Investigations into the influence of donor traits on the performance of fingermark development reagents. Part 1: 1,2-indanedione/zinc chloride

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Abstract

This study outlines the use of 1,2-indanedione/zinc chloride (IND/$ZnCl_2$) to treat fingermarks with a view to observing possible trends that may be present in a donor population. Fingermark samples from 131 donors were treated and subsequently evaluated using the grading scale devised by the Home Office Police Scientific Development Branch (now the Centre for Applied Science and Technology; CAST), UK. Out of a total of 1310 grades assigned, only 6 (0.5 %) returned a score of 0 and 64.6 % of all grades assigned were a 3 or 4. These tests indicated that grades for fingermarks developed within 3 days vary significantly depending on the age of the donor and the washing of hands prior to deposition. Donors who did not wash their hands the hour prior to deposition, or were below the age of 25, were more likely to offer higher grades. With fresh fingermarks, no significant variation in fingermark grades was observed that could be associated with food consumption, sex of the donor or recent use of cosmetics. The results for the treated 1 month old fingermarks agreed with the findings for fresh fingermarks, with the exception of washing of hands. In this case no significant difference was found between samples where donors had and had not washed their hands prior to deposition.

Introduction

It has been observed that the quality of 1,2-indanedione/zinc chloride (IND/$ZnCl_2$) treated fingermarks is not uniform within a donor population, with age, sex, prior activity and diet of the donor all thought to be possible causes of the amino acid variations [1-5]. Although various studies examining the amino acid concentration profile in human sweat have been conducted, these either did not focus on the amino acid variation in latent fingermarks, or had too few donors to be statistically significant [6-12]. As can be inferred from the 1,2-indanedione reaction scheme presented by Petrovskaia *et al.*, the type and amount of amino acid present in the fingermark deposits will affect the observed development intensity [13]. Similar findings have been seen with amino acid spot tests treated with ninhydrin and 1,8-diazafluoren-9-one [14]. Differences in development intensity upon treating fingermarks may be used to discern variations correlating to the donor given the dependence of fingermark composition.

A previous study by Fritz *et al.* used this concept to investigate the effect that the donor traits and habits may have on the development of IND/$ZnCl_2$ treated fingermarks [15]. The grading scheme used in that study was devised by the Home Office Police Scientific Development Branch (now the Centre for Applied Science and Technology; CAST), UK [16]. Assessment of the treated fingermarks from 120 donors indicated that there may be a correlation between the grade and the age of the developed mark, age of the donor and the prior washing of hands. However, no link was found between the food consumption or gender of the donor and the fingermark grade. In that particular study, the age of treated fingermark deposits was investigated rather than letting the samples age prior to treatment. Also, as only one grader assessed the samples, bias could potentially have occurred and not been recognised. Lastly, further donors and testing variables would enhance the potential conclusions that can be drawn.

This paper presents the first half of a study into the variables affecting fingermark detection. As part of a collaborative effort, a panel of 5 graders from institutions around the world were involved in the planning and evaluation of this study. In this first instalment, three questions were proposed to be answered:

1) How does IND/$ZnCl_2$ perform on fingermarks deposited by a large range of donors?
2) How does short-term ageing over one month affect this development?

3) Can any trends in treated fingermarks be observed for specific donor traits and/or habits?

These questions are part of a larger and on-going investigation into the fundamental chemistry of deposited latent fingermarks. As such, the sample collection employed in this study identified possible donor groups for more targeted chemical profiling experiments which are vital to better understand the composition of latent fingermarks.

Aims

The aims of this study were to use a larger donor age range than that employed in the original work by Fritz *et al.*, to include the use of a grading panel, and to determine whether ageing of the deposit affects the grades assigned to samples [15]. In addition, the efficacy of how well the IND/$ZnCl_2$ reagent performs across a varied donor population was investigated, as well as using appropriate statistical methods to evaluate the data.

Materials and method

Chemicals

1,2-Indanedione (Casali; Optimum Technology, Australia), anhydrous zinc chloride (BDH, Australia), 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.

Collection and treatment of latent fingermarks

Fingermark samples were collected over a period of 18 months from locations comprising the Curtin University Bentley campus, primary schools, retirement villages and special interest groups located in Perth, Western Australia. Donors were instructed to gently place uncharged index, middle and ring fingertips onto white copy paper (Fuji Xerox Professional, 80 g/m^2) for 10 seconds, where the middle finger was placed on a printed line separating two squares. These gridlines formed 16 squares and facilitated the collection of a 3-print depletion series of 4 impressions with each hand, where the 3-finger impressions were split down the middle fingermark. Fingermark samples from 131 donors were collected, where all fingermark donors were required to provide information pertaining to their age, biological sex, food consumption, washing of hands and other recent activity (Table 1).

Table 1 Donor information with regards to the number of donors for each variable in the donor study (n=131).

Variable	Grouping	Number of Donors
Biological sex	Male	*67*
	Female	*64*
Age	30 and over	*56 (M:31, F:25)*
	Under 30	*75 (M:36, F:39)*
Food consumption (< 1 hr prior)	Yes	*69*
	No	*62*
Washing of hands (< 1 hr prior)	Yes	*57*
	No	*74*
Recent cosmetics use (< 12 hrs prior)	Yes	*52*
	No	*79*

One half of the split samples were treated with IND/$ZnCl_2$ within 3 days, and the other half 1 month later (stored in a darkened cupboard under controlled laboratory conditions). The IND/$ZnCl_2$ method outlined by the National Centre for Forensic Studies was employed [17-20]. Samples were dipped briefly in the working solution (Table 2) and allowed to air dry before being heat treated for 10 seconds in an Elna laundry press (model Alize, with the highest heat setting used at *ca.* 160 °C) [17, 18].

Table 2 Preparation of the IND/$ZnCl_2$ stock and working solutions.

	Solution	Reagent Preparation
Wet Contact IND/$ZnCl_2$	*IND stock solution*	4 g 1,2-indanedione dissolved in 450 mL ethyl acetate and 50 mL glacial acetic acid
	$ZnCl_2$ stock solution	8 g zinc chloride dissolved in 200 mL absolute ethanol
	Working solution	2 mL zinc chloride stock solution and 50 mL stock solution added to 450 mL HFE-7100 solvent

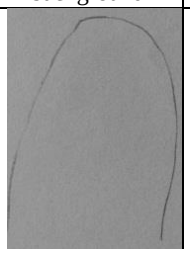
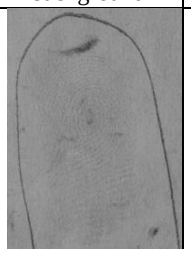
Photography of samples

Samples were photographed using a Nikon D300 camera, equipped with an AF-S Micro-Nikkor lens, mounted on a Firenze Mini Repro tripod and connected to a computer using Nikon's Camera Control Pro Version 2.0.0. Illumination in the luminescence mode was achieved using a Rofin Polilight® PL500 (Rofin, Australia), with an excitation wavelength of 505 nm (40 nm bandwidth) and an orange 550 nm long-pass barrier filter on the camera (Foster + Freeman Schott OG550). The photographic conditions were as follows: focal length 60 mm; shutter speed 1 sec; aperture f/11; sensitivity ISO 200; and auto white balance. Evaluation of the fingermark development was carried out on raw images (unprocessed).

Data distribution and assessment of developed latent fingermarks

262 original sample images and 19 replicates were ranked independently by 5 graders. To reduce the effects of exhaustion, stress, etc., and to make the process less overwhelming, the samples were distributed to the graders in 5 batches. This was implemented by numbering the samples and then randomly assigning them to one of the batches using the random number generator in Microsoft Excel Professional Plus 2010. The images were distributed to fingermark assessors via an online cloud program, Dropbox (v.1.4.8). Each assessor was required to grade their batch of samples, before the next batch of images was released. All treated fingermarks were graded using a 5-point system based on that used by CAST (Table 3) [16].

Table 3 Grading system for developed latent fingermarks based on the CAST scale [16].

Grade	0	1	2	3	4
Friction Ridge Detail Development	No development	Signs of contact, but less than 1/3 of fingermark continuous ridges	1/3 – 2/3 of fingermark continuous ridges	More than 2/3 of fingermark continuous ridges, but not quite a 'perfect' fingermark	Full development; whole fingermark, continuous ridges
Background Development	Heavy background	Heavy background	Medium background	Very light background	No background
Photographic Representation					

Statistical analyses

Cohen's kappa test was used to determine the level of agreement for the paired grades resulting from duplicated images to test for intra-grader consistency [21, 22]. The inter-grader consistency was assessed using the intraclass correlation coefficient (based on the 80 original sample images) [23]. The Wilcoxon signed rank test was used to identify any changes in the median rank from the first to the second assessment to paired data resulting from re-grading the same samples after three years. Independent data (i.e., donor traits and habits) were evaluated using the Mann-Whitney U test. The calculated z value was 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 [24]. Statistical analyses were performed using SPSS version 2.0, and a p-value < 0.05 was taken to indicate a statistically significant association in all tests.

Results and discussion

Fingermark samples from 131 donors representing a range of ages of both biological sexes were collected as part of a collaborative study (Table 1). In a similar manner to the pilot study by Fritz *et al.*, one half of the split fingermark samples were treated with IND/$ZnCl_2$ within 3 days of their collection [15]. However, unlike the pilot study where all samples were regraded 2 years after their treatment, the second half of the donor study samples was treated and subsequently graded one month after the first half. The median grade of 5 fingermark graders was used for all statistical analyses, including the Wilcoxon signed rank test and Mann-Whitney U tests. Repetition of the statistical tests using the average scores of the 5 graders showed no significant difference to the results obtained using median values. The study highlighted the efficacy of IND/$ZnCl_2$, as only 6 samples out of a total 1310 grades given (i.e., 0.5 %) returned a score of 0 and 64.6 % of all grades assigned were a 3 or 4.

Grader variation

As a form of quality control, 19 fingermark images were replicated and graded at different points in time by all graders. 78 % of the duplicated images were graded the same as the originals, which is similar to the result obtained in a preceding study investigating the variability of grading processes (Figure 1) [25]. As before, the intra-donor variation was observed more often in fingermark samples that may be harder to classify (i.e., grades of 2 or 3).

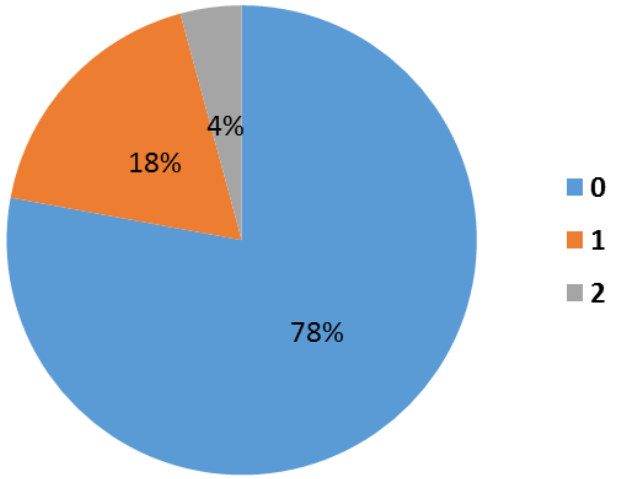


Figure 1 Differences between grades assigned to replicated images.

Cohen's kappa tests were performed (Table 4), indicating that there was good to very good agreement between the original and duplicated grades for each grader (Cohen's kappa value

= 0.683, $p < 0.0005$) [21, 22, 26, 27]. The grades given by grader 1 were consistently lower than those of the other graders, where it is thought that this was due to this grader's much more expansive experience with fingerprints. However, the intraclass correlation coefficient value of 0.847 demonstrated that there was still a strong agreement between all 5 graders. The lower and upper confidence intervals show that, 95 % of the time, the assigned grade will give a correlation between 0.749 and 0.899, further reinforcing the strong level of agreement between the graders.

Table 4 Statistical values gained from the Cohen's kappa test, where the original scores given by each grader were compared to the ones assigned to the duplicates (n=19).

Grader	1	2	3	4	5
Median (original)	2	3	3	3	3
Mean (original)	2.1	2.6	2.9	2.4	3.1
Median (duplicates)	2	3	3	3	3
Mean (duplicates)	2.2	2.8	2.8	2.5	3.3
Std. Dev. (original)	1.10	1.16	0.94	1.22	0.94
Std. Dev. (duplicates)	1.01	0.92	1.01	1.31	0.82
Cohen's kappa value	0.778	0.778	0.849	0.494	0.517
p value	<0.0005	<0.0005	<0.0005	<0.0005	0.001

Ageing of deposited fingermarks

Having established that the fingermarks were graded consistently, statistical approaches could be applied to the sample data. In the pilot study, the relative stability of the IND/$ZnCl_2$-amino acid reaction product was evaluated. In this project, the effect that natural deposit ageing may have on fingermark grades was compared by reference to the results from fresh fingermarks (Table 5).

Table 5 Statistical values calculated using the Wilcoxon signed rank test on the median results of 5 graders.

Age of sample	Fresh	1 month old
Number of Donors	131	131
Median	3	3
Mean	2.86	2.97
Standard Deviation	0.95	0.86
p-value	0.047	
Z score	-1.984	

The results of the Wilcoxon signed rank test indicate that the median grades for the treated fresh fingermarks ($\mu_{1/2}$ = 3, SD = 0.95) are not statistically the same as the median grades for the sample halves treated 1 month later ($\mu_{1/2}$ = 3, SD = 0.86). The Z score of -1.98 is greater than the $z_{critical}$ (-1.96) and the probability of incorrectly having rejected the null hypothesis (p) is 0.047. The closeness of both the Z score and the p value to the decision making values indicates that, while the null hypothesis is rejected, there may not be a strong statistical difference between the medians of the grades attained. This can be seen in Table 6 and Figure 2, where the very similar distribution of grades given for the fingermark samples may suggest that the effect of ageing (at least within this timeframe) is minimal. The stability of latent fingermarks deposited on porous substrates is well established, and this finding is supported by literature where the successful development of fingermark samples over 40 years old has been reported [28].

Table 6 The distribution (%) of grades given for fingermarks treated immediately and after one month.

Grade	Fresh (%)	1 month old (%)
0	1	0
1	15	14
2	21	20
3	30	33
4	33	34

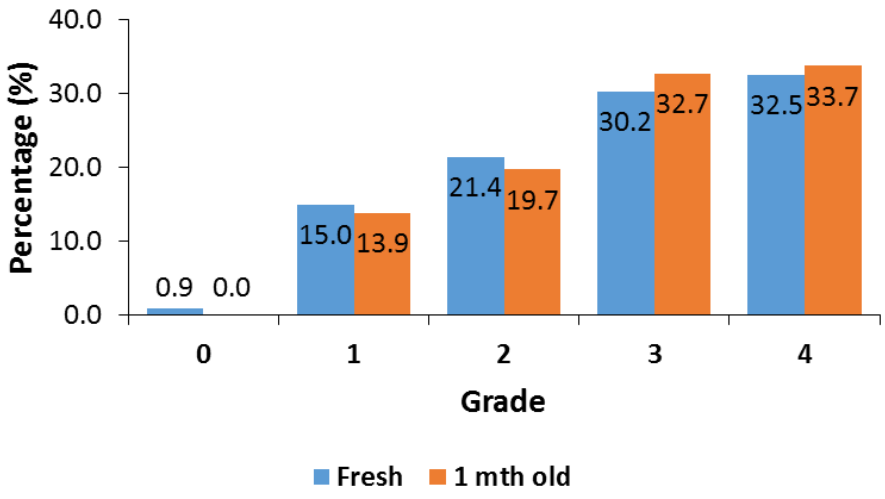


Figure 2 Variability in quality for IND/$ZnCl_2$ developed fingermarks on paper as a function of the age of the sample prior to treatment.

Donor variability

The treatment of fingermark deposits from 131 donors was undertaken to see if this would reinforce the findings of the pilot study. Grades associated with some donor traits could be readily distinguished; for example, the effect of the age of the donor (Figure 3). As Figure 4 demonstrates with the biological sex of the donor, other traits may appear to offer negligible differences. Therefore, the non-parametric Mann-Whitney U test was again used to discern whether there were statistical differences between grades associated with the independent donor traits.

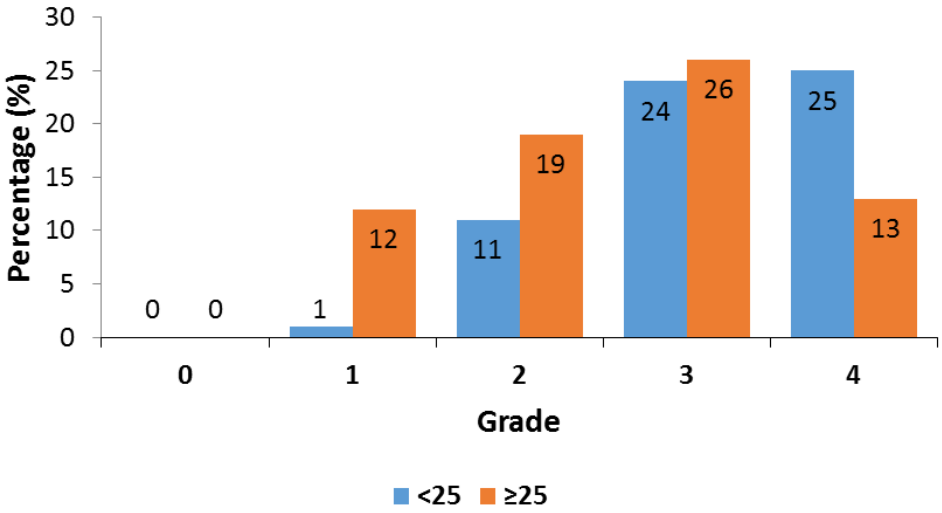


Figure 3 Variability in quality for IND/$ZnCl_2$ developed fingermarks on paper as a function of donor age.

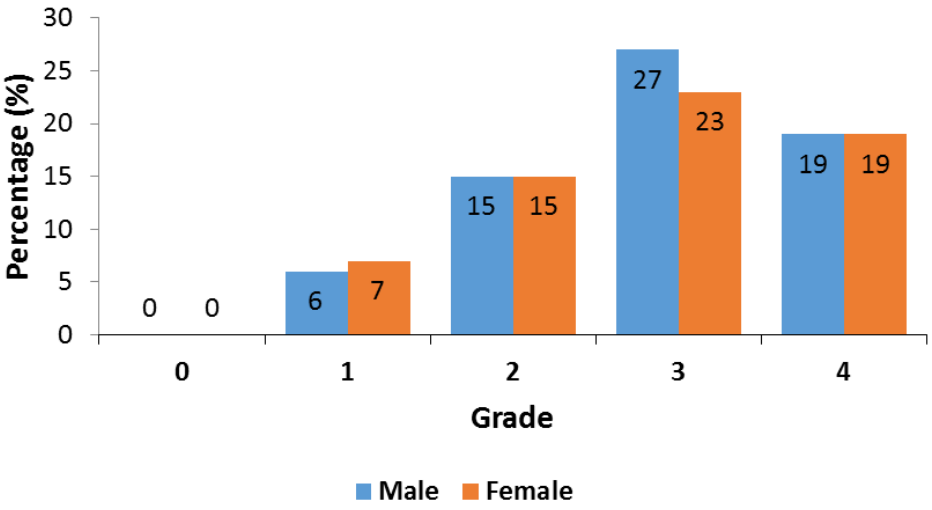


Figure 4 Variability in quality for IND/$ZnCl_2$ developed fingermarks on paper as a function of sex.

The grades given to fresh fingermark deposits indicate that there was a significant difference ($p = 2.7 \times 10^{-4}$, $Z = -3.638$) between donors over ($\mu_{1/2} = 2$, SD = 0.95) and under the age of 25 ($\mu_{1/2} = 3$, SD = 0.81) (Table 7). The Z score is much larger than the critical value ($Z = -1.96$), meaning that the null hypothesis can be rejected with greater than 99.99% confidence. These findings are in agreement with the results of the pilot study, further reinforcing that differences in the amino acid content may exist as a function of donor age.

A significant difference was also found due to the washing of hands within the hour prior to fingermark deposition ($p = 0.041$, $Z = -2.04$), and again this reinforces results from the pilot study. The median values of donors who had washed their hands ($\mu_{1/2} = 3$, SD = 0.93) or not ($\mu_{1/2} = 3$, SD = 0.95) were significantly different and the null hypothesis can be rejected with 97.93 % confidence (Table 7). As amino acids are largely water soluble, it is not surprising that lower grades (i.e., less reaction with IND/$ZnCl_2$) were given to donors who had washed their hands prior to fingermark deposition.

No statistically significant dissimilarity was found between the grades given to treated fresh fingermarks from donors who had or had not recently consumed food (<1 hour, $p = 0.44$), applied cosmetics (<12 hours, $p = 0.65$), or due to the biological sex of the donor ($p = 0.87$) (Table 7). The food consumption and biological sex results once again mirror the pilot study findings.

Table 7 Statistical values gained from Mann-Whitney U tests applied to the fresh fingermark grades and given as a function of the independent variables.

Variable	**Donor age**		**Washing of hands (<1 hr)**		**Biological sex**		**Food consumption (<1 hr)**		**Recent cosmetics (<12 hrs)**	
	25 and over	**Under 25**	**Yes**	**No**	**Male**	**Female**	**Yes**	**No**	**Yes**	**No**
# of Donors	70	61	57	74	67	64	69	62	52	79
Median	3	3	3	3	3	3	3	3	3	3
Mean	2.57	3.20	2.68	3.00	2.85	2.87	2.81	2.92	2.81	2.90
Standard Deviation	0.99	0.79	0.93	0.95	0.95	0.96	0.94	0.96	1.03	0.90
U score	1384.0		1690.5		2109.5		1978.5		1963.0	
Z score	-3.638		-2.040		-0.167		-0.777		-0.449	
p-value	2.7×10^{-4}		0.041		0.868		0.437		0.653	

It is interesting to note that the trends found for the age ($p = 0.04$) and biological sex ($p = 0.46$) of the donor, as well as the recent use of cosmetics ($p = 0.35$), for 1 month old fingermarks agree with those of fresh fingermarks. The washing of hands ($\mu_{1/2} = 3$, SD = 0.89), however, did not have a significant impact on the grade given ($p = 0.085$, $Z = -1.72$) compared to donors who had not washed their hands ($\mu_{1/2} = 3$, SD = 0.83). This is in contrast to the findings with fresh fingermarks, yet the similarity of the median and mean scores (Table 8) indicates that both results were quite similar, where a 10% higher average grade was given in both cases to samples deposited by donors who had not washed their hands.

Though the influence of food consumption was found to be insignificant for grades given to both fresh and 1 month old fingermarks, there was a marked decrease in p value from 0.44 to 0.09 over this time period. An extended experimental timeframe may find a significant difference between fingermarks from donors who have and have not recently consumed food prior to fingermark deposition, for results obtained over one month following deposition. A trend of this nature was observed in the initial pilot study, where it was proposed that certain chemical components present in food may increase the rate of Joullié's Pink degradation [15]. These results suggest that recent food consumption may indeed cause faster decomposition of both reacted and unreacted amino acids. One potential cause could be that food contamination of the fingers may result in higher levels of bacteria on the skin, increasing the rate of amino acid degradation through microbial activity. The cause of this trend should be considered and investigated in greater detail in future studies.

Table 8 Statistical values gained from Mann-Whitney U tests applied to the 1 month old fingermark grades and given as a function of the independent variables.

Variable	**Donor age**		**Washing of hands**		**Biological sex**		**Food consumption**		**Recent cosmetics (<12 hrs)**	
	25 and over	**Under 25**	**Yes**	**No**	**Male**	**Female**	**Yes**	**No**	**Yes**	**No**
# of Donors	70	61	57	74	67	64	69	62	52	79
Median	3	3	3	3	3	3	3	3	3	3
Mean	2.69	3.06	2.82	3.10	2.99	2.97	2.84	3.13	2.87	3.05
Standard Deviation	0.92	0.77	0.89	0.83	0.97	0.74	0.92	0.78	0.95	0.80
U score	1708.0		1758.5		1993.5		1792.5		1864.5	
Z score	-2.086		-1.723		-0.734		-1.691		-0.944	
p-value	0.037		0.085		0.463		0.091		0.345	

Conclusions

Complimentary data to that presented in the previous study by Fritz *et al.* was generated in the present project. Cohen's kappa tests showed good to very good agreement between the original and duplicated grades for each of the 5 graders used in this study (Cohen's kappa value = 0.683, $p<0.0005$). The intraclass correlation coefficient value of 0.847 also demonstrated that there was strong overall agreement between all 5 graders.

A statistically significant difference was found between fingermark samples treated and graded within 3 days of deposition to those treated after one month; however, the actual distribution appeared to be very similar. Out of a total of 1310 grades given, only 6 (0.5 %) returned a score of 0 and 64.6 % of all grades assigned were a 3 or 4. These tests indicated that grades of fingermarks developed within 3 days vary significantly depending on the age of the donor and the washing of hands within one hour prior to deposition. Donors who did not wash their hands the hour prior to deposition, or were below the age of 25, were more likely to result in higher grades in developed fingermarks. No significant variation between the fingermark grade and food consumption, sex of the donor or recent use of cosmetics was observed with fresh fingermarks.

Statistical analyses of samples that were treated after one month agreed with the results of the fresh fingermarks, except in the case of the washing of hands. Here, the Z score (-1.723) and p value (0.085) indicated that there was no dissimilarity between donors who had and had not washed their hands the hour prior to fingermark deposition. While recent food consumption was not found to have a statistically significant effect in these studies, longer timeframes may potentially reveal that treated and untreated amino acids degrade faster after the recent consumption of food, possibly due to increased bacterial action.

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