documents in an appropriate sequence would prove efficient. Hence, below is a recommendation on a structured sequence which would be efficient in the analysis of a hair sample.

Part 1. Before any microscopic examination takes place, the hair should be analysed macroscopically. These characteristics include:

- i) Colour
 - a. Yellow, red, brown, black with mention of shade (light, medium, dark)
- ii) Shaft form
 - a. Straight, arced, wavy, curly, twisted, tightly coiled, crimped
- iii) Shaft length
- iv) Shaft thickness (not accurate measurement)
 - a. Fine, medium, coarse
- v) Proximal end
 - a. Root absence, root presence

Part 2. Following the macroscopic examination is the microscopic examination of features. Although, before examining the characteristics present in the cuticle, cortex and medulla, it is essential to list out any abnormalities and irregularities that are acquired by the hair shaft which include the following:

- i) Artifacts present
 - a. Nits/lice, mould, fungal tunnels, insect bite marks, debris, blood, post-mortem banding
- ii) Damage
 - a. Environmental/chemical damage
 - b. Crushed, burned, cut, broken, frayed, twisted, tangled
- iii) Abnormalities/diseases
 - a. Pili annulati, Trichoschisis, Monilethrix, Trichorrhexis nodosa, Trichorrhexis invaginate, Pili torti, Trichonodosis, Trichoptilosis
- iv) Artificial/cosmetic treatments
 - a. Dyes, hair spray/gel, styling products, bleaches, lighteners

Part 3. Once the acquired characteristics have been analysed, the cuticle, cortex and medulla are next. These features include:

- i) Cuticle
 - a. Cuticle absent, present
 - b. Thickness (thin, medium, thick)
 - c. Outer cuticle margin (flattened, smooth, serrated, cracked, looped, irregular etc.)
 - d. Inner cuticle margin (distinct, indistinct)
- ii) Cortex
 - a. Cellular texture (coarse, medium, fine)
 - b. Ovoid bodies (size, distribution, abundance)

- c. Cortical fusi (size, shape, distribution, abundance)
 - d. Pigmentation (present/absent)
 - i. If absent, steps 'e' to 'i' will not be conducted
 - e. Pigment size (coarse, medium, fine)
 - f. Pigment aggregation (streaked, clumped, patchy)
 - g. Pigment aggregate size (large, medium, small)
 - h. Pigment density (absent, light, medium, heavy, opaque)
 - i. Pigment distribution (uniform, peripheral, one-sided, pigment in cuticle, banded, random or variable, central or medial)
- iii) Medulla
- a. Root present
 - i. Telogen, catagen, anagen, sheathed, follicular tag
 - b. Root absent
 - i. Severed, decomposed, crushed
 - c. Distal ends
 - i. Tapered tips (uncut), rounded or abraded, square cut, angular cut, frayed, split, crushed, broken, singed

Hence, this sequence of examination starts off with analysis of macroscopic characteristics which is followed by any acquire characteristics and finally the analysis of the cuticle, cortex and medulla. The original list from the SWGMAT (2) and ENFSI (1) have not suggested any sequence in which hair should be examined. The sequence provided above also splits the hair analysis into 3 parts. Although, this can potentially induce confirmation bias because based on

the macroscopic and acquired characteristics as the examiner can tend to believe that it would be rare to find two hair samples with the same acquired characteristics. Even though it is rare to find, there are still possibilities and the complete examination needs to occur in order to provide a judgment on the hair. In order to eliminate the possibility of bias it will prove helpful if each part is performed by different examiners as it can mainly aid in reducing any potential confirmation bias. Additionally, this can be paired with blind study techniques where the examiner will not have knowledge about the hair sample and where it was obtained.

This 3-part sequence of examining hair also creates an efficient and effective procedure of analysing hair as the workload can be split into 3 parts and would save time. Additionally, the pigment characteristics have also been included in the cortex characteristics as opposed to the two documents that have kept the pigmentation characteristics separate. The pigments are formed in the cortex which is the reason it has been included within the cortex characteristics. The ENFSI document has also not mentioned blood in their acquired characteristics list. It is essential to mention any blood or other bodily fluids present that have the ability to contain cellular material as it can provide evidentiary value for the case at hand. Hence, the examination of acquired characteristics occur in part 2 of the sequence and if any blood or body fluid is found, it can be sent for DNA testing before any other analysis is done on the shaft.

4. Recommendations

Some of the recommendations regarding hair comparison using microscopy are given below:

- The examiners should first examine the evidence and document their findings before doing any type of comparison with another hair sample (13). This assists in reducing

any external influence on the way the information is processed or the amount of weight it carries (13).

- Blind and double-blind procedures should be utilised wherever possible in order to greatly reduce the amount of potential bias (13). Forensic laboratory scientists should also undergo psychology courses which would train them in identifying bias and eliminating them to improve the judgement and decision-making process (13). This should be done in addition to the procedures followed to reduce bias. It will help in reducing the likelihood of bias as the analysis will not be completely relying on procedures such as blind study techniques in order to prevent bias. Additionally, the ACE-V method should also be utilised which can help create a sequence for examination and reduce any potential bias.
- The use of technology can assist in saving time and making associations much faster than humans, but enough precaution should be taken as the likelihood of finding close non-matches is increased. Additionally, it can create a ranking of potential POI (Person of Interest) which can possibly cause biased expectations in the examiners which would lead to incorrect identifications (13).
- All laboratories, expert witnesses and other personnel involved in the cases should strictly abide by their code of conduct and ethics based on the legislation of their region. This should be done in order to avoid issues such as testimony beyond the examiner's competence and over-stepping conclusions.

Proper note-taking procedures should be followed, and detailed notes should be taken of every examination performed in the laboratory. Additionally, contemporaneous

notes should be taken alongside the examination in order to prevent reconstructive effects bias (17). The use of correct and uniform terminology is also important when reporting the result from a hair analysis. There are 3 outcomes that can be achieved: 1) the known and questioned samples exhibit the same characteristics, 2) the known and questioned samples exhibit different characteristics, 3) no conclusion can be reached regarding the similarities of the questioned and known samples due to insufficient characteristics available (in cases of damaged or partial hair) or due to the hair samples exhibiting both similarities and differences. Similarly, the results should be reported using such terminology and not with words such as “match” as it can sway the jury into believing that the questioned sample has been confirmed of having the same origin as the known sample. Confirmatory tests involving DNA analysis need to be conducted in order to prove the origin of the questioned sample.

In order for microscopic hair examination to advance in the future, a number of problems would need to be addressed:

1. Error rates of microscopic hair examinations: more studies need to be conducted which explore the error rates associated with hair analysis in order to expand the amount of literature in this subject (17). Some examples of such studies are Wickenheiser and Hepworth, and Deedrick et al. (18, 20).
2. A recurring research needs to be conducted regularly in order to statistically evaluate the frequency with which certain hair characteristics are distributed in the population of a region. This should also be gradually upgraded to evaluate the characteristics in all countries.

3. The 3-part sequence should also be tested for its validity and applicability in a forensic laboratory.
4. Training of forensic hair microscopists: the training of hair examiners has proven to play a significant role in the determination of accuracy in of the microscopical hair comparisons they perform. SWGMAT, according to its trace-evidence quality-assurance guidelines, has recommended a 12-month full-time training program for inexperienced examiners (17). Their quality-assurance guidelines also include a proposed curriculum for the training program (17).
5. Proficiency testing of forensic hair examiners: SWGMAT has also recommended that all trace evidence examiners, which includes hair examiners as well, need to complete at least one proficiency test each year. SWGMAT has also proposed guidelines for the preparation, administration, and interpretation of these tests (17).
6. More valid studies and experiments need to be conducted in order to verify the number and type of characteristics that need to be analysed in order to declare a positive identification.

5. Conclusion

Microscopic examination provides evidentiary value and can greatly assist in the efficiency of the hair examination. However, it is not a confirmatory test, but it can support in the elimination procedure of the case samples. In order to advance the use of microscopy for hair analysis various methods such as the evidence line-up and the 3-part sequence of characteristics need to be utilised. These have the capability to greatly reduce the limitations caused by bias as well as increase efficiency of an examination. The recommendations of

further research can also aid in answering some of the unresolved problems in hair examination such as the evidentiary value of certain characteristics and the number of features needed to be analysed in order to come to a conclusion. At present, microscopic analysis can only be used as a presumptive testing method which can further be analysed using DNA as a confirmatory procedure and the joint use of these two methods will prove to be the most accurate and efficient.

Appendix

Handling and recovery of hair evidence

As stated by Edmund Locard, a transfer of materials will occur when two objects come into contact with each other (21). The trace evidence that is transferred between the two objects have the capability to show connections between objects, individuals or location but maximum care should be taken when handling evidence of this nature since they are quite vulnerable to contamination (20). Similarly, hair evidence should also be handled with care as it can be contaminated if correct procedures are not followed.

One of the initial steps in the examination of hairs is the recovery from the scene of crime. Some of the methods used to recover hair from locations such as clothing and bedding items are by scraping, taping, picking and shaking (20). In the case of large areas that have carpets, an appropriate vacuum for the collection of trace evidence can be used (20). These vacuums contain a filtered cannister where all the trace evidence are filtered and easy to recover from a large area. In cases where you may need to record the specific location of the site of recovery, the picking method can be utilised to pick the hairs one by one and record their locations accordingly (20). Regardless of the method used, extra care should always be taken to avoid any contamination to the evidence or cross-transfers that may be caused due to mishandling of the evidence (20).

List of issues involved in hair examination:

1. Subjectivity

The examiner needs to determine not only if two samples are alike but if the similarities outweigh the differences because there will also be differences between the samples of the same individual (22). There is subjectivity involved here because hair cannot be individualised like fingerprints and even though the examination may involve objective methods of analysis, the subjective decision is left with the examiner (22). There are over 20 different characteristics that can be used in microscopic hair analysis but most of the characteristics are subjective (7). For example, characteristics such as pigment distribution (uniform, peripheral, one side, clusters) or the cortical texture (fine, medium, coarse) are very subjective because different examiners might have different descriptions for such characteristics (7).

2. Lack of standards

There is a lack of uniform standards on the number of features that need to be examined in hair to give reliable results (7,22). There are also no standards for regulating the amount of weight given to each characteristic (22). In the case *State of Connecticut v. Chasity West*, which was filed in 2005, the prosecution's expert witness did not explain to the court which of the 25 characteristics he/she claimed to be consistent, any standards for determining whether the samples were consistent, amount of people who could have shared the same combination of characteristics and an explanation to how he/she arrived at the conclusions (22). The lack of standards and the subjective nature creates doubt on the validity of the analysis as a scientific method (22). In the case of Kirk Odom and two other men, they were all convicted for murder which was based on flawed hair analysis and served 22 years in prison for a crime they did not commit (7). They were all later exonerated with the help of DNA testing (7).

There is a critical need for standards in microscopic hair examination in order to prevent false convictions. In order to make hair examination a reliable and consistent method, there needs to be certain standards to decide the minimum number of characteristics that need to be similar between the questioned and known sample and also the amount of weight each characteristic holds.

3. Cognitive bias

The problem of cognitive bias is seen when dealing with subjective interpretations (22). Especially in cases of ambiguity, the decision of the examiner is affected as other information creates a negative influence in their decision (22). For example, any information such as notes from the case or the detective's opinion can influence the decision of the examiner (22). The notes written at the scene of crime will contain every detail of the scene which can cause the examiner to influence his/her opinion about the evidence after seeing the notes or hearing the detective's opinion. For example, if a victim was found dead in his room and it looked like a suicide, the hair examiner does not need to know the detective's opinion who is leaning towards the case being a suicide act. If the information about hair evidence being found next to the victim was released to the examiner, they can be aligned towards believing that the hair most probably belongs to the victim and there might not be proper analysis done on the hair sample.

In a report that had been released by the inspector general of the Department of Justice in 1997 explained various drawbacks such as inaccurate testimony, testimony beyond the competence of the examiners, improperly prepared lab reports, insufficient documentation of

results and a failure to resolve all the allegations of incompetence (17). With regards to the Oklahoma City bombing case, the inspector general found that the examiners conclusion was purely based on speculation and was titled in a way that would incriminate the defendants (17). This is particularly an example of role effect bias where the examiner tried to incriminate the defendants whereas his job is to present all the findings of the case in an appropriate manner (17). This shows the power of bias where the integrity of the whole case gets compromised and even the innocent can be charged with crimes they have not committed.

The misleading nature of hair evidence is sometimes also related to the prosecutor who declares with certainty that there is a match which causes the court to misinterpret the evidence as well (22). In an example from *Bevill v. State* 1990, the prosecutor had stated, "And then you have this hard scientific proof, those hair comparisons" and the expert had boldly stated that, "In my opinion, those hairs came from this man" (22). This shows that both the prosecutor and the expert had overstated in their closing arguments which would cause the court to believe it to be true for a fact.

Closing arguments have a strong impact in court because it sums up the case and provides a conclusion. In the case of *Bevill v. State* 1990, the expert witness overstepped his authority as he is not required to provide his opinion on the case. The expert only needs to present the evidence and show if there are any correlations between the questioned and known samples which would then show the court evidence of occurrence of events or the presence or absence of objects and people at the scene of crime.

4. Testimony and Overstatements

The use of terms “microscopically indistinguishable” and “consistent with” are misleading (22). The evidence at hand might have very little probative value but the use of these words in a testimony can cause the court to believe the fact with certainty. When an expert witness uses the term consistent with, it should also be made clear that the hair evidence could have come from a number of other people as well (22). There have been cases where experts have gone beyond the “consistent with” testimony and stated with absolute certainty that a questioned hair sample belonged to the suspect sample (22). For example, in the Edward Honaker case, the expert testified that the suspect’s hair sample was unlikely to match with anyone other than the defendant. Due to this statement made by the expert, the defendant was sentenced and after 10 years proven innocent with the use of DNA analysis (22).

5. Lack of databases

This is one of the most important and crucial problems as there is no population-based databases (7). This makes it impossible to estimate the probability of any hair characteristic and moreover causes the inability to uniquely identify one person (7). A database devoted to hair characteristics that stores the hair features of the population would be a big step towards individualisation. Just as fingerprints have their own databases in various countries that store fingerprint information of the people, hair should also have their own database in order to start using them for individualisation purposes. It is not possible to show similarities between two hair samples unless there are a certain number of characteristics that match and that would be even more challenging if the questioned sample is present but the available known samples are not showing enough similarities to prove the correlation between the two hair

samples. In these circumstances, a database consisting hair information of a certain population would be beneficial.

6. Foundational validity

Being foundationally valid requires the use of methods that have been subject to empirical testing by multiple groups of scientists in the field (9). These tests should show that the methods used are repeatable, meaning that same results can be achieved if performed again (9). Additionally, the test should also show that the methods are reproducible, meaning that different examiners can reach the same result as the previous examiner (9).

Judge Harry Edwards, who was also the co-chair of the NAS community, explained that he was under the impression that all the forensic science disciplines generally have proper scientific methodology and that crime laboratories follow proven practices in order to ensure validity and reliability of forensic science (3). He later mentioned that he was wrong on what he assumed. An experienced prosecutor who also was a part of the NAS committee mentioned that his views about forensic science had drastically changed in the two years he served for NAS (3). He said that there is not enough genuine science to validate many forensic science disciplines (3).

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