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Forensic identification of urine on cotton and polyester fabric with a hand-held Raman spectrometer

Elizabeth Hager [a], Charles Farber [a], Dmitry Kurouski [a,b,*]

[a] Department of Biochemistry and Biophysics, Texas A&M University, 300 Olsen Blvd., College Station, TX 77843, USA
[b] The Institute for Quantum Science and Engineering, Texas A&M University, TAMU 4242, College Station, Texas, 77843, USA

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ABSTRACT

On-site detection and identification of body fluid samples at crime scenes and in prisons is critical for law enforcement. Current forensic tests for body fluids are highly specific, destructive to potential DNA evidence and time-consuming. Raman spectroscopy (RS) is a label-free, non-invasive and non-destructive analytical technique that provides information about molecular vibrations and consequently chemical structure of the analyzed specimen. These advantages make RS highly attractive for forensic applications. This study demonstrates how RS can be used for confirmatory, non-invasive and non-destructive detection and identification of urine directly on fabrics. This is very important because there have been cases of correctional officers subjected to urine from prisoners. One would envision that conclusive evidence other than eyewitness testimonies will help in potential prosecution of such cases, especially if both urine and DNA can be simultaneously detected. In this study, we show that using a handheld Raman spectrometer we can detect and identify urine in liquid samples, both on cotton and synthetic fabric, as well as on sweat-contaminated clothes. We also demonstrate that RS is capable of detection and identification of urine directly on police uniform. Finally, we show that coupling of partial least squares discriminant analysis with RS allows for high accuracy prediction of urine on all studied types of fabric.

1. Introduction

Forensic investigations typically involve detection and identification of body fluids at a crime scene [1]. The most commonly found body fluid evidence at crime scenes are blood, semen, vaginal fluid, sweat, and urine [2]. Currently available tests for these fluids are often destructive, which is undesirable due to the high importance of DNA that may be present in the sample [3]. Body fluid analysis is also highly specific, which requires utilization of different forensic methods for each body fluid confirmation. This significantly increases the required time and cost of a detailed analysis for a crime scene where several body fluids are simultaneously present. Therefore, a technology that would be sample-general, confirmatory, non-invasive and non-destructive is highly desired.

The ability to positively identify body fluids is not only relevant to forensic science but also law enforcement. For instance, there have been cases of correctional officers being subjected to urine from prisoners [4]. One would envision that conclusive evidence other than eyewitness testimonies would help in potential prosecution of such cases, especially if both urine and DNA are simultaneously detected. Recently, it was shown that it is possible to extract DNA from fresh [5] and dried [6] urine samples. However, a confirmatory and non-destructive detection of urine on fabrics is a challenging task. This is because urine is a heterogeneous body fluid, with compositional variations between different species, people, and even within an individual depending on diet, medications, drugs, hydration, and other factors [7]. Urine is composed of 95% water and 5% metabolites, including urea, salts, and organic compounds [8]. Thus, high concentrations of this body fluid are typically required for confirmatory forensic analysis. Reverse-phase high-performance liquid chromatography (RP-HPLC) alone or in a combination with mass-spectrometry (RP-HPLC-MS) can be used to confirm the presence of urine in the sample of interest [9,10]. However, these approaches are destructive, time consuming and cannot be accessed directly at a crime scene. Other methods available at the crime scene, such as those that use urease and dyes for positive detection, are still destructive to the sample [2].These challenges catalyzed our efforts to search for a portable non-invasive, non-destructive, label-free tool that would provide law enforcement the ability to quickly and accurately identify urine.

Raman spectroscopy (RS) is becoming a popular tool for forensic purposes [11]. RS has been shown to be successful in confirmatory

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* Corresponding author at: Department of Biochemistry and Biophysics, Texas A&M University, 300 Olsen Blvd., College Station, TX 77843, USA.
E-mail address: dkurouski@tamu.edu (D. Kurouski).

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identification of many body fluids, such as blood [12], saliva [13], sweat [14], semen [15], and vaginal fluid [16]. RS has also proven to differentiate between sources of fluids, such as peripheral and menstrual blood [17] and even differentiate mixed fluids [18]. Furthermore, studies of gunshot residue differentiation utilizing RS have shown great potential [19,20]. Another advantage of RS is its portability. Over the last decade, several companies have developed portable Raman spectrometers, which enabled utilization of RS directly in the for applications such as detection and identification of hair dyes [21], illicit drugs [22], rocks and minerals [23,24], and maize pathogens [25].

Using advanced statistics, one can gain further insight from data from chemical analyses such as RS. Coupled to statistics, RS can detect gunshot residues [20,26], distinguish between blood from different species [27], and detect the presence of dyes in hair [21]. Partial least-squares discriminant analysis (PLS-DA) is a type of multivariate statistical method [28] that determines the number of significant components and, in the case of RS, the wavenumbers in the spectra which best explain the differences between different treatments, or classes [29].

In this study, we demonstrate how RS can be used for non-destructive detection of urine directly on fabrics. We show that using a hand-held Raman spectrometer we can detect and identify urine in liquid samples, both on cotton and synthetic fabric, as well as on sweat-contaminated clothing. We also utilize multivariate data analysis to determine whether RS can be used for the quantitative detection and identification of urine [28]. Our results indicate that using PLS-DA coupled to RS allows for high accuracy prediction of the presence of urine on all studied types of fabric.

2. Materials & methods

2.1. Materials

Urea was purchased from Sigma Aldrich (St. Louis, MO, USA). Urine samples were obtained from volunteers in a sample collection kit and used immediately. Cotton samples were Kimtech Kimwipes Delicate Task Wipers (Kimberly-Clark Worldwide, Inc., Roswell, GA). The lab coat (35% cotton and 65% polyester) used was a 40″ Unisex Antimicrobial Labcoat (Spectrum Uniforms, Houston, TX). The uniform shirt was an SS POLYESTER SUPER-SHIRT® in Heather Blue purchased from Blauer Manufacturing Company, Boston, MA.

2.2. Urea solution sample preparation

Super-saturated urea was prepared at 60% weight/weight (w/w) with 40% (w/w) Milli-Q H_2O. Specifically, 60 g of urea was added to 40 mL of Milli-Q H_2O. Aqueous urea solutions of 5–25 mg/mL were prepared by dissolving the appropriate amount of urea in 5 mL of Milli-Q H_2O.

2.3. Sample application to fabrics

Urine was applied to white cotton, blue jeans, a white lab coat, and a blue uniform (polyester) shirt. Specifically, 500μL of urine was deposited onto a portion of fabric and scanned immediately.

2.4. Raman measurements and data analysis

Raman spectra were acquired using a handheld portable Rigaku Progeny spectrometer (Rigaku Analytical Devices, Inc. Wilmington, MA) equipped with a 1064 nm Nd:YAG laser. Spectra from each representative fabric type (cotton and polyester) were obtained at a power of 300mW. Ten spectra were collected from each sample, with ten 8 s exposures. Spectra were baseline corrected by Rigaku software. Obtained spectra had a resolution of 8–11 cm^{-1} and were processed using Grams/AI 7.01 (Thermo Galactic, Salem, NH) for spectral averaging and base-line correction.

2.5. Statistical analysis

Collected Raman spectra were imported into PLS Toolbox 8.5.2 (Eigenvector, Manson, USA) and the mean offset was removed at each wavenumber. Each spectrum was assigned to a class (typically clean or dirty) based on the treatment applied to the fabric before reading. Partial least squares discriminant analysis (PLS-DA) was performed to determine the number of significant components and identify the wavenumbers that best explain the differences between the clean and soiled fabrics. The software used the venetian blinds data partition method to generate the cross-validation dataset. A model was made for each type of fabric and treatment combination.

3. Results & discussion

3.1. Direct forensic analysis of urine in liquid samples

Raman analysis of urine solution with 1064 nm excitation wavelength revealed a single peak centered at 1000 cm^{-1} that corresponds to the stretching of N—C—N bonds of urea [30]. The urine spectrum lacked spectroscopic signatures of proteins, hormones, and other metabolites, which could potentially overcomplicate forensic analysis of this body fluid (Fig. 1A). Based on this evidence, we can conclude that near-IR excitation wavelength ideally suits for confirmatory forensic examination of urine directly probing vibrational signature of urea, which present at 8–25 mg/ml concentrations at physiological conditions.

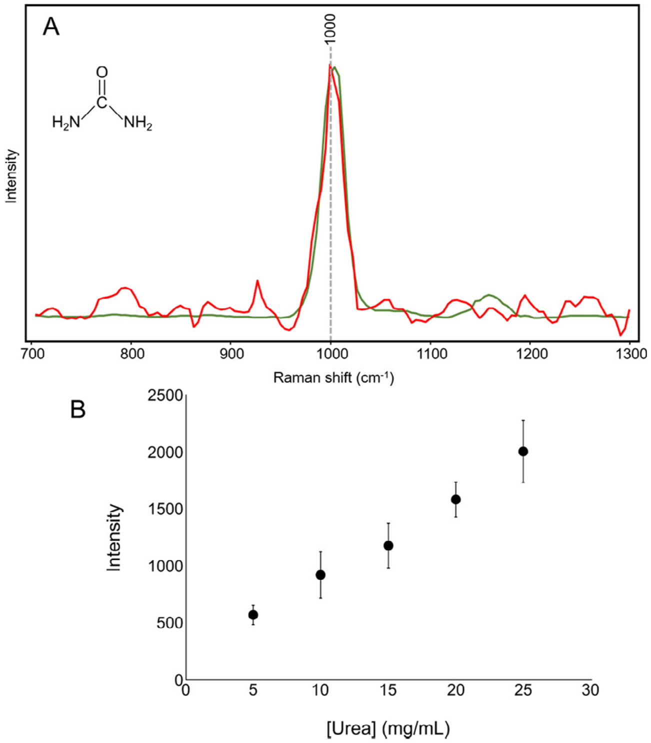

Fig. 1. (A) Averaged Raman spectra of urine (red) and super-saturated urea (green; inset molecule). Spectra were normalized to the 1000 cm^{-1} peak for clarity. (B) A plot of average 1000 cm^{-1} peak intensity as a function of the concentration of urea. The physiological concentrations of urea range from 9.3 to 23.3 mg/mL.

Table 1
Vibrational bands of Handheld Raman Spectra Acquired from Cotton, Polyester, and Urine.

Band	Vibrational mode	Assignment
274	C—C stretching (ring), C—C—C bending (ring) [32]	Polyester
633	Ring deformation, d(C—O—C) [33]	Polyester
703	Ring C—C bend [33]	Polyester
795	Ring deformation [31]	Polyester
858	v(C—O—C) [34]	Polyester
1000	C—N—C stretch [30]	Urea
1095/1096	v(C—O—C), asymmetric; v(C—C) [31]	Cotton; Polyester
1121	v(C—O—C), symmetric, v(C—O—C) ring breathing [31]	Cotton
1288	C—C stretching (ring), C—O stretching [32]	Polyester
1379	$d(CH_2)$ [31]	Cotton
1615	Ring deformation [31]	Polyester
1728	v(C=O) [31]	Polyester

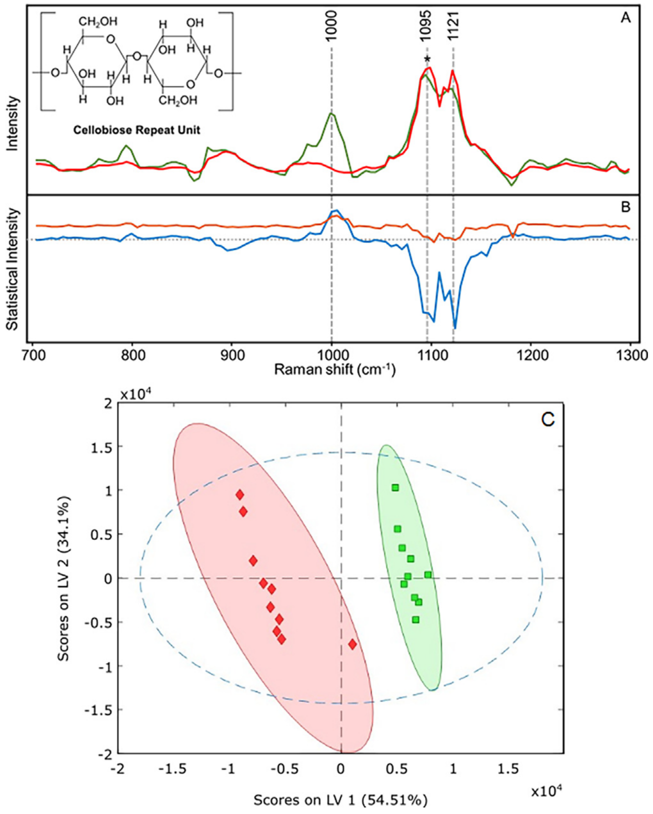

Fig. 2. (A) Averaged Raman spectra of cotton (red) and cotton with urine (green). Due to lack of an internal standard, spectra were normalized to the 1095 cm^{-1} peak, denoted by an asterisk (*), for clarity. (B) Loadings plot of the two latent variables in the Raman spectra of the cotton. Blue is latent variable (LV) 1, orange, LV2. (C) Projection of spectral data into multivariate space, revealing spatial separation between clean (red) and soiled (green) fabrics by the PLS-DA model. Ellipses represent 95% confidence.

Next, we demonstrated that RS enables forensic examination of solution urine samples (Fig. 1B). We observed strong correlation between the intensity of 1000 cm^{-1} band and concentration of urea in the sample. Thus, using RS one can not only confirm urine presence in solution, but also determine its concentration.

3.2. Forensics analysis of urine on cotton fabric

The next step was to test the ability of the hand-held unit to detect the presence of urine on fabrics. As is evident from the previous section, the marker for the presence of urine is the 1000 cm^{-1} peak. We chose undyed cotton as an initial, common, representative fabric that is typically worn under a uniform by both military and law enforcement. From a chemical composition, cotton is a simple fabric that is compositionally >99% cellulose. A typical Raman spectrum of cotton shows vibrational bands at 1095 and 1121 cm^{-1} originating from symmetric and asymmetric C—O—C vibration respectively [31] (Table 1). In the Raman spectrum of cotton with urine applied to it, we observed the urea peak at 1000 cm^{-1} in addition to the discussed above vibrational bands of cellulose (Fig. 2). This indicated that using RS we can confirmatory detect urine presence on cotton fabric.

Next, we used multivariate data analysis to determine whether RS can be used for the quantitative detection and identification of urine on cotton fabric [28,29]. A data set of 10 Raman spectra collected from cotton contaminated by urine and 10 reference spectra of cotton fabric were imported into PLS Toolbox 8.5.2 for statistical analysis. The generated PLS-DA model results are visualized in Fig. 2B and the statistics summarizing the model can be found in Table 2. Multivariate projections are limited to the first 2 latent variables (LVs), except in the case of Fig. 6C, which required all 3 LVs to achieve spatial separation between the clean and dirty fabric.

The two latent variables in the model explain 89% of the total class variation (Fig. 2C). The statistical intensity is the variable importance in projection (VIP) score. More extreme statistical intensity values correspond to the relevance of that wavenumber in predicting class membership [35]. This model identified the cotton peak at 1095 cm^{-1} (LV 1) and 1121 cm^{-1} (LV 1) (Fig. 2B) as the most robust spectral predictors of urine on cotton. The model successfully assigned all 20 spectra to their respective classes in both the calibration and cross-validation. This indicates that coupling of multivariate data analysis with RS allows for a 100% accurate detection and identification of urine on cotton fabric.

3.3. Detection of urine on composite fabric

One may wonder whether prosed spectroscopic approach can be used for confirmatory forensics analysis of urine on composite fabric, which is made from both natural and synthetic polymers. Denim is a good example of such a composite fabric material, as it is a blend of cotton (78%), polyester (20%), and Lycra® (2%). As in cotton, we observed vibrational bands at 1095, representing asymmetric C—O—C vibration, and 1121 cm^{-1}, representing

Table 2
Summary table of the 5 models generated to distinguish between clean and soiled fabrics.

Fabric	# LVs	% class variation explained by:			Total % variation explained	Calibration % Accuracy	CV % Accuracy
		LV1	LV2	LV3			
Cotton	2	54.51	34.19	N/A	88.7	100	100
Jeans	3	64.17	14.53	3.55	82.25	100	100
Uniform Shirt	1	73.75	N/A	N/A	73.57	100	100
Lab Coat (composite fabric)	3	82.20	6.17	5.59	93.96	100	100
Cotton with mixed Fluids	3	50.52	11.34	7.82	69.68	100	95

All models generated had 3 or fewer latent variables (LVs). CV - cross-validation

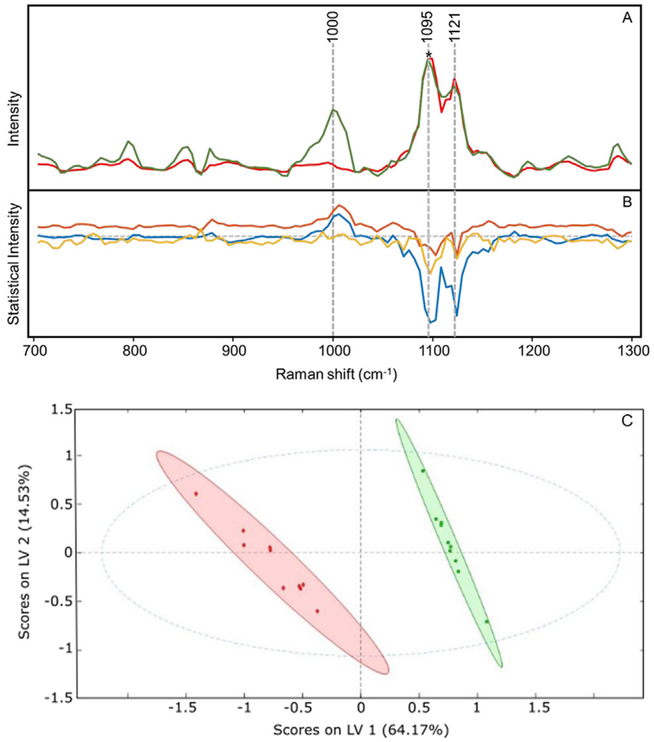

Fig. 3. (A) Averaged Raman spectra of jeans (78% cotton, 20% polyester, 2% Lycra®; red) and jeans with a sample of urine applied (green). Due to lack of an internal standard, spectra were normalized to the 1095 cm^{-1} peak, denoted by an asterisk (*), for clarity. (B) Loadings plot of the three latent variables in the Raman spectra of the jeans. Blue – LV1; Orange – LV2; Yellow – LV3. (C) Projection of jeans data as described in Fig. 2B. Red represents clean spectra; green, soiled.

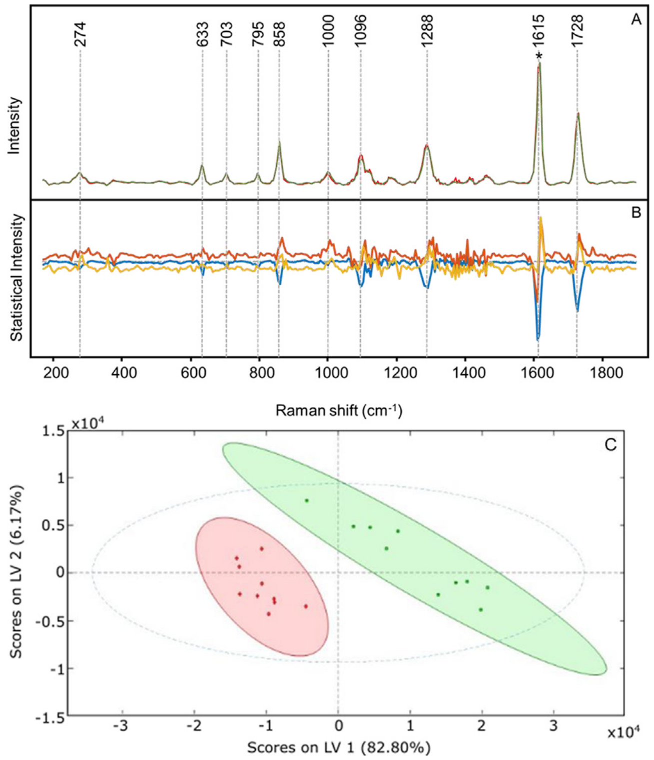

Fig. 4. (A) Averaged spectrum of a lab coat (composite fabric) composed of 35% cotton and 65% polyester with (green) and without urine (red). Due to lack of an internal standard, spectra were normalized to the 1615 cm^{-1} peak, denoted by an asterisk (*), for clarity. (B) Loadings plot of the three latent variables in the Raman spectra investigated fabric. Blue – LV1; Orange – LV2; Yellow – LV3. (C) Projection of composite fabric data as described in Fig. 2B. Red represent clean spectra; green, soiled.

C—O—C symmetric vibration and ring breathing (Fig. 3A). We have not observed vibrational bands associated with polyester or Lycra® (Table 1), likely due to their contributions being too weak to distinguish from those of cotton. Consistently with our previous results, there is a definite indication of the presence of urea after application of urine to the jean sample (Fig. 3A). Statistical analysis confirmed this positive result of the sampled spectra being successfully sorted into distinct between denim with and without urine contamination (Fig. 3B).

As the proportion of polyester in fabric increases, we observe the vibrational bands of this synthetic polymer. Polyester exhibits ring stretching, bending, and deformation in the 200–800 cm^{-1} and 1100–1600 cm^{-1} spectral regions. The polyester spectra also show peaks at 1096 cm^{-1} originating from C—C and C—O—C vibration, and 1728 cm^{-1} corresponding to C=O vibrations. Using RS, we tested urine presence on a lab coat (henceforth referred to as “composite fabric”) composed of 35% cotton and 65% polyester (Fig. 4A). We found no major difference in peak intensities in spectra with and without applied urine. We also found that polyester exhibits a vibrational band around 1000 cm^{-1}, which obscures direct visualization of urea peak on such fabric. This indicates that in a case of composite fabrics with a large (above 65%) percentage of polyester, visual inspection of the urea vibrational band (1000 cm^{-1}) cannot be sufficient for the confirmatory detection of urine.

At the same time, multivariate data analysis is capable for a confirmatory classification of urine-free and urine-contaminated composite fabric with a large (above 65%) percentage of polyester (Fig. 4B and C; Table 2). Our results revealed three variables that explained 94% of the total class variation. The polyester peaks at

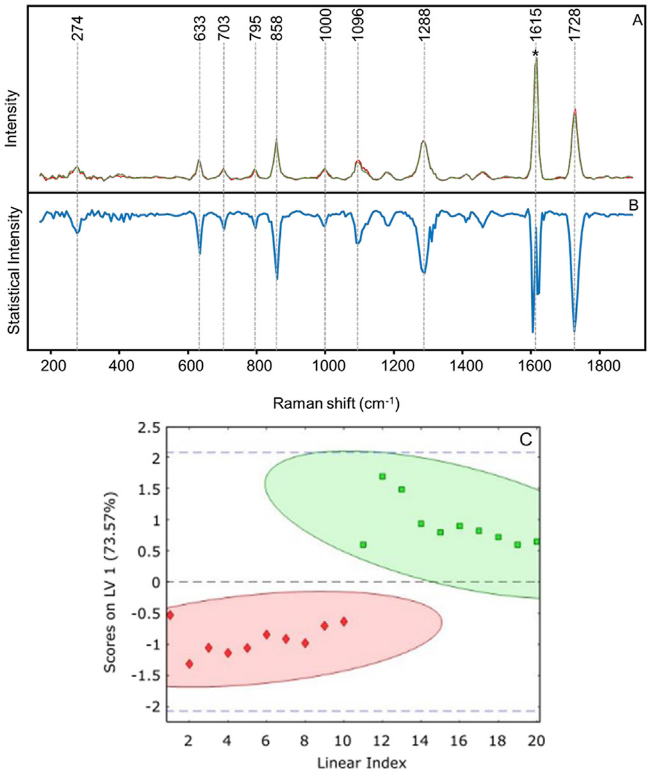

Fig. 5. (A) Averaged spectrum of a uniform shirt (100% polyester; red) and a uniform shirt with a sample of urine applied (green). Due to lack of an internal standard, spectra were normalized to the 1615 cm^{-1} peak, denoted by an asterisk (*), for clarity. (B) Loadings plot of the latent variable in the Raman spectra of a uniform shirt. Blue – LV1. (C) Projection of uniform shirt data as described in Fig. 2B. Data plotted against the linear index, which is the identifier for the individual spectra in PLS_Toolbox, as there was only one latent variable. Red represents clean spectra; green, soiled.

858 cm^{-1} (LV 1), 1096 cm^{-1} (LV 1), 1288 cm^{-1} (LV 1), 1615 cm^{-1} (LV 1), and 1728 cm^{-1} (LV 1) were the strongest predictors of class membership. This indicates that the proposed approach based on multivariate data analysis and RS allows for a 100% accurate detection and identification of urine on composite fabric with a large percentage of polyester.

3.4. Detection of urine on police uniform

To determine whether RS can be used for the confirmatory detection of urine on a police uniform, we applied urine to a sample police uniform, which was composed of 100% polyester. As in the case of composite fabric with large percentage of polyester (discussed above), vibrational bands of this polymer dominate in the Raman spectrum of police uniform (Fig. 5A). Nevertheless, using multivariate data analysis we were able to classify urine-contaminated police uniform with 100% accuracy (Table 2). The model generated to differentiate between the clean and soiled uniform shirt contained a single latent variable, explaining 74% of the total class variation (Fig. 5B). The model identified the polyester peaks at 633 cm^{-1} (LV 1), 858 cm^{-1} (LV 1), 1096 cm^{-1} (LV 1), 1288 cm^{-1} (LV 1), 1615 cm^{-1} (LV 1), and 1728 cm^{-1} (LV 1) as the strongest predictors of class membership.

3.5. Forensic analysis of mixed body fluids on cotton

One may wonder whether proposed spectroscopic approach can have any limitations for the detection of urine with other body fluids simultaneously present on the sample of interest [18]. From the practical perspective of law enforcement, sweat is likely to be found with urine during analysis. Therefore, we investigated if sweat would cause detective interference with the urine-contaminated fabric. The spectra of the cotton shirt soaked with sweat (red spectra; Fig. 6A) showed the same cotton peaks as described previously, but no notable peak at the 1000 cm^{-1} region (green spectra; Fig. 6A). After application of the urine sample, the 1000 cm^{-1} peak appears and is distinct. The model identified the cotton peaks at 1095 cm^{-1} (LV 3) and 1121 cm^{-1} (LV 3) as the most potent predictors of class membership (Fig. 6B and C; Table 2), and was able to distinguish between the two types of samples with high accuracy.

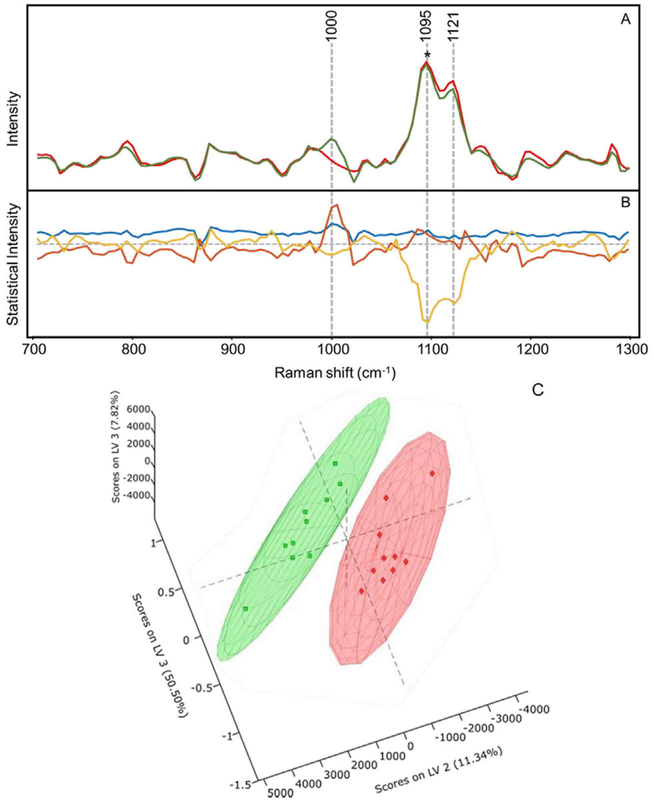

Fig. 6. (A) Averaged spectra of a cotton shirt with sweat (red) and a cotton shirt with sweat and urine (green). Due to lack of an internal standard, spectra were normalized to the 1095 cm^{-1} peak, denoted by an asterisk (*), for clarity. (B) Loadings plot of the three latent variables in the Raman spectra of a cotton shirt with multiple body fluids on it. Blue – LV1; Orange – LV2; Yellow – LV3. (C) 3D projection of composite fabric data as described in Fig. 2B. Red represent cotton contaminated with sweat spectra; green, cotton contaminated with sweat and urine.

4. Conclusion

Detection of a body fluid sample without destroying possible DNA evidence is a necessity for forensic sciences. Current techniques are typically body fluid specific, destructive, and may lead to false-positive results. This study demonstrates that RS can be used for non-destructive, non-invasive, label-free detection of urine directly on fabrics. Using a hand-held Raman spectrometer, we were able to detect urine on cotton, which is mainly composed of cellulose, as well as on complex fabrics made from both natural and synthetic polymers. We also show that coupling RS to PLS-DA allows for high accuracy predictions of urine presence on all studied types of fabric. Additionally, we demonstrate that RS is capable of detection and identification of urine in liquid samples, within physiological ranges. This indicates that RS can be used for quantitative analysis of liquid urine samples directly at a crime scene. Our results also demonstrate that using RS one can detect and identify urine on a sweat-contaminated cotton fabric. Future studies testing the discriminatory capabilities of other body fluids would further enhance the functionality of the proposed approach.

Conflict of interests

The authors declare no conflict of interests.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.forc.2018.05.001.

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