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PAPER

CRIMINALISTICS

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The Differentiation of the Volatile Organic Signatures of Individuals Through SPME-GC/MS of Characteristic Human Scent Compounds

ABSTRACT: Human scent evidence is utilized as an investigative tool through canine scent discriminations based on the premise that human scent is an individualizing characteristic. This study describes the development of what is effectively a human scent barcode consisting of the relative ratios of an individual's "primary odor" compounds utilized to determine a reproducible and individualizing profile which can be stored in a searchable database for a proof of concept of human scent as a biometric measure. Triplicate hand odor samples were evaluated from 10 subjects utilizing solid phase micro-extraction gas chromatography/mass spectrometry (SPME-GC/MS) and compared via Spearman Rank Correlations. Narrowing the compounds considered for each subject to only those common in all three samples, or a subject's "primary odor constituents," produced a greater degree of both individualization and discrimination; at both correlation thresholds of 0.9 and 0.8, the individuals were correctly discriminated and identified in 99.54% of the cases.

KEYWORDS: forensic science, human scent evidence, primary odor, solid phase micro-extraction gas chromatography/mass spectrometry, Spearman Rank Correlations, individualization

Human scent profile analysis and comparison is an area of analytical research which has garnered a great deal of interest in recent times. The medical community's interests lie in the possible use of the volatile organic compounds (VOCs) released by the human body as a diagnostic tool for disease and the forensic community's interest is primarily in the use of human scent evidence as an investigative tool. Human odor has long been cited as a probable parameter containing individualizing characteristics that can be exploited as a biometric measure; however, proof of concept for the instrumental interrogation of this physical characteristic has yet to be achieved.

Genetic Basis for Individualizing Body Scents

A great deal of research focus in recent years has been on determining the link between genetics and body odors. The idea that an individual's body odor is distinctively linked to polymorphic genes within the major histocompatibility complex (MHC) was first suggested in 1974 (1). The MHC consists of polymorphic genes, which contain extreme nucleotide diversity as high as 8.6%, as compared to the nucleotide diversity of the human genome, which has been estimated to be between 0.08% and 0.2% (2). In recent years, a number of studies have been conducted in an attempt to elucidate the genetic link and biological pathway for body odor generation (3–10). Currently, the pathway through which the MHC influences body odors is not known. A model integrating different hypotheses suggests that soluble MHC proteins play a central role in the production of MHC-associated odors (11). MHC molecules may bind to specific allele subsets of peptides, and their volatile metabolites such as carboxylic acids then provide the odorants. Furthermore, MHC genes may alter odor by shaping populations of microbial flora (12). The genetic basis for individualizing body odors has been studied extensively in genetically engineered mice which differ in respect to the genes present in the MHC (13–18). Individual body scents of mice can be altered by modification of a single gene within the MHC (18).

Human Scent Variability Studied Through Canines

The ability of canines to discriminate humans based on their scent has been documented in the scientific literature as early as 1887 (19). George J. Romanes contributed many fundamental observations as to the ability of dogs to scent discriminate among humans such as the human body leaves an individual odor which a dog can distinguish, individual odors can be determined at great distances and under different environmental stresses, and that canines are not deterred from scent discrimination by fragrances.

The ability of dogs to match odor collected from different parts of the body has also been evaluated by several groups producing differing results. Dutch police dogs were shown to be able to match scent collected from hands to scent collected from the crook of the elbow from the same individual 32% of the time, which is greater than 16.7% due to chance alone. These dogs also showed the ability to match odor collected from the hands to scent collected from pants pockets of the same individual (20). Studies in the United Kingdom have also shown that dogs possess the ability to match scent to that

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of an individual taken from various places of the body with a success rate above 80% where that due to chance was 16.7% (21).

Limited research has been conducted into the ability of traditionally trained bloodhounds to discriminate the scent of individuals through trailing (22). Hepper (23) demonstrated that dogs have the ability to distinguish between twins who are nonidentical (ages 3–5 months) and live under the same environmental conditions, i.e., soaps, food, clothing, etc. and identical twins (ages 34 and 50 years) who live under different environmental conditions. In the same study, canines were unable to differentiate between identical twins who live under the same environmental conditions. A more recent evaluation consisted not only of twins but closely related individuals and focused on the variable of genetic relatedness and its effect on bloodhound scent discrimination. It was concluded that environmental odors could affect the odor discrimination, but more importantly to the bloodhound is the genetic odor type of the subject for trailing and scent discrimination purposes (24).

Human Scent Production

Forensically, odor collected from the hand is of primary interest as it is this region of the body where known samples of human scent are most often collected by law enforcement for use by scent discrimination canines in comparing human scent collected from evidentiary items (25). The ability of canines to distinguish the odors of humans collected from the hands over long periods of time (26) suggests that human scent is stable over time, or that portions of an individual's odor profile are stable even though elements of the odor profile may change. Hand odor is a combination of eccrine and sebaceous gland secretions without the involvement of the apocrine gland, which contributes immensely to the malodors generated from the armpit region. Alterations to portions of the odor of an individual may occur due to the influence of illness, the onset of puberty, the menstrual cycle in females, etc. Many of these factors directly affect the apocrine gland. The secretions obtained from the eccrine and sebaceous glands are less likely to be influenced by these changes, thereby more likely to produce the stable odor of an individual.

The production of human scent is a complex process that is yet to be fully understood. It is known that the epidermis (outer) layer of the skin constantly sheds epithelial cells into the environment. The surface of the skin contains about two billion cells, of which 1/30 are being shed daily (approximately 667 cells/sec). The average lifespan of an epithelial cell is approximately 36 h. Dead cells which are shed from the surface of the skin are sometimes referred to as "rafts" which are approximately 14 μ in size and weigh approximately 0.07 μg. The "raft" is composed of one or more dead cells, approximately four microbial bacteria, and body secretions, of which all three components are said to be characteristic to the individual. Each "raft" is also said to be surrounded by a vapor cloud, which results from bacterial action upon the cells (27). Studies conducted by the National Institute for Medical Research in London have shown that there is a current of warm air which surrounds the human body (28). The air current is approximately one-third to one-half inch thick and it travels up and over the body at a rate of 125 feet per minute. Analysis of the air current on the surface of the human body showed that it contained four to five times as many "germs" as the air in the rest of the sampling room. The "germs" come from the bacteria that are shed off with dead skin cells, larger flakes of skin fall to the ground but smaller ones are drawn up into the current. These currents can also be visualized running along the outside of clothing. The warm air currents are said to carry the "rafts" from the body into the surrounding area allowing for the deposit of human scent in the environment.

The idea that human scent is produced through bacterial action on dead skin cells and secretions is the most common depiction of the creation of human odor. Other studies have suggested that odor is formed very quickly, supporting the idea that odor production is due to simple bond cleavage as opposed to a complex bacterial action (29). Comparisons of the extracts of axillary sweat collected from both males and females showed qualitative similarities in the volatile organic acids present, suggesting a similar origin and mechanism for odor production in men and women (30).

Eccrine and Sebaceous Glands

The eccrine glands can be found throughout the body, with the highest densities found in the palms of the hands and the soles of the feet. In a normal individual, eccrine glands are capable of secreting up to 2–4 L of fluid per hour. Pure eccrine secretions have been shown to be white in appearance (31), and typically composed of 98% water, but it also contains various organic and inorganic components (32). Eccrine sweat originates in the extracellular fluid and, therefore, reflects the chemistry of blood plasma (33).

The sebaceous glands are usually located in body regions where hair is present, including the face and scalp. Sebaceous glands produce secretions called sebum, which consists of glycerides, free fatty acids, wax esters, squalene, and cholesterol. A wide variety of organic compounds can be found in the sebum, which can be influenced by an individual's diet and genetics. This milky white secretion hydrates and preserves the natural health of the outermost layers of skin and also plays a role in the odor signal (34). The hydrolysis of human sebum results in the formation of a mixture of fatty acids, and the amount of free fatty acids in sebum can vary but averages between 15% and 25% (32). Investigations into the biochemical uniqueness of skin lipids have suggested that slight differences in the overall composition of the sebaceous fatty acid mixture could lead to unique individual odors in humans (35).

Instrumental Evaluation of Human Scent

The ability of canines to discriminate individuals on the basis of human scent is rooted in the hypothesis that human scent is stable over time and distinguishable between individuals. Scientific research into the ability of canines to distinguish between individuals based on their scent supports this theory (20–24,26). The authors have developed distinguishing terminology for the different categories of components present in a human scent profile: the "primary odor" of an individual contains constituents that come from within and are stable over time regardless of diet or environmental factors; the "secondary odor" contains constituents which also come from within and are present due to diet and environmental factors; and the "tertiary odor" contains constituents which are present because they were applied from the outside (i.e., lotions, soaps, perfumes, etc.) (36). Until now, there has been limited research as to the VOCs which comprise human scent and their usefulness in distinguishing analytically between individuals (36–40).

One of the first steps in determining the viability of utilizing human scent as a biometric measurement is studying the frequency of occurrence of compounds extracted in human scent across a large population to determine the variability of human scent profiles among individuals. In a previous study by the authors (40), a large scale survey of the VOCs present in the headspace above collected hand odor samples was conducted across a 60 subject population. Solid phase micro-extraction combined with gas

chromatography/mass spectrometry (SPME-GC/MS) was utilized for the extraction and analysis of the hand odor samples with Spearman Rank Correlations utilized for comparisons of human odor compounds among individuals. Spearman Rank Correlations were demonstrated to be a viable method of data handling and a high degree of distinction was attained among the subjects. This study also demonstrated that a high degree of variability was present among the population and clearly demonstrated the importance for determining a human odor baseline on an individual basis. The purpose of this study is to determine the utility of "primary odor" components determined on an individual basis as a proof of concept for attaining a reproducible and individualizing profile for a human scent biometric measure consisting in effect of a barcode representing the relative ratios of the individual's primary odor compounds, which will then be compared among the group as well as a previously compiled database of hand scent profiles to further evaluate the ability to instrumentally utilize human scent as a biometric measurement.

Materials and Methods

Materials

Supercritical fluid extraction (SFE) using methanol modified carbon dioxide was used as a pretreatment for the gauze resulting in an analytically clean collection medium (36,40). Gauze pads were DUKAL brand, sterile, 2 × 2, 8ply, gauze sponges (Dukal Corporation, Syosset, NY). The vials used to hold the gauze were 10-mL glass, clear, screw top vials with PTFE/Silicone septa (Supelco, Bellefonte, PA). The extraction solvent for the pretreatment of the gauze pads by supercritical fluid extraction was supercritical grade carbon dioxide (Air Products, Allentown, PA). The methanol used as the modifier for the pretreatment of the gauze pads was HPLC grade (Fisher Scientific, Pittsburgh, PA). The soap used by the subjects to wash the hands and forearms was Natural, Clear Olive Oil Soap from Life of the Party (North Brunswick, NJ).

Pretreatment of Gauze Pads by Supercritical Fluid Extraction

The equipment used was an ISCO Model 260D Syringe Pump with an SFX 2-10 Supercritical Fluid Extractor. Each supercritical fluid extraction began by filling the plastic extraction vessel with two pieces of sterile gauze pads. The optimum SFE conditions developed to extract organic volatile compounds from sterile absorbers included: a 30-min static extraction time followed by a 10-min dynamic extraction time with an extraction temperature of 130°C, a pressure of 4500 psi, and the direct spiking of 500 μL HPLC grade methanol into the extraction vessel (36). These samples were analyzed by similar SPME-GC-MS procedure for compound identification and quantification employed to analyze scent samples described later in the text.

Method for Hand Odor Sampling

Ten subjects were evaluated: five males and five females ranging in age from 17 to 28 years old. The subjects were volunteers from a multi-national university campus and were not asked to alter or maintain any facet of their diet or routine prior to sampling. The sampling protocol has been utilized previously (40) and was as follows: 30 sec of washing the hands and forearms with olive oil-based soap, 2 min of rinsing the areas with cool water, 2 min of air drying, followed by 5 min of rubbing the palms of the hands over the forearms. A pre-treated 2 × 2 sterile gauze pad was then removed from the 10 mL glass vial using tweezers previously rinsed with a 10% bleach solution and placed in the palms of the subjects' hands. The subjects then sampled themselves by holding the pretreated gauze between the palms of their hands, walking outdoors for 10 min and then resealing the sample back into the 10 mL glass vial. The period of time for sampling and method of sampling through holding the material was modeled after the Netherlands National Police (KLPD) method for the collection of human scent for line-up investigations (41). Three samples were collected from each subject nonsequentially throughout a 12 h period. All samples were stored in the 10 mL vials at room temperature, and allowed to sit for approximately 24 h prior to headspace extraction. These storage conditions were chosen to simulate the conditions under which odor is collected for canine evaluation purposes, and no attempt was made to control microbial interactions with the substrate as it may make contributions to the overall odor profile.

Headspace Extraction and Analysis of Hand Odor Samples (SPME-GC/MS)

Divinylbenzene/Carboxen on Polydimethylsiloxane (CAR/DVB on PDMS) 50/30 μm fibers (Supelco) were used to extract the VOCs from the headspace of the vials containing the scented gauze. Exposures were conducted at room temperature for 21 h, which was previously determined to be the optimal extraction time for hand odor samples (40). The samples were then analyzed by GC/MS using an Agilent 6970 GC with a 5973 MS and the column used was an HP5-MS, 30 m, 0.25 μm, 0.25 mm with helium as the carrier gas (flow rate: 1.0 mL/min) was used for the separation and analysis of the analytes. The analytes were desorbed in the injection port of the GC with an inlet temperature of 250°C. The GC method can be found elsewhere (40) and has a total run time: 33 min. The mass spectrometer used was an HP 5973 MSD with a quadrapole analyzer in full scan mode (mass range: 50–550). The compounds were identified through standard comparison as well as the utilization of the NIST 98 mass spectral library. The criterion for the identification of compounds was based on the quality of the detected peak which was set at greater than or equal to 70%. All gauzes were pretreated using SFE and extracted using the SPME-GC/MS method prior to use to assure human scent compounds had been removed prior to sampling individuals.

Spearman Correlation Coefficient Comparisons

After the extraction and analysis of the collected odor sample, the previously reported human compounds determined to be present in the headspace of all of the intra-day hand odor samples for each subject were isolated and, within each sample, the compounds were ranked according to their peak areas in ascending fashion for each subject. These ranked data arrays were then compared using the Spearman Correlation, as seen in Eq. 1 below, where d is the difference between the ranked compounds and n is equal to the number of compounds being compared.

$$r_3 = \frac{6\sum d^2}{n(n^2-1)} \quad (1)$$

The Spearman rank correlation coefficient is a nonparametric method that can be utilized in numerous contexts since there is no assumption about the distribution of the variables. The interpretation of the obtained results is similar to Pearson's r, for the

coefficient ranges from −1 to +1. Therefore, it supplies information as to the strength and direction of relationships. Thus, in this study, the possible correlation between the chemical profile (set of VOCs) of a single individual with a population is investigated (42).

Results and Discussion

Correlations Utilizing All of the Human Scent Compounds Present in the GC/MS Profile

Across the three hand odor samples collected from each of the 10 subjects, 37 previously reported human volatile compounds were extracted. The compounds ranged in functionality, including: acids (5.41%), alcohols (13.51%), aldehydes (32.42%), alkanes (29.73%), ketones (8.11%), and acid-esters (10.81%). Of the 37 compounds detected, six have been previously identified by the authors to be high frequency occurring VOCs detected in collected hand odor samples (40): 2-furancarboxaldehyde, 2-furanmethanol, phenol, nonanal, decanal, and hexanedioic acid-dimethyl ester. Additionally, seven VOCs detected among the 10 subject population have been previously determined by the authors to be medium frequency compounds detected in collected hand odor (40), including: propanedioic acid-dimethyl ester, 6-methyl-5-hepten-2-one, octanoic acid-methyl ester, dodecane, undecanal, 6,10-dimethyl-5,9-undecadiene-2-one, and tetradecane.

TABLE 1—*Correlation outcomes from intra-day hand odor samples.*

All Previously Reported Human Scent Compounds Considered

	Type I Errors	Type II Errors	Total Errors	Overall Percent Discriminated
0.9	52	0	52	88.05
0.8	41	4	45	89.66
0.7	28	7	35	91.95

Human scent profiles contain a varying number of compounds depending on the subject being analyzed. As several variables are being measured within each person, multivariate data is produced. Multivariate data can be used for differentiation between samples where each is characterized by a set of measurements. In this case, the samples are the individual's scent profile that is represented by a set of volatile compounds. Correlation tests are used to determine relationships between two or more variables.

Spearman correlation coefficient comparisons were conducted utilizing all of the human scent components detected in the headspace of the hand odor samples. Three samples from 10 subjects were considered producing 435 possible pairings (for 30 intra-day samples). For the purposes of a clear distinction between the two types of errors in statistical analysis, it is important to note that type I errors reject a null hypothesis even though it is true while type II errors retain a null hypothesis even when it is false. When considering the types of errors present in the study, retaining or excluding a null hypothesis refers to the exclusion or inclusion of a sample's chemical profile to the identity of the given subject. As summarized in Table 1, when considering a correlation threshold of 0.9, the individuals were correctly distinguished and identified in 88.05% of the cases (52 type I errors). When considering a correlation threshold of 0.8, the individuals were correctly distinguished and identified in 89.66% of the cases (4 type II, 41 type I errors). When considering a correlation threshold of 0.7, the individuals that were correctly distinguished and identified increased to 91.95% (7 type II, 28 type I errors). The high number of type 1 errors demonstrates the importance of determining a human scent baseline measurement for each subject.

Correlations Utilizing the Primary Odor Components Determined for Each Subject

Narrowing the compounds for each subject to only those determined to be present in all three intra-day samples collected, what is termed here as an individual's "primary odor," results in 24

TABLE 2—*Primary odor components of samples collected from the hands. [Correction added after online publication 2 Dec 2009: Table replaced with higher resolution image.]*

		Subject, Sample																													
R.T.	Compound Name	F1,1	F1.2	F1,3	F2,1	F2,2	F2,3	F3,1	F3,2	F3,3	F4,1	F4,2	F4,3	F5,1	F5,2	F5,3	M1,1	M1,2	M1,3	M2,1	M2,2	M2,3	M3,1	M3,2	M3,3	M4,1	M4,2	M4,3	M5,1	M5,2	M5,3
3.86	Pyridine													X	X	X															
4.68	2-Butenal, 2-metbyl-													X	X	X															
5.70	Butanaic acid	X	X	X										X	X	X															
6.50	2-Fnrancaibaxaldebyde	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
7.20	2-furanmethanol													X	X	X							X	X	X	X	X	X			
8.32	Nonane													X	X	X															
9.03	Propanedioic acid, dimethyl ester																X	X	X										X	X	X
9.72	Benzaldehyde	X	X	X										X	X	X							X	X	X						
10.24	Phenol	X	X	X	X	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
11.26	Benzyl Alcohol				X	X	X																X	X	X						
12.37	l,6-Octadien-3-ol, 3,7-dimethyl-																						X	X	X						
12.38	Undecane																												X	X	X
12.47	Nonanal	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
12.82	Octanoic acid, methyl ester																X	X	X	X	X	X									
13.42	2-Nonenal,(E)-																														
13.89	2-Decanone	X	X	X										X	X	X				X	X	X									
14.02	Dodecane	X	X	X							X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
14.12	Decanal	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
14.42	Nonanoic acid, methyl ester																X	X	X	X	X	X							X	X	X
14.76	Hexanedioic acid, dimethyl ester				X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X				X	X	X
15.49	Tridecane	X	X	X										X	X	X							X	X	X						
15.62	Tetradecane	X	X	X										X	X	X	X	X	X				X	X	X						
17.82	5,9-undecadien-2-one,6,10-diniethyl	X	X	X	X	X	X							X	X	X	X	X	X	X	X	X	X	X	X						
18.87	Dodecanoic acid													X	X	X															

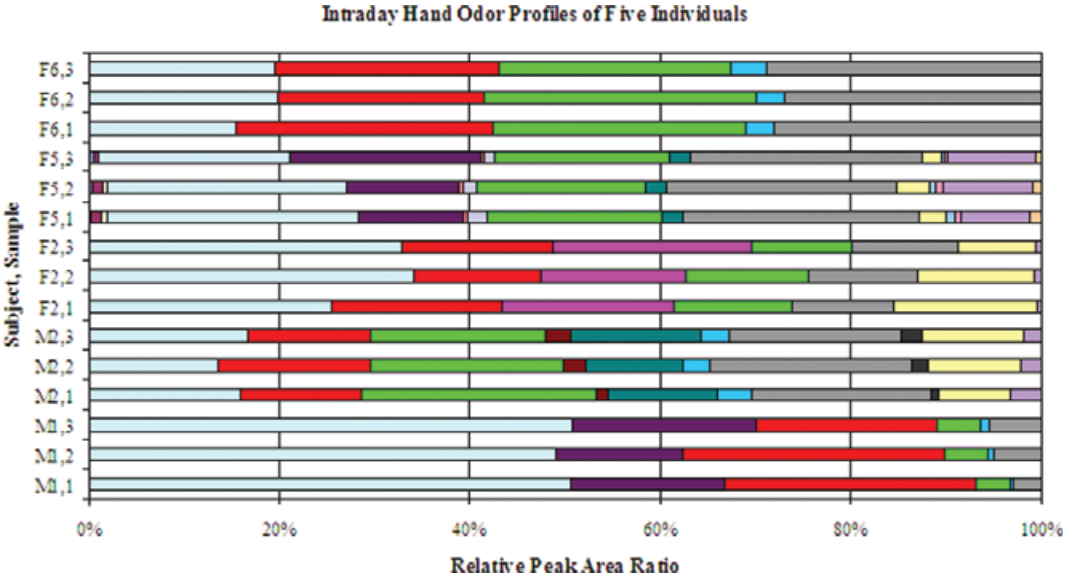

FIG. 1—*Color odor charts from five individuals. [Correction added after online publication 2 Dec 2009: Figure resolution improved.]*

compounds across the 10 subjects. Table 2 displays the primary odor compounds, with corresponding color codes and subject. Figure 1 demonstrates, in semi-quantitative fashion, the relative ratios of the peak areas of the color-coded human compounds extracted in the headspace above the collected hand odor samples for the intra-day samples collected from the 10 subjects. When running the Spearman correlation using only the 24 primary odor components, the results produce a significantly decreased number of type 1 errors. As summarized in Table 3, when considering a correlation threshold of 0.9 and 0.8, the individuals were correctly discriminated and identified in 99.54% of the cases (2 type 1 errors). At a correlation threshold of 0.7, 100% of the individuals were correctly discriminated and identified. The significant reduction and elimination of type 1 errors (depending on the correlation threshold utilized) validates the importance of determining a human scent baseline measurement on an individual basis referred to here as an individual's "primary odor profile."

A total of 24 compounds were determined across the 10 person population to be part of a "primary odor profile"; this assortment of compounds encompasses: acids (8.33%), alcohols (16.67%), aldehydes (25%), alkanes (25%), ketones (8.33%), and acid-esters (16.67%). There was no total loss of a compound class during the total VOC reduction to only those present in a primary odor profile; however, the predominance of the functional groups has shifted with alkanes and aldehydes now comprising 50% of the compounds considered. Aldehydes have been reported to be features of human odor due to oxidative degradation of sebaceous secretion components (43) which are the product of glands which cover the majority of the human body and thus are significant contributors to overall body odor. The aldehydes reported here to be part of an individual's "primary odor" have been detected across diverse areas of the human body: 2-methyl-2-butenal (hand [43]), 2-Furancarboxaldehyde (hand [36,40]), benzaldehyde (hand [36,40,44,45]), blood (46), human cadaver (47), nonanal (hand [36,40,44,45,48,49]), arm (39,43), breath (50), human cadaver (47), E-2-nonenal (hand [36,40]), armpit (33,43,51), and decanal (hand [39,44,48,52]) arm (43,49), human cadaver (47).

TABLE 3—*Primary odor correlation outcomes from intra-day hand odor samples.*

Primary Odor Component Compounds Considered				
	Type I Errors	Type II Errors	Total Errors	Overall Percent Discriminated
0.9	2	0	2	99.54
0.8	2	0	2	99.54
0.7	0	0	0	100

Twelve of the 24 "primary odor" components have been previously reported by the authors to be detected in high (H) and medium (M) frequency in collected hand odor samples (40): 2-furancarboxaldehyde (H), 2-furanmethanol (H), phenol (H), nonanal (H), decanal (H), hexanedioic acid-dimethyl ester (H), propanedioic acid-dimethyl ester (M), octanoic acid-methyl ester (M), dodecane (M), undecanal (M), 6,10-dimethyl-5,9-undecadiene-2-one (M), and tetradecane (M). Although 6-methyl-5-hepten-2-one was previously reported by the authors to be a compound occurring at a medium frequency in collected hand odor samples (40), it was not determined to be part of the "primary odor" constituents of the subjects studied. This ketone was detected in only one of the thirty samples evaluated in this study and has been reported elsewhere as a low frequency compound in the headspace above the forearm skin of females (49). Tetradecane was also previously reported to be present in the headspace above the forearm skin of females as a high frequency compound (49) which was also determined here to be a constituent of "primary odor."

E-2-nonenal was detected in this study to be present as a "primary odor" constituent in 2 out of 10 subjects who ranged in age from 17 to 28. This compound was previously extracted in 25% of the hand odor samples from a 60 subject population whose ages also ranged from 17 to 28 years of age (40). The detection of E-2-nonenal in this study is in agreement with previous studies of the volatile components of hand and armpit odor conducted by the authors (40,51) and illustrates that E-2-nonenal is not a suitable odor marker of individuals over the age of 40 as previously reported by Haze (43).

Correlation of 10 Subject Population Against a Library

The possibility of matching individual odor profiles from a human scent database (HSD) library was then evaluated. The 10

TABLE 4—*Correlation outcomes of 10 subject primary odor constituents compared to a population.*

Primary Odor Components Against Population Library				
	Type I Errors	Type II Errors	Total Errors	Overall Percent Discriminated
0.9	2	20	22	99.34
0.8	2	201	203	93.89
0.7	0	634	634	80.91

subject intra-day sampling population was then compared using the Spearman Rank Correlation against a library containing hand odor profiles obtained from 52 individuals (26 males, 26 females). The hand odor samples inputted into the HSD library were collected and extracted following the same procedure listed in the Methods Section. Across the 52 individuals, there were 63 previously reported human scent compounds present in the headspace of the collected hand odor samples. However, the compounds considered for comparison were restricted to the 24 compounds determined to be primary odor components through analysis of multiple samples collected from the 10 subjects in this study.

The 10 subject intra-day sample population was combined with the 52 subject library, resulting in 3321 possible pairs. As summarized in Table 4, when considering a correlation threshold of 0.9 the individuals were correctly distinguished and identified in 99.34% of the cases (20 type II, 2 type I errors), considering a correlation threshold of 0.8 the individuals were correctly discriminated and identified in 93.89% of the cases (201 type II, 2 type I errors), and at a correlation threshold of 0.7, the individuals that were correctly discriminated and identified decreased to 80.91% (634 type II Errors). Table 5 displays the top six correlation results for each of the intra-day subject samples when compared to the 52 subject hand odor database. In all except two of the cases the top three matches across the total of 82 samples considered within the comparison were the other intra-day samples collected from that individual.

The 52 subject library which originally contained 63 compounds was reduced to 24 compounds determined to be "primary odor" constituents by the 10 subject population presented in this study. Since this reduction in compounds utilized by the library was not done on an individual basis, it is likely that some "primary odor" compounds were included and others excluded for these individuals. The importance of determining a human odor baseline on an individual basis is critical in determining which VOCs are significant for determining identity. It is apparent that the discrimination power of this technique can be enhanced through evaluating multiple samples from a subject prior to inputting into the human scent database.

TABLE 5—*Top six correlations of intra-day subject samples to 52 library samples.*

Female 1 (F1)						Male 1 (M1)					
F1,1	1.000	F1,2	1.000	F1,3	1.000	M1,1	1.000	M1,2	1.000	M1,3	1.000
F1,2	0.909	F1,3	0.936	F1,2	0.936	M1,3	0.955	M1,3	0.982	M1,2	0.982
F1,3	0.873	F1,1	0.909	F1,1	0.873	M4,2	0.936	M1,1	0.936	M1,1	0.955
M12	0.760	M12	0.840	M12	0.774	F7	0.809	F21	0.802	F21	0.797
M2,1	0.727	M1,1	0.721	F8	0.740	F29	0.786	F7	0.796	F29	0.795
M1,1	0.725	M25	0.720	M25	0.705	F21	0.775	F29	0.795	F7	0.782
Female 2 (F2)						Male 2 (M2)					
F2,1	1.000	F2,2	1.000	F2,3	1.000	M2,1	1.000	M2,2	1.000	M2,3	1.000
F2,2	0.964	F2,1	0.964	F2,2	0.893	M2,2	0.964	M2,1	0.964	M2,1	0.952
F2,3	0.857	F2,3	0.893	F2,1	0.857	M2,3	0.952	M2,3	0.939	M2,2	0.939
M17	0.550	M17	0.617	M17	0.650	F13	0.818	F13	0.769	F13	0.727
F27	0.539	F27	0.600	F27	0.636	F1,1	0.727	M9	0.709	M26	0.679
M14	0.518	M13	0.576	M14	0.609	F22	0.720	M26	0.697	M5,3	0.650
Female 3 (F3)						Male 3 (M3)					
F3,1	1.000	F3,2	1.000	F3,3	1.000	M3,1	1.000	M3,2	1.000	M3,3	1.000
F3,3	1.000	F3,3	0.900	F3,1	1.000	M3,2	0.912	M3,3	0.967	M3,2	0.967
F3,2	0.900	F3,1	0.900	F3,2	0.900	M3,3	0.824	M3,1	0.912	F18	0.831
M4,2	0.643	M4,1	0.643	M4,2	0.643	F6	0.760	F18	0.835	M3,1	0.824
M10	0.631	F27	0.631	M10	0.631	M34	0.759	M34	0.798	M34	0.741
F27	0.607	M10	0.607	F27	0.607	F23	0.757	F6	0.760	F6	0.735
Female 4 (F4)						Male 4 (M4)					
F4,1	1.000	F4,2	1.000	F4,3	1.000	M4,1	1.000	M4,2	1.000	M4,3	1.000
F4,3	0.900	F4,3	0.900	F4,2	0.900	M4,2	0.943	M4,3	0.943	M4,2	0.943
M7	0.821	M7	0.714	F4,1	0.900	M4,3	0.886	M4,1	0.943	M4,1	0.886
F4,2	0.700	F4,1	0.700	M7	0.786	F26	0.869	F26	0.845	F26	0.821
M22	0.607	M22	0.536	M22	0.560	M13	0.845	M13	0.798	M29	0.750
F9	0.560	F9	0.464	F9	0.536	M29	0.821	M10	0.798	M13	0.750
Female 5 (F5)						Male 5 (M5)					
F5,1	1.000	F5,2	1.000	F5,3	1.000	M5,1	1.000	M5,3	1.000	M5,3	1.000
F5,2	0.993	F5,1	0.993	F5,2	0.932	M5,3	0.967	M5,2	1.000	M5,2	1.000
F5,3	0.921	F5,3	0.932	F5,1	0.921	M5,2	0.967	M5,1	0.967	M5,1	0.967
M3,1	0.694	M3,1	0.697	M3,1	0.664	M14	0.794	F29	0.855	F29	0.855
M31	0.666	F16	0.668	F16	0.647	F29	0.791	M18	0.825	M18	0.825
F16	0.665	M3,2	0.661	M24	0.634	F15	0.780	F25	0.804	F25	0.804

Conclusions

Human scent is a distinguishing characteristic that has yet to be fully exploited by the scientific community. The present study has demonstrated that human scent can be measured instrumentally and utilized to both identify and distinguish individuals. The current study has validated the utility of human scent as a biometric measurement which previously was only possible through the use of specially trained canines.

The method developed for the instrumental analysis of collected human hand odor samples employed SPME-GC/MS and Spearman Rank Correlations for profile comparisons and has been shown to be a reproducible means to obtain an individualizing human scent profile. Prior to the correlations, the ''primary odor'' profile constituents were assigned a rank order which effectively yielded an individualized barcode for each subject that was used for the comparisons. Although the ''primary odor'' components were determined to be similar across gender, sufficient variability exists which allowed for successful instrumental differentiation of the individuals tested. The correlation comparisons of the collected human scent samples revealed new insights into human scent identity.

Through this study, the value of determining ''primary odor'' constituents on an individual basis for assessing human scent identity has been determined, and proof of concept has been demonstrated for the employment of ''primary odor'' constituents as a human scent biometric measure. When considering all human scent compounds present in a profile, the high number of type 1 errors demonstrated the importance of determining a human scent baseline measurement for each subject. Utilizing the primary odor barcodes of the subjects, 99.54% were discriminated and identified amongst the group studied and a value of 99.34% was achieved when compared against a previously compiled human scent database.

While previous studies by the authors indicated the discriminating potential of human scent compounds (40), a degree of uncertainty remains as to whether human scent identity lies within the relative ratio of the common compounds between individuals, the presence of compounds which have a high variation between people, or whether it requires a combination of both of these factors. This study has demonstrated that utilizing the relative ratio patterns of a combination of compounds which vary in degree of frequency of detection in hand scent profiles can produce a distinguishable human scent profile. The feasibility of determining a standard set of volatile compounds which are sufficiently discriminating for the differentiation and matching of human scent profiles using a computer database has also been achieved through the utilization of the 24 compounds presented here.

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