Article

Variability in Visualization of Latent Fingermarks Developed with 1,2-Indanedione–Zinc Chloride

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Abstract: Amino acid variability in sweat may affect the ability of amino acid-sensitive fingermark reagents to successfully develop all latent fingermarks within a large population. There has been some speculation that age, gender, or prior activity may be the cause for differences in the amino acid profile within a population.

Latent fingermarks from 120 donors were collected and treated with 1,2-indanedione–zinc chloride. Grades were given to treated samples based upon their initial color and resultant luminescent properties. Degradation of developed prints over three years was also assessed by regrading all samples and comparing the results to the initial grade.

Statistical analyses, such as the Mann-Whitney U test, revealed that there was a correlation between the grade and the age of the developed print, age of the donor, and the washing of hands. However, no link was found between the food consumption or gender of the donor and the grade.

Introduction

A range of chemical reagents can be used to visualize latent fingermarks. These reagents react with the deposited fingermark residue consisting of both foreign compounds and secretions from skin glands. These secretions are produced by the eccrine, apocrine, and sebaceous glands, of which only the eccrine gland

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is present on the palms and fingers [1]. The eccrine gland is responsible for the presence of amino acids (AA) in latent fingermarks; amino acids are the target of many commonly used fingermark reagents for porous surfaces. The quality of the print is thus directly related to the quantity of AA present. One such reagent is 1,2-indanedione–zinc chloride (referred to as IND-Zn in this article, although IND/$ZnCl_2$ or INDZ are also commonly used), which is extensively used in countries with drier climates, such as Australia [2]. It reacts via a similar pathway to produce a colored product that has been referred to as Joullié's pink [3]. The advantage of IND-Zn is that, although it gives relatively poor visible color when compared to ninhydrin, the reaction product is highly luminescent, offering excellent contrast and sensitivity. It has been noted that the quality of IND-Zn-treated fingermarks is not uniform within a donor population, with age, gender, prior activity, and diet thought to be possible causes of amino acid variation [4–8]. Although various studies examining the AA concentration profile in human sweat have been conducted, these either did not focus on the AA variation in a population or had too few donors to be statistically significant [4, 9–14].

In this pilot project, latent fingermarks from 120 donors of both genders drawn across a wide age range (10–65 years old) were collected on paper and treated with IND-Zn. The intention was to examine what variability in response there might be and to investigate appropriate statistical methods to interpret the data. The knowledge gained on the detectability of latent fingermarks by IND-Zn also provides guidance as to how larger scale trials can be carried out in the future.

This project was undertaken in collaboration with high school students in Perth, Western Australia as part of the BioGenius Challenge of Western Australia. The Challenge takes high school students into research laboratories, giving them the opportunity to work alongside scientists. Forensic science in practice depends heavily upon the underlying molecular and physical sciences and thus provides an excellent opportunity to present those sciences in an interesting and engaging context. There is also the potential to explore the social and ethical issues associated with science and the broader community. It has been on this basis that forensic science has increasingly appeared in secondary education science curricula both in Australia and around the world. This project exposed the students to the research process: ethics approval, experimental design, sample collection, data collection, data interpretation, and communication of results.

Materials and Methods

Chemicals

1,2-Indanedione (CASALI/Optimum Technology, Australia), anhydrous zinc chloride (BDH, USA), ethyl acetate (Univar analytical, Australia), glacial acetic acid (CSR chemicals, Australia), absolute ethanol (CSR chemicals, Australia), and HFE-7100 (1-methoxynonafluorobutane, 3M Novec, Australia) were all used as received and were of analytical reagent grade unless otherwise stated.

Preparation of Reagent Solution

IND-Zn reagent was prepared as recommended by the Australian Federal Police (AFP) and as described in various other studies [2, 15–17]. The preparation of stock and working solutions for the IND-Zn formulation is summarized in Table 1.

Solution	Reagent Preparation
1,2-Indanedione stock solution	4 g 1,2-indanedione dissolved in 450 mL ethyl acetate and 50 mL glacial acetic acid
Zinc chloride stock solution	8 g zinc chloride dissolved in 200 mL absolute ethanol
Working solution	2 mL zinc chloride stock solution and 50 mL 1,2-indanedione stock solution added to 450 mL HFE-7100 solvent

Table 1

Preparation of 1,2-indanedione stock solutions and working solution.

Collection of Latent Fingermarks

Latent fingermarks were collected on white copy paper (Fuji Xerox Professional) from 120 donors. The conditions were kept as natural as possible with no “charging” of fingermarks (rubbing of fingers on sebum-rich body parts) prior to deposition. Donors were instructed to gently place fingertips onto the substrate. As well as collecting the fingermarks, donors were asked to provide information concerning their age, gender, and activity 30 minutes prior to sample collection, with respect to food consumption and washing of hands. Samples were typically treated within 24 to 36 hours of deposition. The details of the donors are given in Table 2. Samples were stored in a locked cabinet in a temperature regulated office environment (20–24 °C).

Variable	**Total**	**Gender**		**Age**		**Food Consumption**		**Washing of Hands**	
Grouping	-	Male	Female	Over 25	Under 25	Yes	No	Yes	No
Number of Donors	120	60	60	69 (M:37, F:32)	51 (M:23, F:28)	28	92	30	90

Table 2

Donor information with regards to the number of donors for each variable.

Development of Latent Fingermarks Using IND-Zn Methods

IND-Zn treatment was carried out as described by the AFP [15]. Samples were briefly dipped in the working solution and allowed to air-dry before being heat-treated for 10 seconds with an Elna laundry press at 160 °C.

Photography of Samples

Samples were photographed using a Nikon D300 camera mounted on a Firenze Mini Repro tripod and connected to a computer using Nikon Camera Control Pro version 2.0. Treated samples were photographed in luminescence mode (Table 3) using a Rofin Polilight PL500 (Rofin, Australia), with an excitation wavelength of 505 nm and an orange camera filter attachment (550 nm barrier filter).

	Luminescence Mode
Focal Length/mm	60
Exposure Mode	Manual
White Balance	Auto
Shutter Speed(s)	2
Aperture	f/11
Sensitivity	ISO 200

Table 3

Photographic conditions for luminescence mode photographs.

Visual Analysis of Developed Latent Fingermarks

Treated fingermarks were graded using a 5-point system based on that used by the United Kingdom Home Office Police Scientific Development Branch (HOPSDB) (Table 4). Later adjustments of the images were performed on Adobe Photoshop CS5 version 12.0.

Grade		Description
0	No development	No visible ridge detail
1	Weak development	Signs of contact, but less than 1/3 of fingermark visible as continuous ridges
2	Medium development	1/3–2/3 of fingermark visible as continuous ridges
3	Strong development	More than 2/3 of fingermark visible as continuous ridges, but not quite a "perfect" fingermark
4	Full development	Whole fingermark visible as continuous ridges

Table 4

Grading system for developed latent fingermarks.

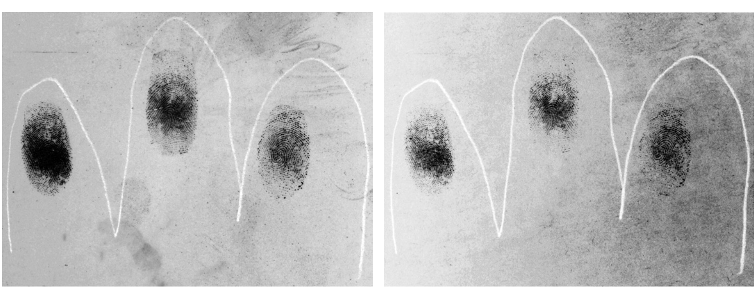
Statistical Analyses

Statistical analysis is required to determine whether there are significant differences in the grades obtained for different variables such as donor age, gender, and so forth. It cannot be assumed that the grades resulting from the analysis of the developed fingermarks are normally distributed. Distribution-free or nonparametric statistical tests, which do not make prior assumptions of the underlying distribution of the data, are more appropriate [18–21]. The nonparametric equivalent of the parametric independent *t* test is a Mann-Whitney U test [19], whereas the equivalent of a paired *t* test is the Wilcoxon signed rank test [20]. In this research, the aging studies required the use of the Wilcoxon signed rank test, because the same samples were re-examined and therefore paired (i.e., in 2009 and 2012). All other data are independent of each other and thus the Mann-Whitney U test was applied. The calculated z value is compared to the critical value, which is ±1.96 at the 95% confidence level, where $z_{calc} > z_{critical}$ indicates that the difference is significant [21]. The calculated probability values (*p* values) below 0.05 indicate that the null hypothesis can be rejected with greater than 95% confidence. The Mann-Whitney U test and Wilcoxon signed rank test analyses were performed with IBM's SPSS Version 2.0 and Microsoft Excel's Sigma-XL add-on.

Results and Discussion

It can be difficult to observe subtle changes when viewing IND-Zn-treated fingermarks in photoluminescence mode because of their very strong luminescence. To make the grading process simpler, all luminescent photos were transformed to negative black and white images. The grades were recorded from the resulting images. A typical example of a freshly developed sample and its state after three years of aging is shown in Figure 1.

The main interest of this study lay with the variation with which an amino acid-sensitive reagent develops latent fingermarks (and therefore the relative amounts of amino acid in sweat within a population). To be significant, this requires a large data set and hence statistical analyses were used rather than reviewing individual results. To this effect, the Mann-Whitney U test and the Wilcoxon signed rank test were employed to investigate the variation in fingermark grades and therefore the relative amounts of amino acids present within a population. Although this study was commenced as a school project, the wealth of information that could be gained by further analyses of the results quickly became apparent. This preliminary research will also form the basis of a much larger donor study, where the fingermark samples will be graded by international collaborators and the population size will be increased to at least 300 donors.



(a) *(b)*

Figue 1

Photographs (negative) of a luminescent fresh fingermark sample (a) and after three years (b). Photographs taken with a Nikon D300 camera in luminescence mode; focal length: 60 mm, shutter speed: 2 seconds, aperture: f/11.

Statistical Analyses

Aging of Developed Fingermarks

As depicted in Table 5, the Wilcoxon signed rank test indicates that the grades obtained from freshly deposited and treated fingermarks [median ($\mu_{1/2}$) = 3, standard deviation (SD) = 1.12] are statistically dissimilar to the grades obtained three years later ($\mu_{1/2}$ = 2, SD = 1.19). The Z score of -8.35 is much greater than the $z_{critical}$ (-1.96) and the probability of incorrectly having rejected the null hypothesis (p) is 1.3E-07. The $\mu_{1/2}$ value shows the median grade that was achieved for that variable, with the SD indicating how close the spread of all the grades are to this median value (lower SD indicates that the grades lie closer together). The large Z score and low *p* value attained in the Wilcoxon signed rank test indicate that there is a substantial and significant statistical difference between the grades attained.

Variable	Age of Sample		
	Fresh		Aged
Number of Donors	120		120
Median	3		2
Mean	2.74		1.89
Standard Deviation	1.12		1.19
p value		1.3E-07	
Z score		-8.35	

Table 5

Statistical values calculated using the Wilcoxon signed rank test.

Although nonparametric tests do not require the calculation of means and standard deviations, the values have been included. They give a better indication of the distribution of the grades than the integers of the medians can give. In addition to the results from the statistical techniques, the number of donors contributing to a fingermark grade of zero increased from 0 in 2009 to 13 in 2012. Also, 40 samples achieved the highest grade of 4 in 2009, which decreased to 12 in 2012 (Table 6). These results suggest that degradation of the Joullié's pink complex is occurring as a function of time. Previous research also suggests that treated samples degrade over time, especially when the reagent formulation does not include zinc [22, 23]. However, the extent of the decomposition is not described in these studies.

Grade	Number of Donors, 2009	Number of Donors, 2012
0	0	13
1	23	40
2	25	26
3	32	29
4	40	12

Table 6

The distribution of the fingermark grades in samples from 2009 and 2012, in reference to the number of donors.

Donor Variability

As previously stated, the quality of IND-Zn-treated fingermarks is not uniform within a donor population, with age, gender, prior activity, and diet thought to be possible causes of amino acid variation [4–8]. Figure 2 is an example where the difference is easily visualized. However, in Figure 3, the differences are much more difficult to observe. In order to deduce meaningful information from the data, rigorous statistical analyses have to be applied.

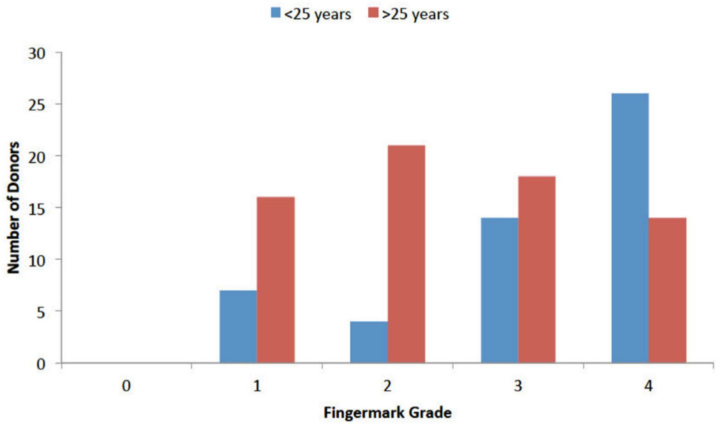


Figure 2

Variability in quality for IND-Zn-developed fingermarks on paper as a function of donor age.

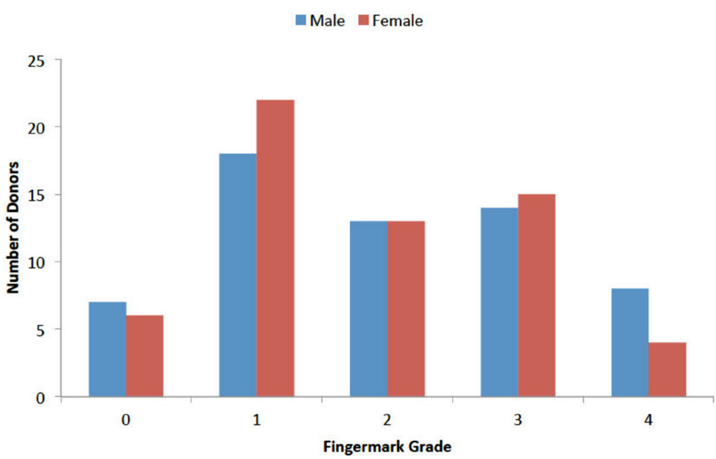


Figure 3

Variability in quality for IND-Zn-developed fingermarks on paper as a function of gender.

To this effect, the nonparametric Mann-Whitney U test was used to evaluate whether statistically significant disparity exists between the nonpaired variables of gender, donor age, food consumption, and washing of hands (Tables 7 and 8).

Variable	Donor Age		Washing of Hands		Gender		Food Consumption	
	Over 25	Under 25	Yes	No	Male	Female	Yes	No
# of Donors	69	51	30	90	60	60	28	92
Median	2	4	2	3	3	3	3	3
Mean	2.44	3.16	2.20	2.92	2.67	2.82	2.61	2.78
Standard Deviation	1.09	1.19	1.01	1.21	1.25	1.13	1.07	1.20
U Score	1101		880.5		1741.5		1166	
Z Score	-3.63		-2.95		-0.32		-0.79	
p value	2.9E-04		3.2E-03		0.75		0.43	

Table 7

Statistical values gained from Mann-Whitney U tests, with the fresh luminescent fingermark grade given as a function of the independent variables.

Variable	Donor Age		Washing of Hands		Gender		Food Consumption	
	Over 25	Under 25	Yes	No	Male	Female	Yes	No
# of Donors	69	51	30	90	60	60	28	92
Median	1	3	1	2	2	2	1	2
Mean	1.48	2.45	1.43	2.04	1.97	1.82	1.54	2.00
Standard Deviation	1.01	1.19	1.01	1.21	1.25	1.13	1.07	1.20
U Score	959.5		968.5		1681.5		994	
Z Score	-4.39		-2.39		-0.64		-1.89	
p value	1.2E-05		1.7E-02		0.52		0.06	

Table 8

Statistical values calculated from Mann-Whitney U tests, with the aged luminescent fingermark grade given as a function of the independent variables.

The initial (2009, fresh) grades from donors over ($\mu_{1/2}$ = 2, SD = 1.09) or under ($\mu_{1/2}$ = 4, SD = 1.07) the age of 25 were found to be significantly different (*p* = 2.9E-04, Z = -3.63) (Table 7). The Z score is -3.63, which greatly exceeds the critical z value of -1.96, and the null hypothesis can be rejected with greater than 99.97% confidence. In effect, the statistics support the idea that there are significant differences between donors over or under the age of 25. In this study, 34 out of the 51 donors under the age of 25 were aged 15 years or younger. Previous research suggests that there is a marked difference, especially within the lipid fraction, between the chemical profile of children's and adults' latent fingermarks [5, 7, 8, 24, 25]. Buchanan et al. explained that surface lipids in children occur because of the epidermis (outer skin layer), rather than sebaceous glands in adults, with sebaceous secretions increasing following puberty [26]. Further, Williams et al. found that the main difference between adults' and children's deposits were the relative ratios of compounds and that proteins, themselves comprised of various amino acids, make up less than 1% in children, but around 5% in adults [25].

A similar statistically significant disparity was observed in the initial fingermark grades as a function of the donors washing their hands ($\mu_{1/2}$ = 2, SD = 1.16) or not ($\mu_{1/2}$ = 3, SD = 1.09, p = 3.2E-03, Z = -2.95) prior to fingermark deposition (Table 7). Again, the very low p value and the large Z score indicate with greater than 99.6% confidence that there is a significant difference in grades obtained for those donors washing their hands compared with those with unwashed hands. This is to be expected, because more of the water-soluble amino acids are present on the surface of unwashed hands and are thus transferred and available to react with IND-Zn.

There appears to be no statistically significant dissimilarity between recent food consumption or handling or not (p = 0.43) or gender of the donor (p = 0.75) as a function of the fresh fingermark grade (Table 7). For instance, there did not appear to be a contribution to the fingermark grade from either food residues or the gender of the donor. With regards to the food consumption, this only refers to donors who physically handled food with their hands and not the effect their diet has on fingermark deposits. Although there is speculation that the gender may affect the chemical composition of fingermark deposits, no conclusive results have been found in the literature, and the Mann-Whitney test outcomes in this respect are not surprising.

All binary combinations of the variables were also investigated, by comparing subgroups (age and gender, e.g., female donors over 25 and under 25, etc.). No significant interactions were found, with the exception of the washing of hands, which was found to override the age of donor factor. Washing would remove appreciable quantities of amino acid and hence diminish the age-related difference. This implies that the washing of hands is more crucial to the fingermark grade and overrides the observed age differential.

All of the trends that were found for the initial grades above were also observed in the grades from independent variables for three-year-old (2012) fingermarks (Table 8). However, in the case of food consumption and handling, the aged prints returned a higher grade if the donors had not consumed food, as opposed to those who had eaten recently. This would suggest that chemical components transferred onto the hands from the food increases the rate of degradation for the Joullié's pink complex. The specific chemistry that may account for this is as yet unknown.

Conclusion

This study reinforces that variation of amino acids in sweat exists within a population and is detectable through examination of 1,2-indanedione-treated latent fingermarks. Treatment with IND-Zn allowed initial grading for fingermarks observed in both absorbance and luminescence mode. Although 81% of the samples achieved grades of 2 or above in luminescence mode initially, this decreased to 56% after the treated samples had aged three years. The Mann-Whitney U test and Wilcoxon signed rank test in conjunction with the Z score were utilized as statistical tools to assess fingermark grade variation. These tests indicated that grades of developed fingermarks vary significantly between fresh and aged fingermarks, the age of the donor, and the washing of hands prior to deposition. Donors below the age of 25 offered superior grades, as did donors who did not wash their hands. However, the washing of hands prior to fingermark deposition was found to override the observed age of donor variation. No significant difference between the fingermark grade and food consumption or the gender of the donor was observed.

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