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Chemical Analysis of Sexual Lubricant Residue: A Comparison of Medical Examination Swabs Analyzed Using Spectroscopic Techniques

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Abstract: Sexual assault kits are the standard method for collecting and preserving sexual assault evidence. During the sexual assault examination, swabs are commonly used to collect bodily fluids as sexual assault evidence from the vagina, anus, mouth, and skin. The type of fiber swab used during collection can greatly influence the recovery of the substrate. In cases where lubricant residue may be present, it would be useful to identify the swab type that would be the most efficient in the collection of lubricant residues. In this study, four types of swabs with different fibers (i.e., cotton, polyester, rayon, and foam) with sexual lubricants present, were extracted in various solvents. The extracts were analyzed using attenuated total reflectance Fourier-transform infrared (ATR-FTIR) and Raman spectroscopy. The Pearson correlation coefficient (PCCs) test was applied to determine a pairwise comparison between swab lube extracts and the standard lubricant reference. Visual comparisons of the lubricant reference, blank fiber swab, and the fiber lubricant extract were used to determine peak overlap, significance, and matrix interference.

Keywords: forensic lubricants; optimal swab; sample collection; ATR-FTIR; Raman spectroscopy

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1. Introduction

Swab collection is an effective technique for collecting biological evidence for forensic analysis. The collection and extraction efficiency of swabs have been investigated but primarily for the collection and extraction of biological cells for DNA analysis. However, considering that lubricant residue can also be collected with biological cells, it is necessary to determine how lubricant components can be collected with these types of swabs [1,2]. The collection and extraction efficiency of swabs can vary depending on the chemical composition, morphology, and construction of the swab.

Although swab types may differ, the general anatomy of the swab consists of a shaft, with the fiber coiled onto the shaft as the fiber tip. The construction of the swab tip is either a flat padded cushion (i.e., the sleeve of a porous material around the shaft), flocked (i.e., multi-length fibers attached to an adhesive-coated surface), or wound (i.e., one long fiber around the shaft) [3]. The fiber type determines the absorption capability of the swab.

One research group investigated the collection, extraction, and recovery of DNA via five different swab fiber types: cotton, rayon, polyester, foam, and nylon. The research group determined that cotton, polyester, and rayon swabs were tightly wound, while foam swabs were more openly structured [4]. However, Bruijns et al. [3] were not able to determine a direct correlation of the extraction or collection efficiency with the swab material. This is likely because they were collecting epithelial cells and trying to extract enmeshed cells from the fiber using solvents. However, this entanglement issue may not be a problem for the collection and extraction of lubricant residues.

In forensic sample collection, cotton fiber swabs are primarily used because they are inexpensive and versatile in what they can absorb [5–7]. Cotton is a long chain polymer that is mainly composed of cellulose and other natural fibers [8]. Cotton and rayon are primarily hydrophilic due to the number of hydroxyl groups on the polymer. Similarly, polyester and polyurethane foam fibers are hydrophilic due to the presence of polar C=O functional groups [3]. Due to their hydrophilicity, these swab fibers are highly used because they attract a variety of different molecular species during the absorption process.

Various types of samples, such as explosives [9], fingerprints [10], epithelial cells [11,12], bodily fluids [13], and sexual lubricant evidence [14] have been collected using various swab types. However, cotton swabs are often used as an inexpensive option to collect many types of trace evidence [15]. Most swab optimization studies have been conducted to determine the most appropriate swabs to enhance forensic DNA recovery. Hedman et. al. [6] evaluated thirteen different brands of cotton, nylon, and foam swabs of various head sizes and shapes by sampling a 2 cm $\times$ 2 cm saliva stain on wood, window glass, and ridged plastic with equivalent DNA yields. To determine which swab produced a higher recovery, multi-factor analysis of variance (MANOVA) and interaction plots were evaluated. It was determined that although all of the fiber swab types produced identical DNA recoveries when sampling from a smooth/non-absorbing surface, the foam fiber swab produced higher DNA yields when sampling from wood and ridged plastic. In a similar study, cotton, and nylon swabs were compared for collecting post-coital vaginal and seminal samples [16]. It was determined that the nylon swabs produce a higher yield of extracted female and male DNA. Although many swab studies [17–19] have explored swab fiber type for DNA collection, the optimal swab for recovering sexual lubricant residues has not been investigated. Due to the increase in condom usage during sexual assault [20], it is important that the fiber swab type is suitable for effectively collecting sexual lubricant residues.

Several studies have focused on identifying instruments that are capable of screening sexual lubricants [21–23]. Spectroscopy has been a common technique used to detect the presence of lubricant residues. In particular, Fourier-transform infrared (FTIR) spectroscopy has frequently been used for lubricant analysis. Cho et al. [24] purchased thirty-five condoms and identified the primary chemical components of the lubricants present using FTIR. The majority of the condoms tested were polydimethylsiloxane (PDMS) lubricated condoms, while three were classified as water-based due to the presence of polyethylene glycol (PEG) and glycerin absorption peaks.

Another study was done to establish a protocol for forensic analysis of sexual lubricants [25]. Diffuse reflectance infrared Fourier-transform spectroscopy (DRIFTS) was used to analyze fifty-eight condoms that were extracted in hexane and methanol (MeOH). However, the research team observed a significant amount of interference caused by the broad intense alcohol absorption peak from the MeOH solvent. Therefore, gas chromatography/mass spectrometry (GC-MS) was explored as a complementary instrument [25]. Although GC/MS has been considered a valuable technique, sample preparation can be extensive and co-elution of the lubricant components is possible due to the complex nature of sexual lubricants [26].

This has led to the exploration of Raman spectroscopy as a complement to the FTIR analysis of lubricant samples because hydroxide functional groups do not interfere in the resulting spectra. Coyle et al. [27] used Raman spectroscopy to screen for the presence of silicone-based lubricants on sterile cotton swabs. Twenty-four blank cotton swab samples were averaged and compared to the silicone-based lubricated swabs. The cotton blank swabs contained characteristic peaks at 1120 (glycosidic link), and 2894 cm^{-1} (CH2 band), while swabs with PDMS produced discriminating peaks at 490 (Si-O-Si) and 708 cm^{-1} (Si-C). The resulting spectra demonstrated that the blank cotton swabs did not contain any apparent silicone-related vibrations and thus Raman spectroscopy can be used to detect the presence of silicone-based lubricants.

Although studies [27–29] have analyzed sexual lubricants on swabs, swab suitability for lubricant collection/extraction, has yet to be presented in the literature. It is critical that an optimal fiber swab is determined for sexual lubricant recovery and identification. The objective of this study was to determine if extracted lubricants from a swab could be associated with the known reference sample using FTIR and Raman spectroscopy.

2. Materials and Methods

2.1. Materials

One personal bottled water-based lubricant [Classic Erotica Crazy Girl® Wanna Be Daring™ Anal Ease lubricant (aka Crazy Girl, W0125)] and one condom silicone-based lubricant [Trojan™ Double Ecstasy™ lubricated condoms (aka Double Ecstasy, C0112)] were selected from the National Center of Forensic Science Sexual Lubricant Database for this project [21,30,31]. Puritan sterile cotton swabs (Puritan™ 25806 1WC FDNA, Guilford, ME, USA), Puritan sterile polyester swabs (Puritan™ 25806 2PD, Guilford, ME, USA), Puritan sterile rayon swabs (Puritan™ 25806 2PR TT, Guilford, ME, USA), McKesson rayon swabs (McKesson™ 24-808, Irving, TX, USA), Puritan sterile polyurethane foam swabs (Puritan™ 25-1805, Guilford, ME, USA), McKesson cotton swabs (McKesson™ 24-1062S, Irving, TX, USA), and Cardinal Health polyester swabs (Cardinal Health™ A5005-1, Irving, TX, USA) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). A summary of the swabs used in this study is displayed in Table 1 to show type, manufacturer, and shaft composition.

Table 1. Overview of the swabs used.

Fiber Type	Manufacturer	Model Number	Shaft Material
Polyester	Puritan	25-806 2PD	Plastic
Polyester	Cardinal Health	A5005-1	Plastic
Rayon	Puritan	25-806 2PR TT	Plastic
Rayon	McKesson	24-808	Paper
Foam	Puritan 5571	25-1805 1PF RND	Plastic
Foam	Puritan 5621	25-1805 1PF RND	Plastic
Cotton	Puritan	25-806 1WC FDNA	Wood
Cotton	McKesson	24-106-2S	Wood

High performance liquid chromatography grade hexanes, MeOH, and methylene chloride (DCM), purchased from Thermo Fisher Scientific, were used for extracting lubricant samples from the swabs [23]. Polyethylene glycol 400 was purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as an instrument standard and calibrator.

Solvent safety: DCM is classified as a hazardous airborne pollutant and carcinogen, and the United States Environmental Protections Agency has proposed to prohibit most industrial and commercial uses, as well as limit the manufacturing and distribution of the solvent [32]. Considering the safety concerns, potential alternatives that can be used instead of DCM for extraction purposes are 2-methyltetrahydrofuran, toluene, ethyl acetate, and methyl tert-butyl ether [33].

2.2. Extraction Process

To prepare the lubricated swabs, a sterile swab was removed from its package and weighed to record the mass of the swab before and after lubricant was added. Approximately sixty-two milligrams (mg) of lubricant were applied onto the swab. This was done by rubbing the lubricant onto the twirling swab to ensure an even distribution of lubricant on the swab. Both water-based and silicone-based lubricants were used to investigate the variability of lubricant collection. The swab was then dried for 30 min before obtaining the pre-extraction mass. After drying, the swab tip was then placed in 700 µL of the respective solvent (Hexane, MeOH, a 1:1 mixture of DCM-MeOH); subsequently, the swab shaft was removed. The wooden shaft was cut from the swab tip with a clean pair of scissors.

The plastic shafts were removed by bending the swab shaft over the tube edge while the larger foam swabs were cut with scissors into smaller pieces to fit into the microcentrifuge tubes. The swab fiber was agitated in the solvent by a vortex mixer for 30 s. Seventy-two lubricated swab samples were prepared with a combination of variables (Table 2). Samples were prepared in duplicate to determine sample variability.

Table 2. Illustration of the combination of variables for the extraction of the lubricant from each swab fiber type. This list of variables presented here was replicated for the other three fiber types.

Sample	Solvent	Fiber Type	Swab Manufacturer	Lubricant Source
1	Hexane	Polyester	1	Silicone-based Condom
2	DCM/MeOH	Polyester	1	Silicone-based Condom
3	MeOH	Polyester	1	Silicone-based Condom
4	Hexane	Polyester	1	Water-Based Bottle
5	DCM/MeOH	Polyester	1	Water-Based Bottle
6	MeOH	Polyester	1	Water-Based Bottle
7	Hexane	Polyester	1	Blank
8	DCM/MeOH	Polyester	1	Blank
9	MeOH	Polyester	1	Blank
10	Hexane	Polyester	2	Silicone-based Condom
11	DCM/MeOH	Polyester	2	Silicone-based Condom
12	MeOH	Polyester	2	Silicone-based Condom
13	Hexane	Polyester	2	Water-Based Bottle
14	DCM/MeOH	Polyester	2	Water-Based Bottle
15	MeOH	Polyester	2	Water-Based Bottle
16	Hexane	Polyester	2	Blank
17	DCM/MeOH	Polyester	2	Blank
18	MeOH	Polyester	2	Blank

Non-lubricated swabs were extracted using the same extraction process for each fiber type to create swab blank extracts. Following the same extraction process as the lubricant swab samples, the lubricant references were made by solubilizing sixty-two mg of the neat lube into 700 µL of each of the three solvents. Each extracted sample, lubricant reference, and sample blank were analyzed in triplicates via Raman and FTIR spectroscopy.

2.3. Instrumental Parameters

2.3.1. ATR-FTIR Spectroscopy

Fourier-transform infrared spectroscopic (FTIR) analysis was performed on a Jasco 6600 Fourier-transform infrared spectrometer (Jasco Corporate, Hachioji-shi, Tokyo, Japan) with a germanium/potassium bromine beam splitter and an attenuated total reflectance (ATR) accessory. A mid-IR scan range of 400–4000 cm^{-1} with 64 scans at a resolution of 4 cm^{-1} was used to collect spectra. Quality control of the instruments was monitored by running an air background before the sample analysis. A thin film of the sample was prepared by pipetting a 5 µL aliquot onto the crystal twice with drying in between, to concentrate the sample.

2.3.2. Raman Spectroscopy

All sample extracts were analyzed using a Horiba XploRA™ Plus Raman spectrometer (Horiba Instruments Incorporated, Irvine, CA, USA) with a laser wavelength of 785 nm and a charge-coupled detector (CCD). A silicon wafer was used to calibrate the instrument across three different gratings (600, 1200, 1800 g/mm). The wavelength of incident light was found using the full spectral range function to ensure that the appearing peak was near the expected 520 nm reference peak. The full instrumental parameters are provided elsewhere [23]. All samples were homogenized before pipetting 5 µL onto an aluminum-covered microscope slide for analysis. The slide was covered with aluminum foil to

minimize the presence of silicone vibrational bands from the glass slide in the resulting spectra. The objective lens was adjusted to focus the laser onto the extracted sample. After the sample was dried onto the slide, Horiba Scientific's LabSpec 6 software was used to collect the Raman spectroscopic data with a 1200 g/mm grating from 50 to 2000 cm^{-1} within a 20 s acquisition time. The baseline was automatically corrected using Horiba's flat line correction. The cosmic ray removal tool was used with a threshold of 10 bandwidths. Each sample was run in triplicate and the spectra were averaged to obtain one representative spectra. The reference lubricant (5 µL) standards were pipetted onto an aluminum slide and analyzed in triplicate.

2.4. Statistical Analysis

The data was base-peak normalized using Microsoft Excel (version 2310). The normalized data were separated into solvent-based matrices, resulting in three matrices for FTIR and Raman spectroscopy. All of the six data matrices were subjected to Pearson correlation coefficient calculation (PCC). PCC is a statistical technique that provides a similarity measurement for pairwise comparisons by measuring the linear relationship between two variable sets (i.e., spectra). Pairwise PCC scores were calculated for two comparison sets: (1) between the extracted blank swab reference (EBR) and the extracted lubricated swab (ELS) and (2) between the extracted lubricant reference sample (ELR) and the ELS. A stronger correlation between the ELS and ELR will result in a score closer to 1. Correlation scores between 0.8 and 1 (+/−) are considered strong correlations, 0.4 to 0.79 (+/−) are moderate correlations, and correlations below 0.3 ranging from low correlation to no correlation [34,35].

Six (6) spectra were collected for each of the trace EBR and ELS samples with each instrument. Three (3) spectra were collected for the bulk ELR samples. Two inter-sample pairwise comparison sets were collected for each swab substrate. For the EBR–ELS comparison sets, there were 36 PCCs calculated and averaged. The ELR–ELS comparison sets had 18 PCCs that were calculated and averaged (refer to Supplementary Table S1). These two comparison sets were then compared using a Student's *t*-test to determine if there was a significant difference between the two comparison sets. If the resulting *p*-value was less than 0.05 then there was a significant difference, if it was greater than 0.05 then there was no significant difference at a 95% confidence interval. If there was a significant difference determined and the ELR–ELS yielded a higher average PCC score than the ELS–EBR average score, then lubricant residue was indicated, and it was denoted as "lubricant preferred." If there was a significant difference and the average ELS–EBR was higher, then it was considered "swab preferred." If the two groups could not be differentiated then it was considered statistically similar and classified as "insignificant." This comparison was conducted for each swab substrate that contained one of the two lubricant samples and was extracted using one of the three solvents. Intra-sample comparisons were not conducted in this study.

In conjunction with the PCC score calculations, a visual comparison of the spectra for the extracted lubricant reference (ELR), the control extracted blank swab (EBR), and extracted lubricant swab (ELS) were conducted to understand and explain the similarity score results. Significant peaks of the lubricant references were identified and monitored to ensure that the lubricant was recovered after extraction from the swab and that any matrix interference from the swab was minimal.

3. Results

Forensic laboratories often use a direct comparison methodology when determining if an unknown sample may have been associated with a known reference [36]. Therefore, that methodology was employed here to provide forensic laboratories with a way of determining if lubricant residues have been collected by the swab submitted for analysis. In this study, the spectrum of the extract from the swab with the lubricant present was compared to the spectra of the extract from a blank swab reference and the extract from

the known lubricant reference. This comparison determined which reference the extracted "unknown" sample most resembled and yielded a quantifiable similarity metric. Thus, allowing forensic laboratories to readily screen for the presence of lubricant residues despite any containments coming from the swab substrate.

3.1. FTIR Spectroscopy Screening for Lubricant Residues

3.1.1. The Extraction of Silicone-Based Condom Lubricants

The spectra of the C0112 MeOH ELR primarily consisted of peaks at 795 cm^{-1}, 1014 cm^{-1}, and 1257 cm^{-1} which were used to monitor the extraction of the lubricant through this portion of the study. These peaks are attributed to Si–C stretching, Si–O–Si symmetric stretching, and C–H_3 bending, respectively. A visual comparison of the C0112 reference (gray line) extracted in MeOH from different fiber swabs is shown in Figure 1.

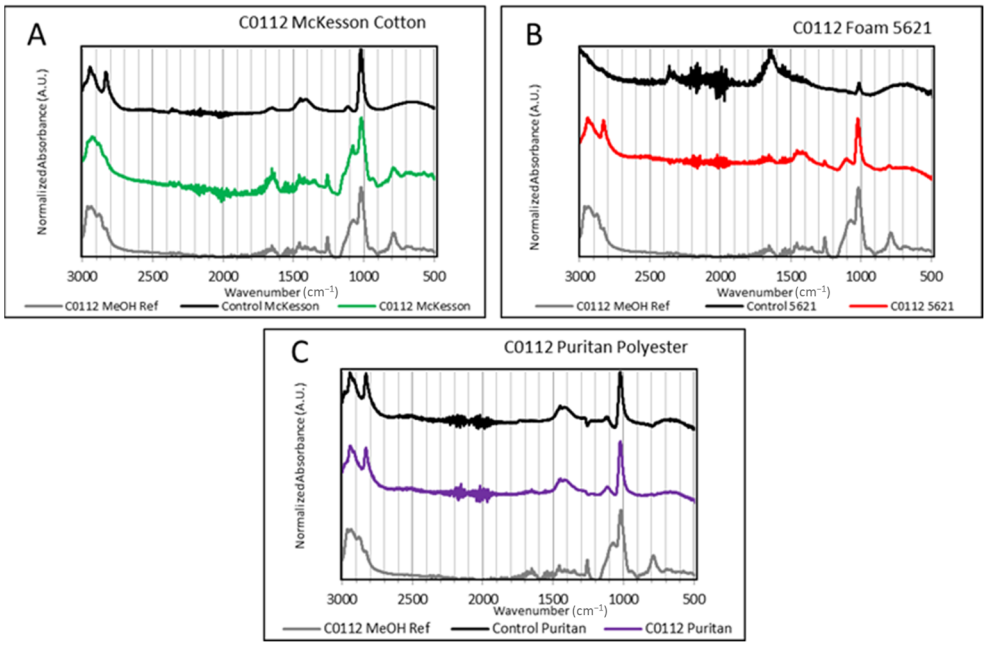

Figure 1. Comparative spectra of blank swab extract (black lines), the C0112 silicone-based lubricant reference (gray lines), and the lubricant extracted by MeOH from the swab in the designated color. The extract from the (**A**) McKesson cotton swab is the green line, (**B**) foam 5621 swab is the red line, and (**C**) Puritan polyester swab is the purple line. Remaining spectra presented in Figure S1.

Upon visual comparison, the C0112 McKesson cotton ELS closely resembled the C0112 ELR spectra with peaks at 780 cm^{-1}, 1014 cm^{-1}, and 1257 cm^{-1}, which indicated that major components of the lubricant had been extracted (Figure 1A). The peak at 1011 cm^{-1} is present in all three spectra and could not be used in the determination of attributed source for the ELS. However, there is a peak at 1069 cm^{-1} in the ELR spectrum that is present in the ELS spectrum indicating that lubricant was present. Additionally, there were more peaks shared between the ELS and the ELR spectra explaining the strong PCC scores.

The C0112 ELS spectra from the McKesson cotton swab were compared to the ELR, and the average PCC score was 0.816 ± 0.022 (Table 3, Methanol). The EBR vs. ELS PCC scores were calculated and averaged to be 0.717 ± 0.22. The Student's *t*-test yielded a *p*-value of 0.027, which is less than the a = 0.05 indicating that the two comparison sets were significantly different. The average PCC score for the ELS–ELR comparison was highest indicating that the presence of the lubricant on the swab was preferred.

Table 3. The average PCC scores, standard deviation, and *p*-values for ELR vs. ELS spectral comparisons and EBR vs. ELS spectral comparisons from the FTIR when the swab was extracted by either the hexane, methanol or DCM/MeOH solvent. Spectral data for MeOH spectra are presented in Figures S1–S4. NOTE: green = "lube preferred", yellow = "insignificant", red = "swab preferred".

FTIR Data				
		Methanol		
Sample	**ELR v. ELS**	**EBR v. ELS**	***p*-Value**	**Determination**
W Cotton McK	0.747 ± 0.033	0.528 ± 0.013	9.15×10^{-5}	Lube Preferred
W Cotton Pur	0.734 ± 0.038	0.601 ± 0.026	0.019	Lube Preferred
W Foam 5571	0.826 ± 0.017	0.708 ± 0.04	0.011	Lube Preferred
W Foam 5621	0.728 ± 0.023	0.559 ± 0.036	1.00×10^{-3}	Lube Preferred
W Poly CH	0.879 ± 0.004	0.807 ± 0.012	0.003	Lube Preferred
W Poly Pur	0.861 ± 0.006	0.684 ± 0.043	3.60×10^{-5}	Lube Preferred
W Rayon Mck	0.584 ± 0.048	0.852 ± 0.040	0.001	Swab Preferred
W Rayon Pur	0.685 ± 0.002	0.801 ± 0.018	1.96×10^{-5}	Swab Preferred
C Cotton McK	0.816 ± 0.022	0.717 ± 0.022	0.027	Lube Preferred
C Cotton Pur	0.843 ± 0.016	0.774 ± 0.018	0.07	Insignificant
C Foam 5571	0.748 ± 0.030	0.668 ± 0.050	0.155	Insignificant
C Foam 5621	0.592 ± 0.044	0.741 ± 0.035	0.016	Swab Preferred
C Poly CH	0.657 ± 0.045	0.616 ± 0.118	0.6	Insignificant
C Poly Pur	0.662 ± 0.044	0.754 ± 0.053	0.1261	Insignificant
C Rayon Mck	0.336 ± 0.009	0.849 ± 0.014	1.47×10^{-20}	Swab Preferred
C Rayon Pur	0.309 ± 0.001	0.824 ± 0.012	2.39×10^{-28}	Swab Preferred
		Hexane		
Sample	**ELR v. ELS**	**EBR v. ELS**	***p*-Value**	**Determination**
W Cotton McK	0.017 ± 0.327	0.579 ± 0.288	1.00×10^{-3}	Swab Preferred
W Cotton Pur	0.445 ± 0.241	0.839 ± 0.006	0.004	Swab Preferred
W Foam 5571	0.385 ± 0.381	0.703 ± 0.070	0.05	Swab Preferred
W Foam 5621	0.270 ± 0.444	0.516 ± 0.097	0.151	Insignificant
W Poly CH	0.269 ± 0.290	0.622 ± 0.058	0.015	Swab Preferred
W Poly Pur	0.329 ± 0.337	0.758 ± 0.020	0.006	Swab Preferred
W Rayon Mck	0.380 ± 0.438	0.815 ± 0.027	0.013	Swab Preferred
W Rayon Pur	0.325 ± 0.395	0.832 ± 0.024	0.003	Swab Preferred
C Cotton McK	0.565 ± 0.025	0.421 ± 0.024	0.003	Lube Preferred
C Cotton Pur	0.576 ± 0.015	0.457 ± 0.027	0.007	Lube Preferred
C Foam 5571	0.487 ± 0.061	0.560 ± 0.105	0.362	Insignificant
C Foam 5621	0.406 ± 0.065	0.559 ± 0.072	0.049	Swab Preferred
C Poly CH	0.637 ± 0.030	0.522 ± 0.071	0.064	Insignificant
C Poly Pur	0.715 ± 0.018	0.594 ± 0.059	0.022	Lube Preferred
C Rayon Mck	0.470 ± 0.120	0.764 ± 0.044	0.003	Swab Preferred
C Rayon Pur	0.582 ± 0.029	0.242 ± 0.050	1.60×10^{-7}	Lube Preferred
		DCM/MeOH		
Sample	**ELR v. ELS**	**EBR v. ELS**	***p*-Value**	**Determination**
W Cotton McK	0.663 ± 0.032	0.614 ± 0.026	0.344	Insignificant
W Cotton Pur	0.642 ± 0.019	0.579 ± 0.012	0.106	Insignificant
W Foam 5571	0.491 ± 0.108	0.681 ± 0.123	0.059	Insignificant
W Foam 5621	0.635 ± 0.038	0.619 ± 0.043	0.779	Insignificant
W Poly CH	0.699 ± 0.049	0.501 ± 0.128	0.222	Insignificant
W Poly Pur	0.747 ± 0.018	0.706 ± 0.015	0.283	Insignificant
W Rayon Mck	0.729 ± 0.040	0.704 ± 0.047	0.672	Insignificant
W Rayon Pur	0.661 ± 0.051	0.709 ± 0.028	0.433	Insignificant
C Cotton McK	0.798 ± 0.018	0.755 ± 0.015	0.268	Insignificant
C Cotton Pur	0.670 ± 0.054	0.768 ± 0.017	0.108	Insignificant
C Foam 5571	0.407 ± 0.156	0.672 ± 0.053	0.015	Swab Preferred
C Foam 5621	0.445 ± 0.088	0.632 ± 0.046	0.025	Swab Preferred
C Poly CH	0.517 ± 0.032	0.605 ± 0.119	0.222	Insignificant
C Poly Pur	0.643 ± 0.014	0.833 ± 0.016	4.76×10^{-6}	Swab Preferred
C Rayon Mck	0.914 ± 0.001	0.623 ± 0.066	7.21×10^{-8}	Lube Preferred
C Rayon Pur	0.875 ± 0.003	0.544 ± 0.020	8.84×10^{-17}	Lube Preferred

The C0112 foam 5621 ELS spectra contained the same functional groups as the C0112 ELR spectra, but at different relative intensities (Figure 1B). The peaks at 795 cm^{-1}, 1257 cm^{-1} were less intense while the peak near 1014 cm^{-1} peak was more resolved resulting in two peaks at 1022 and 1096 cm^{-1}. This may indicate that all of the major lubricant components were not extracted (i.e., incomplete extraction) or that extraction of other components from the swab substrate occurred. Based on the Student's *t*-test comparison, the C0112 foam 5621 ELS–ELR PCC scores were statistically different from the ELS–EBR scores (p = 0.016), but the mean PCC score for the ELS–EBR pairwise comparison was higher, resulting in a "swab preferred" designation.

Conversely, the C0112 Puritan polyester ELS sample had a diverse set of replicate spectra. Three (3) of the spectra looked like the EBR (Figure 1C) and three of the spectra resembled the ELS with the presence of all four major lubricant peaks. There were also some indistinguishable peaks between 1450 and 1500 cm^{-1} that were seen in all three spectra (EBR, ELR, and ELS). The mean PCC score for the ELR–ELS comparison was 0.662 ± 0.044 and the mean PCC score for the EBR–ELS was 0.754 ± 0.053. Both averages were moderate, but lower than an acceptable score of 0.8. According to the Student's *t*-test, the two pairwise comparison sets (EBR vs. ELS and the ELR vs. ELS) were statistically similar with a p-score > 0.05 (Table 3, Methanol).

The "lubricant preferred" designation was only observed with one of the two cotton substrates. For the remaining six substrate samples, the result was either "swab preferred" or statistically similar. This is likely due to the nonpolar composition of the silicone components present in the condom lubricant, which does not readily extract into MeOH as a solvent (Table 3, Methanol). Therefore, these results may not be due to the substrate specifically but the composition of the substrate and the poor extraction capability of the solvent [23].

More "lubricant preferred" designations were observed with the hexane solvent which is likely due to the better extraction capability of the solvent for silicone-based lubricants (Table 3, Hexane). The extracted lubricant was readily observed for both cotton swab substrates. Additionally, one of the polyester substrates and one of the rayon substrates yielded the same designation. One concern was that the average PCC score for these "lubricant preferred" determinations was lower than expected at approximately 0.6 (Table 4). The hexane solvent yielded the lowest positive "lubricant preferred" determinations for the FTIR analysis, in comparison to methanol (0.799) and the DCM-MeOH system (0.895). These results were even lower than the average Raman spectroscopy positive determinations, which all were greater than 0.8.

Table 4. The mean PCC scores for "lubricant preferred" designations for ELR–ELS and EBR–ELS comparison sets when extracted by each solvent on both instruments.

Instrument	Solvent	Mean ELR–ELS PCC	Mean EBR–ELS PCC
FTIR	Hexane	0.610	0.429
	MeOH	0.799	0.658
	DCM-MeOH	0.895	0.584
	FTIR Mean	0.755	0.576
Raman	Hexane	0.878	0.386
	MeOH	0.872	0.593
	DCM-MeOH	0.898	0.599
	Raman Mean	0.884	0.558

The rayon swab substrates were the only samples that resulted in "lubricant preferred" designations when samples were extracted with the DCM-MeOH solvent system (Table 3). They resulted in high PCC scores, at approximately 0.9, indicating low interference from the substrate and high correlation with the same peaks in the known ELR spectra. One of the polyester swabs and two of the foam swab substrates resulted in "swab preferred" designa-

tions while the other swab samples resulted in an "insignificant" designation. Considering that this solvent system should extract polar and nonpolar components, the expectation was that more "lubricant preferred" designations would have been observed. However, it was confirmed that more interference components from the substrate were extracted, yielding the lower ELR–ELS average PCC scores and higher EBR–ELS PCC scores.

3.1.2. The Extraction of Water-Based Lubricants

The W0125 reference lubricant was extracted in MeOH and resulted in two broad discriminating unresolved peaks at 1029 and 1100 at cm^{-1}. These peaks correlate to a O–CH_3 stretch and C–O stretch, respectively. There was also a broad peak at 2870 cm^{-1} with a shoulder at 2920 cm^{-1}, which correlates to a –OH stretch. Figure 2 shows the comparison of the W0125 ELS against the ELR (gray line) and the EBR of three different swab substrates (black lines).

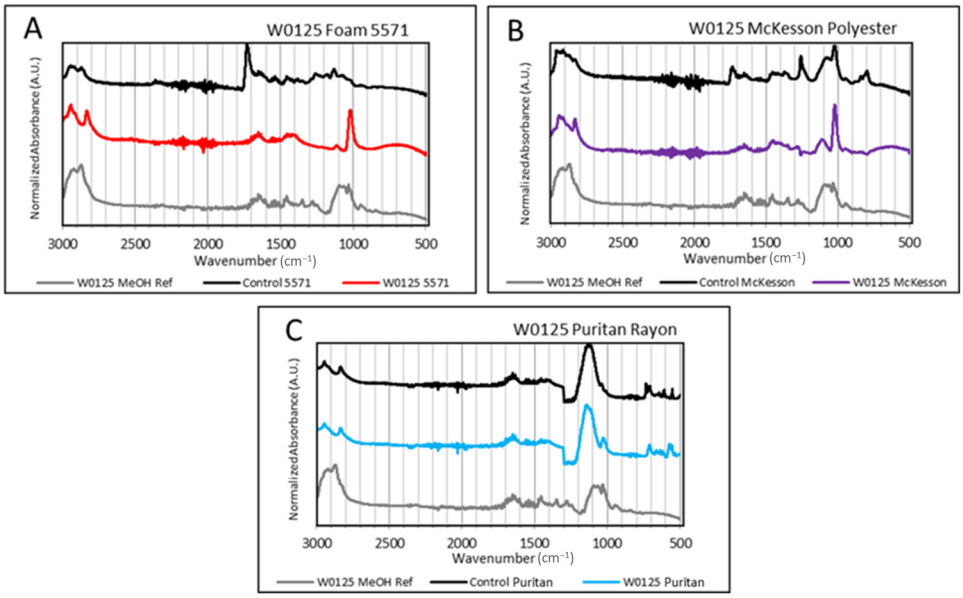

Figure 2. Comparative spectra of blank swab extract (black lines), the W0125 water-based lubricant reference (gray lines), and the lubricant extracted by MeOH from the swab in the designated color. The extract from the (**A**) foam 5571 swab is the red line, (**B**) Cardinal Health polyester swab is the purple line, and (**C**) Puritan rayon swab is the blue line. Remaining spectra presented in Figure S2.

The foam 5571 W0125 ELS spectrum showed two primary peaks that indicated the presence of the lubricant in the extract, at 1029 and 1100, where the 1100 cm^{-1} peak was significantly smaller than the same peak in the ELR (Figure 2A). It is important to note that the EBR spectra varied for this sample set. Two (2) of the EBR replicate spectra looked different from the ELR spectra (Figure 3, replicate D and E), which was expected; however, the other four (4) spectra looked like the ELR spectra although the intensities differed (Figure 3). However, the Student's *t*-test revealed that the two comparison sets were significantly different (*p*-value = 0.011). W0125 foam 5571 swab spectra were more similar to the W0125 ELR than the foam EBR spectra. The mean PCC score for the ELR–ELS spectra was 0.826 ± 0.017 and for the EBR–ELS spectra was 0.708 ± 0.040, resulting in the designation "lubricant preferred". This was likely due to the presence of the lubricant peaks in three of the six replicates of the ELS spectra.

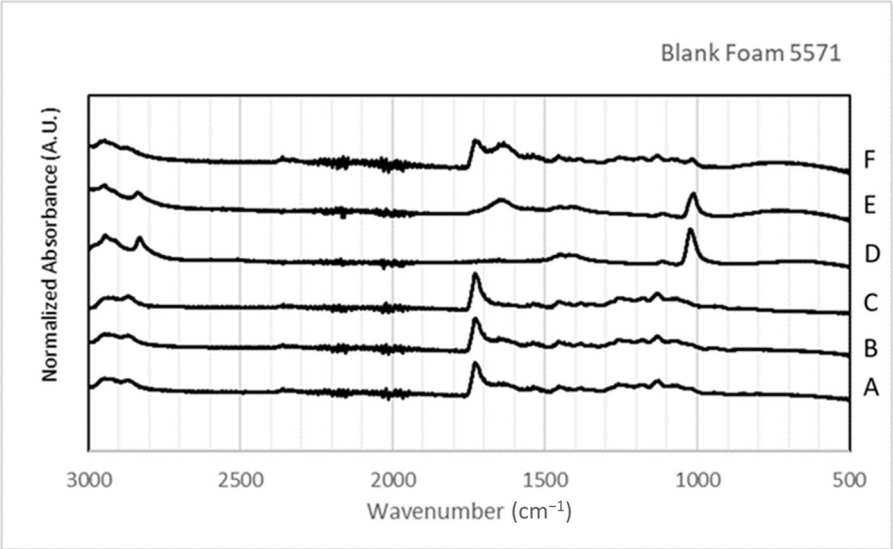

Figure 3. FTIR replicates (6) of the blank foam 5571 swabs extracted in methanol to show the variation of the resulting spectra.

The Cardinal Health polyester W0125 ELS spectra consisted of more resolved peaks at 1029 cm^{-1} and 1100 cm^{-1}. Again, the 1100 cm^{-1} peak was relatively smaller in comparison to the ELR, but the peak at 1029 cm^{-1} was more intense. The Cardinal Health polyester EBR spectra contained peaks near 1029 and 1100 cm^{-1} as well, so there is no clear indication that the observation of these peaks in the ELS was primarily from the lubricant through visual comparison (Figure 2B). The Student's *t*-test revealed that the comparison between the ELR–ELS spectra was significantly different from the EBR–ELS spectra (p = 0.003). The mean ELR–ELS PCC was 0.879 ± 0.004 and the EBR–ELS mean PCC was lower at 0.807 ± 0.012 (Figure 3). The ELR and the EBR looked quite similar except for one EBR peak at 1260 cm^{-1}. Although the influences from the lubricant were small, the resulting ELS spectra still looked more like the ELR spectra because the one EBR peak was not present in the ELS. However, the one difference in the EBR spectra may be the reason why the ELS–EBR average PCC scores were almost as high as the ELS–ELR average scores.

The W0125 rayon McKesson ELS spectra consisted of small peaks between 500 and 800 cm^{-1} and a broad peak at 1126 cm^{-1} with a shoulder peak at 1028 cm^{-1} (Figure 2C). There were also two smaller peaks located around 2830 cm^{-1} and 2950 cm^{-1}. This spectrum looked like the McKesson rayon EBR spectra, aside from the one shoulder peak at 1021 cm^{-1}, which appears to come from the lubricant. Upon the Student's *t*-test analysis, it was determined that there was a significant difference between the W0125 rayon McKesson ELS–EBR and the ELS–EBR comparison sets (p = 0.001), Table 3, Methanol. Based on the average PCC scores, the ELS spectra were more similar to the EBR spectra since the ELS–EBR PCC mean (0.852 ± 0.040) was higher than the ELS–ELR PCC mean (0.584 ± 0.048). Despite small influences from the lubricant in the resulting ELS spectra, the ELS spectra still looked like the EBR spectra.

The "lubricant preferred" designation was observed for all water-based lubricant samples extracted from the cotton, foam, and polyester swab with methanol. However, the rayon swabs yielded a "swab preferred" designation, since the ELS was more similar to the EBR (Table 3, Methanol).

With the hexane solvent, none of the water-based lubricant samples were designated as "lubricant preferred" due to the incomplete extraction of the lubricant components in the solvent (Table 3, Hexane). This was because the nonpolar hexane solvent does not extract polar compounds effectively and the water-based lubricant contains primarily polar compounds, therefore the lubricant components did not dissolve in the hexane solvent. The Student's *t*-tests results revealed that the W0125 ELR–ELS and EBR–ELS comparison

sets were significantly different, but the EBR–ELS average PCC was highest, resulting in a "swab preferred" designation (Table 3, Hexane). This designation was observed for all swab substrates indicating that the swab used was not important because the lubricant compounds could not be detected.

The DCM-MeOH solvent also provided different results from the MeOH solvent for the water-based lubricants. Surprisingly, the W0125 ELR–ELS and EBR–ELS comparison sets were indistinguishable from one another resulting in an "insignificant" designation for all swabs substrates (Table 3, DCM-MeOH). This was unexpected because DCM-MeOH contains polar and nonpolar attributes, and it was expected that the polar W0125 lubricant residues would be extracted by this solvent system as seen in previous research [23]. However, the ELS spectra generally looked more like the EBR spectra, regardless of the swab substrate, resulting in moderate PCC averages for all samples.

3.2. Raman Spectroscopy Screening for Lubricant Residues

3.2.1. The Extraction of Silicone-Based Condom Lubricants

The C0112 ELR Raman spectrum contained the following bands at 475, 709, 1036, and 1458 cm^{-1} attributed to Si–O–Si, Si–C, C–O stretching, and CH_3 bending, respectfully (Figure 4, gray lines). The 1036 and 1458 cm^{-1} bands were observed in the ELR, ELS, and the EBR (Figure 4) for each set of spectra. Considering that these bands were observed in the EBR, their source is unknown. It is likely that these bands were from the MeOH solvent that may not have fully evaporated, or they were from the swab substrate, or they are common stretches that are present in many components.

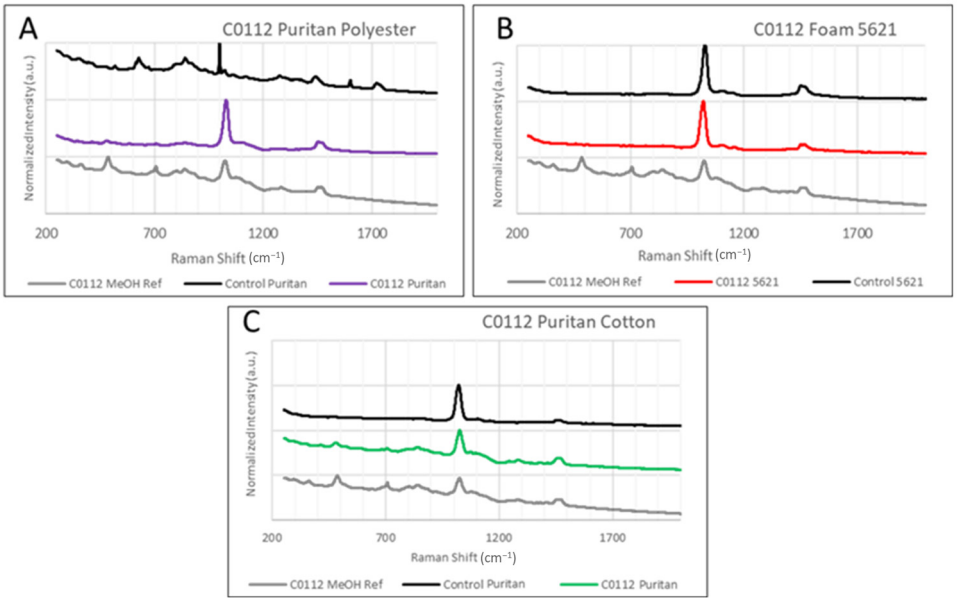

Figure 4. Comparative spectra of blank swab extract (black lines), the C0112 silicone-based lubricant reference (gray lines) and the lubricant extracted by MeOH from the swab in the designated color. The extract from the (**A**) Puritan polyester swab is the purple line, (**B**) foam 5621 swab is the red line, and (**C**) Puritan cotton swab is the green line. Remaining spectra presented in Figure S3.

The C0112 Puritan polyester ELS spectra contained the 1036 and 1458 cm^{-1} bands, but the 475 and 709 cm^{-1} bands were absent (Figure 4A). The Student's *t*-test results illustrated that there was no significant difference between the C0112 Puritan polyester ELS–EBR and the ELS–ELR comparison sets (*p*-score = 0.086), (refer to Table 5, Methanol).

Table 5. The average PCC scores, standard deviation, and *p*-values for ELR vs. ELS spectral comparisons and EBR vs. ELS spectral comparisons from the Raman instrument when the swab was extracted by either the hexane, methanol or DCM/MeOH solvent. NOTE: green = "lube preferred", yellow = "insignificant", red = "swab preferred".

Raman Data				
		Methanol		
Sample	**ELR v. ELS**	**EBR v. ELS**	***p*-Value**	**Determination**
W Cotton McK	0.820 ± 0.008	0.485 ± 0.105	5.48×10^{-6}	Lube Preferred
W Cotton Pur	0.846 ± 0.008	0.778 ± 0.013	0.021	Lube Preferred
W Foam 5571	0.702 ± 0.055	0.611 ± 0.095	0.254	Insignificant
W Foam 5621	0.775 ± 0.019	0.662 ± 0.034	0.015	Lube Preferred
W Poly CH	0.837 ± 0.013	0.551 ± 0.029	4.67×10^{-10}	Lube Preferred
W Poly Pur	0.851 ± 0.009	0.838 ± 0.011	0.651	Insignificant
W Rayon Mck	0.836 ± 0.008	0.406 ± 0.231	6.89×10^{-6}	Lube Preferred
W Rayon Pur	0.837 ± 0.009	0.586 ± 0.026	6.29×10^{-9}	Lube Preferred
C Cotton McK	0.952 ± 0.001	0.594 ± 0.142	1.40×10^{-5}	Lube Preferred
C Cotton Pur	0.921 ± 0.012	0.712 ± 0.038	2.45×10^{-7}	Lube Preferred
C Foam 5571	0.672 ± 0.053	0.550 ± 0.104	0.133	Insignificant
C Foam 5621	0.773 ± 0.063	0.652 ± 0.066	0.105	Insignificant
C Poly CH	0.981 ± 0.001	0.601 ± 0.053	1.13×10^{-11}	Lube Preferred
C Poly Pur	0.837 ± 0.016	0.768 ± 0.021	0.086	Insignificant
C Rayon Mck	0.892 ± 0.005	0.468 ± 0.240	1.02×10^{-5}	Lube Preferred
C Rayon Pur	0.895 ± 0.007	0.676 ± 0.063	0.001	Lube Preferred
		Hexane		
Sample	**ELR v. ELS**	**EBR v. ELS**	***p*-Value**	**Determination**
W Cotton McK	0.897 ± 0.006	0.872 ± 0.006	0.293	Insignificant
W Cotton Pur	0.785 ± 0.020	0.679 ± 0.115	0.141	Insignificant
W Foam 5571	0.794 ± 0.021	0.954 ± 0.002	0.001	Swab Preferred
W Foam 5621	0.522 ± 0.028	0.754 ± 0.048	0.001	Swab Preferred
W Poly CH	0.793 ± 0.022	0.139 ± 0.013	6.95×10^{-16}	Lube Preferred
W Poly Pur	0.818 ± 0.024	0.891 ± 0.01	0.091	Insignificant
W Rayon Mck	0.522 ± 0.028	0.542 ± 0.112	0.772	Insignificant
W Rayon Pur	0.788 ± 0.025	0.684 ± 0.050	0.067	Insignificant
C Cotton McK	0.794 ± 0.024	0.909 ± 0.003	0.007	Insignificant
C Cotton Pur	0.924 ± 0.001	0.723 ± 0.115	0.003	Lube Preferred
C Foam 5571	0.947 ± 0.001	0.965 ± 0.001	0.008	Swab Preferred
C Foam 5621	0.952 ± 0.001	0.410 ± 0.075	1.04×10^{-11}	Lube Preferred
C Poly CH	0.769 ± 0.060	0.189 ± 0.090	2.33×10^{-9}	Lube Preferred
C Poly Pur	0.844 ± 0.061	0.827 ± 0.058	0.82	Insignificant
C Rayon Mck	0.952 ± 0.001	0.467 ± 0.070	6.41×10^{-11}	Lube Preferred
C Rayon Pur	0.460 ± 0.084	0.508 ± 0.142	0.625	Insignificant
		DCM/MeOH		
Sample	**ELR v. ELS**	**EBR v. ELS**	***p*-Value**	**Determination**
W Cotton McK	0.620 ± 0.134	0.498 ± 0.123	0.369	Insignificant
W Cotton Pur	0.882 ± 0.003	0.585 ± 0.007	5.28×10^{-17}	Lube Preferred
W Foam 5571	0.885 ± 0.004	0.679 ± 0.085	0.001	Lube Preferred
W Foam 5621	0.902 ± 0.007	0.872 ± 0.004	0.245	Insignificant
W Poly CH	0.910 ± 0.005	0.693 ± 0.032	8.58×10^{-6}	Lube Preferred
W Poly Pur	0.906 ± 0.005	0.295 ± 0.259	3.08×10^{-8}	Lube Preferred
W Rayon Mck	0.811 ± 0.038	0.532 ± 0.068	9.36×10^{-5}	Lube Preferred
W Rayon Pur	0.869 ± 0.020	0.588 ± 0.036	4.22×10^{-7}	Lube Preferred
C Cotton McK	0.986 ± 0.001	0.804 ± 0.091	0.001	Lube Preferred
C Cotton Pur	0.786 ± 0.099	0.714 ± 0.089	0.437	Insignificant
C Foam 5571	0.790 ± 0.046	0.611 ± 0.86	0.009	Lube Preferred
C Foam 5621	0.937 ± 0.002	0.894 ± 0.004	0.003	Lube Preferred
C Poly CH	0.979 ± 0.001	0.751 ± 0.032	5.79×10^{-9}	Lube Preferred
C Poly Pur	0.986 ± 0.001	0.126 ± 0.360	3.81×10^{-10}	Lube Preferred
C Rayon Mck	0.739 ± 0.107	0.593 ± 0.083	0.155	Insignificant
C Rayon Pur	0.837 ± 0.038	0.632 ± 0.047	0.002	Lube Preferred

Similarly, the C0112 foam 5621 ELS spectra contained bands at 1036 and 1458 cm^{-1} which were also present in the ELS and EBR spectra. However, the ELS looked more similar to the EBR spectra, which contained only those two peaks as well (Figure 4B). The ELR had additional peaks that were not present in the ELS. This indicated that the C0112 lubricant was not efficiently extracted from the foam 5621 swab. Three of the six replicates looked similar to the EBR spectra and the other three resembled the ELR spectra. The ELR–ELS mean PCC score was 0.773 ± 0.063 whereas the EBR–ELS mean PCC score was 0.652 ± 0.066. Even though the ELR–ELS average score was slightly higher, the variance within the sample set made it indistinguishable from the EBR–ELS average score based on the Student's *t*-test (Table 5, Methanol). Therefore, it may be difficult to determine the presence of lubricant residue on this type of substrate.

The C0112 Puritan cotton ELS spectra consisted of the same aforementioned bands as in the C0112 ELR, but the 475 cm^{-1} band was relatively less intense (Figure 4C). There was a 1036 cm^{-1} band in the C0112 ELR spectra. The 1036 cm^{-1} band in the EBR spectra may have influenced the ELS spectra, resulting in the band being more intense relative to the rest of the spectra. Although the ELS had a similar profile to the ELR, there were influences from the EBR. The Student's *t*-test confirmed that there were significant differences between the ELS–EBR set and ELS–ELR set ($p = 2.45 \times 10^{-7}$). The average PCC scores of ELS–EBR (0.712 ± 0.038) and ELS–ELR (0.921 ± 0.012) sets were compared, and it was determined that the C0112 Puritan cotton ELS spectra favored the ELR spectra, resulting in a "lubricant preferred" designation (Table 5, Methanol) despite the similarities to the EBR.

All cotton and rayon samples resulted in a "lubricant preferred" designation for the condom samples extracted from the swab by methanol (Table 5, Methanol). Both foam samples and one of the polyesters swab samples provided poor results leading to a "statistically similar" designation.

Alternative findings were observed in the hexane solvent (Table 5, Hexane). Only one sample of each fiber type (cotton, foam, rayon, and polyester) was designated as "lubricant preferred". The other samples' comparison sets were indistinguishable from one another resulting in a "insignificant" designation. However, the foam 5571 swab resulted in a "swab preferred" designation due to the ELS spectra being more similar to the EBR spectra.

The DCM-MeOH solvent provided the best results in comparison to the other solvents under Raman analysis. All of the polyester and foam samples looked similar to the ELR spectra, which resulted in the desired designation (Table 5, DCM-MeOH). However, one of the cotton samples' and one of the rayon samples' comparison sets were statistically similar.

3.2.2. The Extraction of Water-Based Lubricants

The methanol-extracted W0125 lubricant reference showed distinct bands at 849, 1028, 1278, 1472, and 1687 cm^{-1} relating to the C–O–C, C–O, O–CH_3, CH_3 deformation, and C=O stretches, respectively (Figure 5, gray lines).

All of these bands were observed in the W0125 Cardinal Health polyester ELS spectra at relatively the same intensities as the bands in the ELR spectra (Figure 5A). The Student's *t*-test confirmed that there were distinct differences between the ELS–ELR and EBR–ELS spectra since the ELS–ELR average PCC score (0.837 ± 0.013) was higher than the ELS–EBR average PCC (0.551± 0.029) score (Table 5, Methanol). Based on these results, it could be determined that the lubricant could be extracted and associated with the known reference.

Similarly, the foam 5621 ELS spectra contained the above specified bands suggesting that lubricant was present after the swab was extracted with methanol. However, the 1687 cm^{-1} band was relatively less intense than the same band in the ELR spectra (Figure 5B). The 1028 cm^{-1} band was more intense relative to the other bands in the ELS spectra and may have been influenced by the substrate. Three of the ELS replicates appeared to be more similar to the EBR spectra and the other three resembled the lubricant reference spectra. Still, the Student's *t*-test showed that the W0125 ELS–ELR and the ELS–EBR were significantly different ($p = 0.015$). The W0125 ELS–ELR average PCC score

was 0.775 ± 0.019 and the ELS–EBR PCC score was 0.662 ± 0.034, resulting in a "lubricant preferred" designation (Table 5, Methanol).

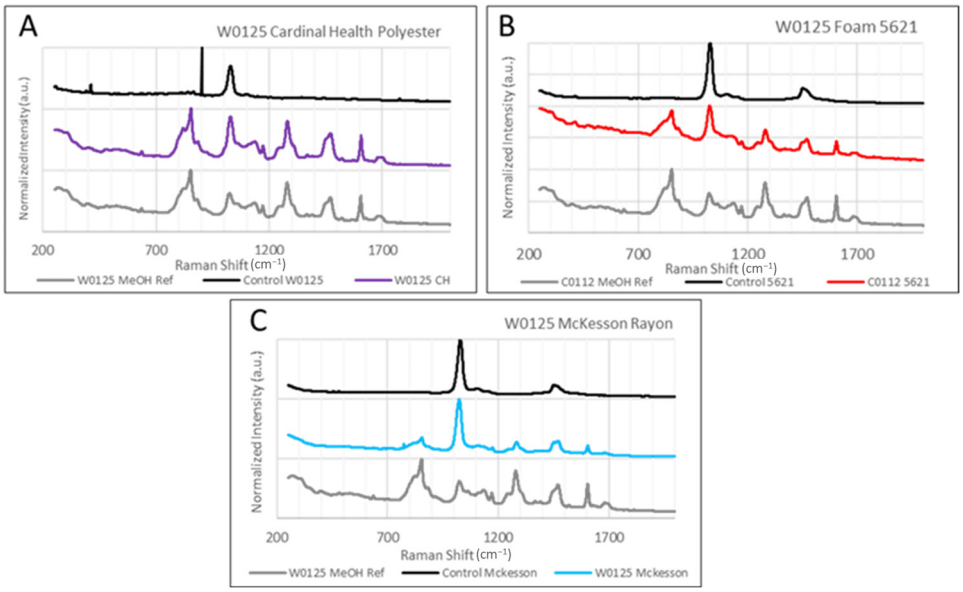

Figure 5. Comparative spectra of blank swab extract (black lines), the W0125 water-based lubricant reference (gray lines) and the lubricant extracted from the swab in the designated color. The extract from the (**A**) Cardinal Health polyester swab is the purple line, (**B**) foam 5621 swab is the red line, and (**C**) McKesson rayon swab is the blue line. Remaining spectra presented in Supplemental Figure S4.

The rayon McKesson ELS spectra contained the same bands as the W0125 ELR reference but at a relatively lower intensity. The band at 1028 cm^{-1} looked more similar to the same band in the rayon McKesson EBR spectra indicating interference from the swab (Figure 5C). The EBR spectra had a band at 1472 cm^{-1} that also influenced the ELS spectra. This band in the ELS spectra appeared to have equal influence from the EBR and ELR spectra. Five of the replicates resembled the ELR spectra and one replicate looked like the EBR spectra. The Student's *t*-test illustrated that the rayon McKesson ELR–ELS and EBR–ELS were significantly different (Table 5). The ELR–ELS PCC score (0.836 ± 0.008) was higher than the EBR–ELS PCC score (0.406 ± 0.231), indicating a "lubricant preferred" designation.

All of the cotton and rayon ELS samples extracted with methanol resulted in a "lubricant preferred" designated. One of the polyester and foam samples comparison sets could not be differentiated (Table 5, Methanol).

It was expected that the extraction of the water-based lubricants using hexane would not result in many positive responses. One of the polyester swab samples was designated as "lubricant preferred". Both of the foam samples were "swab preferred" due to the similarity of the spectra between the ELS and EBR (Table 5, Hexane).

The DCM-MeOH-extracted water-based lubricants analyzed using Raman spectroscopy provided the strongest results. Most of the samples resulted in the desired designation (Table 5,DCM-MeOH). However, one of the cotton and one of the foam samples were designated as "statistically similar". The DCM-MeOH samples resulted in the highest average PCC, when a positive "lubricant preferred" designation was observed, regardless of the substrate and the lubricant used (Table 4).

4. Discussion

The averaged PCC scores for each comparison set (EBR–ELS and ELR–ELS) are shown in Table 3 for each sample analyzed. The FTIR analyzed samples (Table 3, Methanol) demonstrated that the "lube preferred" designations were assigned to more samples extracted in MeOH than the other solvents. The "lube preferred" designation was observed for all water-based lubricant samples extracted from the cotton, foam, and polyester swab

with methanol. However, the rayon swabs containing water-based lubricant yielded a "swab preferred" designation since there was very little contribution from the lubricant, likely due to the rayon coating being more soluble than the lubricant (Table 3, Methanol, Figure 2). The results of the silicone-based lubricants were largely different, where most of the results yielded a "swab preferred" designation. This is because silicone-based lubricants do not readily solubilize in the methanol solvent. Therefore, it is likely that any coatings or fillers within the swab substrate material would yield a stronger FTIR spectrum vs. the lubricant spectrum.

Hexane FTIR results were opposite of the methanol results (Table 3, Hexane). None of the water-based lubricant samples were designated as "lube preferred" due to the incomplete extraction of the polar lubricant components in the non-polar solvent. It was observed that regardless of the swab substrate extracted in hexane, most of the samples resulted in "swab preferred" designations. This indicated that the swab used was not important because the lubricant compounds could not be detected. However, there were four silicone-based samples that yielded a "lube preferred" designation: two on the cotton swab, one on the polyester swab, and one on the rayon swab. The foam/silicone-based swabs yielded either an "insignificant" or a "swab preferred" designation. The substrate was preferred in the resulting spectra due to the substrate being more soluble in the hexane solvent than in the non-polar solvent.

For the DCM-MeOH-extracted samples, the W0125 ELR–ELS and EBR–ELS comparison sets were indistinguishable from one another resulting in an "insignificant" designation for all swab substrates (Table 3, DCM-MeOH). This was unexpected because DCM-MeOH contains polar and nonpolar attributes, and it was expected that the mid-polar/polar W0125 lubricant residues would be extracted more by this solvent system as seen in previous research [23]. However, the ELS spectra generally looked more like the EBR spectra, regardless of the swab substrate, resulting in moderate PCC averages for all samples. Similar results were observed for the silicone-based lubricants, where most of the results yielded an "insignificant" or "swab preferred" result. Only the rayon swabs with the silicone-based lubricant yielded a "lube preferred" result. This was the one solvent for which both versions of the rayon swab yielded a "lube preferred" designation.

The Raman data yielded more "lube preferred" designations across all three solvents. All of the cotton and rayon ELS samples that contained either the silicone-based or water-based lubricant and were extracted with methanol resulted in a "lubricant preferred" designation. These samples had strong ELR–ELS PCC scores (>0.80) and the EBR–ELS scores were generally less than 0.80. One of the polyester and foam samples comparison sets could not be differentiated due to moderately strong PCC scores (Table 5, Methanol).

It was expected that the extraction of the water-based lubricants using hexane would not result in any positive responses, similar to what was observed with the FTIR results. This was generally observed although one of the polyester swab samples was designated as "lubricant preferred". Both of the foam samples were "swab preferred" due to the similarity of the spectra between the ELS and EBR (Table 5, Hexane). The silicone-based lubricants yielded four "lube preferred" samples just like the IR results and the remaining samples yielded a statistically similar designation. However, the four that had a "lube preferred" result via the Raman sample were not the same four that had the same designation under IR, except the Puritan cotton swab which had a positive result under both instruments.

The extraction of water-based and silicone-based lubricants from the swabs using the DCM/MeOH solvent yielded the most "lube preferred" designations when analyzed via Raman spectroscopy (Table 5, DCM-MeOH). Most of the samples resulted in the desired designation. Those that had this designation had an average PCC score for the ELR–ELS comparison score of 0.898 and the associated EBR–ELS comparison was 0.599. However, one of the cotton and one of the foam 5621 samples were designated as "insignificant" for the water-based lubricants and one of the cotton and one of the rayon samples were "insignificant" for the silicone-based swab samples. The DCM-MeOH samples resulted

in the highest average PCC, when a positive "lube preferred" designation was observed, regardless of the substrate and the lubricant used (Table 4).

Based on these results, the extraction of water-based lubricants from a cotton swab had the "lube preferred" designation when methanol was used as the solvent under FTIR or Raman spectroscopy. Mixed results were observed for the other three swabs using methanol via the two instruments. However, Raman spectroscopy provided positive results for most of the water-based lubricants regardless of the swab but methanol was important for extracting most of the water-based lubricant components for detection by the Raman instrument. The most consistent results were observed for components extracted from the cotton swab and the rayon swab. The silicone-based lubricants did yield some positive responses when hexane was used as the solvent when either IR or Raman spectroscopy was used (i.e., four positive results). However, five and six positive responses, out of eight, were observed using Raman spectroscopy with either methanol or DCM/MeOH solvents, respectively. This was the most observed for any combination including hexane and IR spectroscopy, which has been the traditional protocol used in screening for the presence of silicone-based lubricants. Methanol and DCM/MeOH solvents did not generate a lot of positive responses considering the non-polar nature of the lubricant components. The cotton and rayon swabs yielded the most "lube preferred" designations for the silicone-based lubricants. Cotton and polyester were the best swabs across all combinations for the water-based lubricants.

In a real world setting, understanding the swab that presents the least interference with spectroscopy techniques would be helpful in providing a positive result when screening. Conducting one-on-one comparisons, between the extracted lubricant and a swab and lubricant reference, as presented in this paper, will allow any laboratory to determine if there is a lubricant present on the unknown swab even if the lubricant is a water-based lubricant. The collection/recovery of lubricant residues from a victim or their clothing can introduce biological cells to the residue recovered and potentially interfere with spectroscopy screening of these residues. Although this aspect of sexual assault evidence can occur, the focus of this paper was on the collection of lubricants from non-human sources.

5. Conclusions

Raman spectroscopy provided more "lubricant preferred" designations, irrespective of the solvent or type of swab used. There was less interference from the swab material on the resulting ELS spectra. This was in comparison to the FTIR results which only provided strong indicators of the presence of lubricants for water-based lubricants extracted with a methanol solvent regardless of the swab material. The use of Raman spectroscopy for lubricant screening can provide valuable information for the forensic laboratory when determining how they will process sexual assault samples. Considering the extraction solvents explored, MeOH and DCM-MeOH extracted more lubricant components from the swab substrate, producing high PCC correlations regardless of swab type. Cotton appears to produce minimal interference under all of the conditions and provided consistently reliable results, followed by rayon and polyester fiber swabs. Foam did not yield optimal outcomes and is not advisable for sexual lubricant sample collection due to the potential of yielding false negative results. Additional research may be useful in determining if the foam fiber swab morphology is responsible for the matrix interference or if cutting the foam swab into smaller pieces affected the results.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/forensicsci3040045/s1, Figure S1: Comparative spectra of blank swab extract (black lines), the C0112 silicone-based lubricant reference (gray lines) and the lubricant extracted by MeOH from the swab in the designated color. The extract from the (A) Puritan cotton swab is the green line, (B) Foam 5571 swab is the red line, (C) Cardinal Health polyester swab is the purple line, and (D,E) Puritan rayon and McKesson rayon swab is the blue line; Figure S2: Comparative spectra of blank swab extract (black lines), the W0125 water-based lubricant reference

(gray lines) and the lubricant extracted by MeOH from the swab in the designated color. The extract from the (A) and (B) Puritan and McKesson cotton swab is the green line, (C) Foam 5621 swab is the red line, (D) Puritan polyester swab is the purple line, and (E) McKesson rayon swab is the blue line; Figure S3: Comparative spectra of blank swab extract (black lines), the C0112 silicone-based lubricant reference (gray lines) and the lubricant extracted by MeOH from the swab in the designated color. The extract from the (A) McKesson rayon swab in blue line, (B) McKesson cotton swab is the green line, (C) Foam 5571 swab is the red line, (D) Puritan rayon swab is the blue line, and (E) Cardinal Health polyester swab is the purple line; Figure S4: Comparative spectra of blank swab extract (black lines), the W0125 water-based lubricant reference (gray lines) and the lubricant extracted by MeOH from the swab in the designated color. The extract from the (A,B) McKesson and Puritan cotton swab in green line, (C,D) Puritan and McKesson rayon swab is the blue line, and (E) Foam 5571 swab is the red line; Table S1: An example of the pairwise PCC comparison sets that were calculated for each sample. Table S1A shows the PCC scores for the EBR-ELS samples. Table S1B shows the ELR-ELS PCC scores. The PCC score for each comparison set was then compared using a student t-test to determine similarity. The results to the Student's *t*- test are shown in Table C.

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