Date: 10/16/03

Department of Forensic Biologo

Condom Trace Evidence Analysis Manual

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I. Detection of Polydimethylsiloxane (PDMS) by Fourier Transform Infrared Spectrometry (FT-IR)

A. General guidelines

- 1. All reagents are available pre-made and quality control checked. Do not use supplies that have not been quality control checked.
- 2. In general, semen analysis (AP, sperm identification, or p30 ELISA) is done prior to lubricant analysis. The analysis of amylase and other body fluids should also precede lubricant analysis.
- 3. Scissors and other cutting instruments must be cleaned with 10% bleach followed by 70% ethanol and dried before each cutting of a swab, including blanks. They must not contact any "greasy" or silicone-based lubricants before sampling the swabs. Clean scissors between samples. New razor and scalpel blades must not be used without first being cleaned as they may be coated with a lubricant.
- 4. An adhesive is used to attach cotton to the stick of a swab. These adhesives may produce an infrared spectrum, even at low concentrations, that can interfere with the spectrum of polydimethylsiloxane (PDMS). Therefore when removing a portion of the cotton material, do not contact the stick with the scissors
- To reduce the amount of purge time required to reduce the absorption peaks due to carbon dioxide and ambient moisture, to no breathe into the sample compartment when changing samples. Use the compartment window when possible and make sample changes quickly.
- 6. Negative controls are processary to detect any interfering peaks from the extraction solvent, scissors, swabs, and/or any other extraneous contaminants.
- 7. Perform ALL mullylene chloride extractions under an operating chemical fume hood, not a biohagarchood.

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- B. Analysis by Fourier Transform infrared spectrometry
 - 1. Start-up
 - a. Startup of system, OMNIC E.S.P. software, and diagnostics check

NOTE: The procedure assumes some knowledge of the Windows® operating system.

- 1. NOTE: The IR bench is left on at all times to maintain stability within the instrument and eliminate warm-up. Turn on the computer and monitor. Check that the Nicolet Magna-IR 550 Series II bench is turned on and ready to collect spectra. The Power indicator light, at the top front left of the instrument, should be lit. If the indicator light is not lit, check the power switch at the rear of the instrument and the power ord to ensure that the connections are intact.
- 2. A prompt for the network connection appears. Enter John login name and password.
- 3. Double click the OMNIC E.S.P. icon on the computer desktop.
- 4. Click the Collect option on the menu bes
- Click on Advanced Diagnostics.

This verifies that the components of the instrument are operating properly. If there are any components with a red slash brough it, refer to the **Troubleshooting** section.

6. Click the Report button. Click the View More button. Click the Print button. Click the Return button twice. Click the close window button at the top right of the window.

Store this report in the FT-IR Instrument Usage Log, Maintenance, & Diagnostics Records binder under the Diagnostics section.

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Experiment setup b.

- 1. In the Experiment title bar, which is just above the toolbar, make sure Lubricants Analysis method is selected.
- 2. In the FT-IR Instrument Usage Log, Maintenance, & Diagnostics Records binder under the Usage Log section, enter the date, instrument start time, purpose of usage, and your initials.

NOTE: Remember to fill in the *Time Ended* column when finished with the system along with any comments concerning the instrument's operation.

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c. Verification of collection parameters

1. To verify that the collection settings are correct, click the Collect option on the toolbar and select Experiment Setup.... The following list contains the parameter settings for the collection of spectra. Compare each value with those listed below.

Collect

No. of Scans: 32 Resolution: 2

Data spacing: 0.482cm⁻¹ Final format: Absorbance

Correction: None

☐ Automatic atmospheric correction

☐ Preview data collection

File Handling

✓ Save interferograms

Background Handling

• Collect background after 18 minutes

Main

Bench

Gain: Autogain Velocity: 0.6329

Aperture: 69

Min/Max

Sample Compartment: Main

Detector: DTGS KBr

Beamsplitter: KBr

Source: IR

Accessory: None

Window material: None

Spectral Range

Saved: 4001-400

☐ Single beam

□ Tone

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Quality

Select View: Spectrum

e opectrum		
	Sensitivity	
✓ Use spectral quality checks	-	
✓ Spectrum checks		
□ Peaks present		
✓ Totally absorbing peaks	50	
✓ Fringes or channeling	5	
□ Derivative peaks	N/A	
✓ Baseline error	5	
✓ CO2 levels	50	
✓ H2O levels	70	G
	Nanus	•
Level		
Happ-Genzel		
on: Mertz	Y.O.	
interferogram	10.	
at start of collection	<u>ጉ</u> ፣	
	,	
ntout from Advanced Dagnos	stics provides th	e neces

Advanced

Zerofilling: 1 Level

Apodization: Happ-Genzel Phase correction: Mertz ☐ Single-sided interferogram

□ Reset bench at start of collection

Diagnostics

The printout from Advanced Dagnostics... provides the necessary information needed to determine that all systems are running properly.

2. If the settings are correct, the software is ready to collect spectra. Proceed to the section Collection of spectra.

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2. Collection of spectra

a. Removing and placing IR cards in the sample holder

- 1. On either side of the sample chamber are two levers. Flip them up.
- 2. Open the window to the sample chamber by sliding the cover toward the back of the instrument and remove or place any IR cards in the sample holder.
- 3. Close the window by sliding the cover toward the front of the instrument. Flip both levers down.

NOTE: The levers are kept in the raised position when the cover sopen so that the purge is maintained in the rest of the instrument.

b. Collection of the background spectrum

A background spectrum should be collected every ten (10) minutes or as necessary. Certain actions on the part of the analyst may require the collection of background spectra more frequently.

- 1. The sample chamber should be empty. If not remove any IR cards in the sample chamber following the instructions in the section **Removing and placing IR cards in the sample holder**.
- 2. Click the Col Bkg (Collect Background) button on the toolbar.
- 3. A Confirmation window wears. It reads:

Background

Please prepare to collect the background spectrum

Click OK.

4. A Confirmation window appears after the background has been collected. It reads:

Data collection has stopped.

Add to Window 1?

Click No.

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Collection of spectra from blank IR cards

This section describes the collection of spectra from the IR cards before application of the samples. These negative controls show that the IR cards do not contribute to the PDMS spectrum. Every IR card is checked before sample application! Discard any cards that contain detectable contaminants!

- Place a blank IR card (3M Type 61 polyethylene) into the chamber following the instructions 1. in the section Removing and placing IR cards in the sample holder.
- 2. Click the Col Smp (Collect Sample) button on the toolbar.
- For the spectrum title, type: card blank. Click OK.

 A Confirmation window appears which --3.
- 4.
- 5.

Please prepare to collect the sample spectrum.

Click OK.

A Confirmation window appears after the spectrum has been collected. It reads: 6. Data collection has stopped.

Add to Window 1?

Click Yes.

NK CARD SPECTRUM FOR CONTAMINANTS. 7.

The spectrum stould appear similar to Figure 1 on the following page.

- Change the display limits by selecting Display Limits... in the View menu and set the X-Axis 8. Limits from a Start: value of 1300 to an End: value of 700. Click OK.
- 9. Click on the View menu and select Full Scale.
- The spectrum (1300-700cm⁻¹) should appear similar to the spectrum on the following page. 10. The two peaks between 750-700cm⁻¹ are from polyethylene.

If similar, remove the IR card and continue to step 11.

If there are any other peaks, remove the card and **discard** it. Start at step 1 with a new card.

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- 11. Remove the blank card from the sample chamber. Store the IR card in a clean, dry place until the sample is ready to be applied to the card.
- 12. Select the spectrum card blank from the list. Once the spectrum title is highlighted, press the Cut button on the toolbar.
- 13. Repeat steps 1-12 until all the blank cards have been checked for contaminants (change the Display Limits back to 4000-400cm⁻¹).

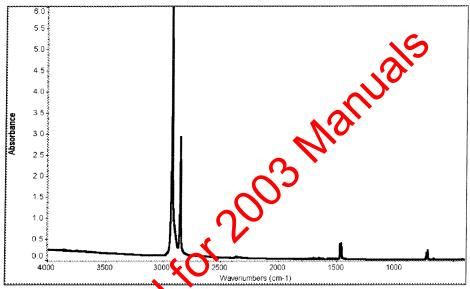


Figure 1a: Spectrum of objective IR card in the 4000-400cm⁻¹ region.

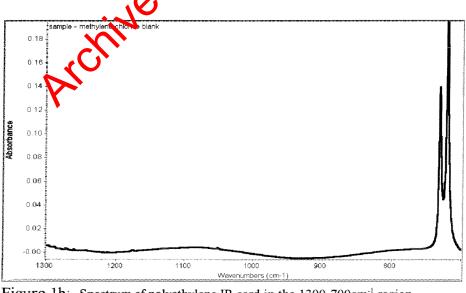


Figure 1b: Spectrum of polyethylene IR card in the 1300-700cm⁻¹ region.

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d. Preparation of extraction negative (methylene chloride blank), positive control, and sample extracts

The purpose of the extraction negative control (reagent blank) is to verify that the solvent does not contain any infrared active species that may interfere with the detection of PDMS. The purpose of the positive control is to demonstrate the effectiveness of the extraction.

NOTE: These steps **MUST** be performed under a chemical fume hood.

- 1. Prepare an extraction negative tube by labeling an empty 1.5mL microcentrifuge tube as such.
- 2. Obtain a positive control tube and note the lot number on the extraction worksheet.
- 3. With a fine tipped marker, label each checked, blank IR care with the appropriate identifier (extraction negative, positive control, or sample identifier (FB number and swab type)). Check off the appropriate box on extraction worksheet

NOTE: Each card must contain the date and time it was prepared and the initials of the individual preparing the cards.

- 4. Place all tubes in the order listed on the extraction worksheet.
- 5. Place the IR sample cards in the carder as the tubes. Have a witness check the tubes and cards and initial the extraction worksheet.
- 6. Pipet approximately 5mL Chethylene chloride into a labeled, working stock tube.

NOTE: Whenever pipetting methylene chloride, the pipet tip must be "conditioned" by pipetting the solvent up and down about 5-10 times before dispensing. If this is not done, the methylene chloride will leak out of the tip immediately.

- 7. Pipet 75μ L of methylene chloride into each tube. Ensure that the entire sample is covered in solvent. Close each tube tightly to minimize evaporation.
- 8. Mark the level of the meniscus on the side of each tube with a fine tipped marker.

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e. Deposition of samples onto IR sample cards

NOTE: This **MUST** be performed under a chemical fume hood.

- 1. Place each IR sample card onto an elevated surface, ensuring that the membrane does not touch any other surface or solution.
- 2. Vortex the appropriate sample tube for 15 seconds.

NOTE: Check the meniscus of the PDMS standards to ensure that the solvent levels are not lower than were previously marked. Add sufficient methylene chloride to raise the level to the previously marked meniscus for samples that have experienced solvent loss. Vortex the affected sample tube(s) for 15 seconds.

With a pipet and an aerosol resistant tip (ART), take $3-5\mu L$ of the extract and place **onto the center** of a labeled IR card. Allow the solvent to dry. Take another $3-5\mu L$ of the extract and place onto the same part of the IR card. Allow the solvent to dry. Repeat until all of the extract has been delivered to the card. Repeat for each card labeled for samples and blanks.

NOTE: It is important that most of the selvent is concentrated at the center of the IR card window because the infrared beam passes through near the center (adjacent to the red laser beam). This area must contain a significant amount of the sample for an accurate spectrum to be collected.

4. After the methylene chloride has evaporated, wait 10 minutes to allow residual moisture to evaporate. Once dry, the samples are ready for IR analysis.

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f. Collection of control and sample spectra

- Collect a new background spectrum. The background level should be checked every ten 1. minutes, or more frequently, if required.
- 2. Begin to collect spectra for each card. Follow the instructions in the section Removing and placing IR cards in the sample holder.
- 3. Place an IR card with a sample into the chamber.
- 4. Click the Col Smp (Collect Sample) button on the toolbar.
- The Collect Sample window appears. It reads: 5. Enter spectrum title:

For the spectrum title, enter the sample identifier as it poears on the IR card and the worksheet. Click OK.

A Confirmation window appears which reads 6.

Sample

Please prepare to collect the sample spectrum.

Click OK.

A Confirmation window appear for the spectrum has been collected. It reads: 7.

Data collection has stopped.

Add to Window 1?

Click Yes.

from the sample chamber and place the next IR sample card into the 8. Remove the IR chamber.

Repeat sample collection steps 4-8 for all sample cards.

After collecting spectra for all sample cards, the spectra should be baseline corrected. For the 9 Automatic Baseline Correction function to baseline correct the entire spectrum, the X-Axis Limits (i.e., wavenumbers) must extend from 4000-400cm⁻¹. If the X-Axis Limits are not from 4000-400cm⁻¹, then select Display Limits... in the View menu and enter 4000 as the Start: value and 400 as the End: value.

NOTE: Automatic baseline correction must be performed with the spectrum displayed as an Absorbance spectrum.

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- 10. From the Edit menu, choose Select All. All the spectra should now be displayed in red.
- 11. Click on Process on the menu bar and select Automatic Baseline Correct.

Spectra that have been baseline corrected have an asterisk (*) preceding the name of the spectrum.

- 12. The original uncorrected spectra should be deleted from the window by using the select and cut method. Click on the down arrow to the right of the sample name window. A pull-down list of all the spectra in the window will be displayed. Click on each name and cut each one (scissor function) that is not baseline corrected (spectra without asterisks).
- 13. Save the baseline corrected spectrum:
 - a. Click on File and select Open....
 - b. Click the button Create New Folder and name it with the appropriate extraction set date and time. Click off the folder.
 - c. Close the Open window.
 - d. Select all of the baseline corrected spectra to save into the folder. From the Edit menu, choose Select All. All of the spectra should be displayed in red. Click on File and select Save As....
 - e. Open the extraction set folder for the appropriate extraction. Name the file with the extraction date and time and click Save. Close the Open window.
- 14. Proceed with the labeling and storage of IR cards.
- 15. Continue with the Analysis of Spectra section.

g. Labeling and storage of IR cards

- 1. On the outside of a coin envelope, print the case number, type of sample, analyst's initials, and date on which the IR card was prepared. This envelope is used to store the IR card after the spectrum has been collected.
- 2. Seal the envelope with tape and initial and date the seal.
- 3. Store the sample card in a secure place at room temperature.

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h. Storage of spectral data

The following printouts (hard copies) must be kept with each case file:

- positive PDMS control
- extraction negative
- all casework samples

Digital archives (computer) can be deleted after the case file has been reviewed AND after a period of six months after the arrival of the evidence in the laboratory.

3. Analysis of spectra

The purpose of the analysis is to provide results concerning the identity of any compounds detected in casework samples. The analysis includes overlaying the sample and RDMS standard spectra and performing spectral subtraction and library searches, if needed.

In order to analyze one spectrum at a time, each spectrum will be neved to its own analysis window.

- 1. Highlight the spectrum to be analyzed by opening the sample name window (down arrow to the right of the sample name) and clicking on the name of the selected spectrum.
- 2. Using the "cut" function (scissor icon), cut the selected spectrum.
- 3. Move the spectrum to a new window by clicking on the Window menu. Select New Window. Name the new window with the appropriate sample identifier. Click OK. A blank window should appear.
- 4. Hit "Paste." The selected spectrum should appear in the window.

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a. Standard PDMS spectrum

The spectrum of PDMS should appear similar to the spectrum below. There should be four peaks that are around 1262, 1096, 1020, and 801cm⁻¹.

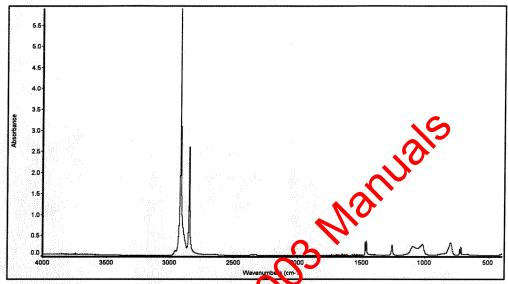


Figure 2a: Spectrum of PDMS in the 4000 400cm⁻¹ region

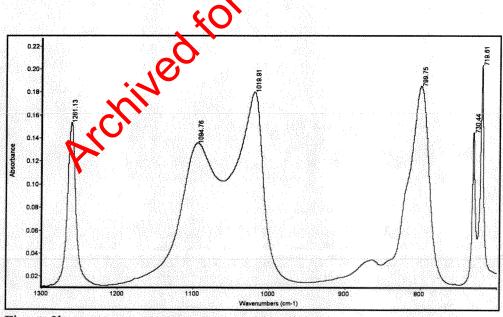


Figure 2b: Spectrum of PDMS in the 1300-700cm⁻¹ region.

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b. Subtraction of spectra

Overlapping peaks are possible. Absorption bands that are partially overlapped may appear as a broad band with two maxima or with a principal maximum and smaller second maximum on either face of the principal peak. Frequently, overlapping peaks appear as an absorption band with a shoulder.

One spectrum may be subtracted from another in instances where an interfering component may be present in a sample. The spectrum of the interfering component is subtracted from the sample spectrum with a normalizing factor which is user defined. For example, if a sample spectrum contains the cotton swab glue and the lubricant PDMS then the spectral features that arise from this glue can be removed to reveal PDMS more clearly. Spectral subtraction must be done with BOTH spectra in absorbance format.

- 1. Retrieve the suspected interfering component spectrum from the library. From the Analyze menu, select Library Manager....
- 2. Click on Search Libraries. Click on PDMS standards
- 3. Select the standard spectrum (previously poted) tandard spectrum with the highest match value) and click the Add to Window button.
- 4. In the Add to Window window, select Add to Window1 and click the Add button. Close the Library Manager window.
- 5. From the Edit menu, select Select All. This will select all of the spectra present in the window. As an alternative, press Ctrl+W.
- 6. Press the Absorbance button to convert the sample spectrum to an absorbance scale.
- 7. Change the display limits to 1400-750cm⁻¹ by selecting Display Limits... in the View menu, and set the X-Axis Limits from a Start: value of 1400 to an End: value of 750. Click OK.

NOTE: If the interfering component is glue from a cotton swab, there may be an absorption peak at approximately 1373cm⁻¹. This peak can be utilized as a reference peak in the subtraction process. This peak is NOT unique to cotton swab glue, however, its absence in a PDMS spectrum makes it a practical reference peak.

- 8. Click the Full Scale button.
- 9. From the Process menu, select Subtract....

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- 10. Ensure that the sample spectrum is the top spectrum in the window. If not, click the double-headed arrow button at the right to swap the spectra.
- 11. Adjust the Factor bar by sliding the button up or down the scale until the reference peak at 1373cm⁻¹ disappears (or flattens). The Coarser and Finer buttons can be used to increase/decrease the normalizing factor.
- 12. Once the interfering component has been removed from the sample spectrum, add the spectrum to a new window. Select the Add to new window and click the Add button.
- 13. A New Window window appears which reads: Enter a title for the window:
- 14. For the title of the new window, type Subtraction result. OK. The subtracted spectrum will appear in a new window.
- 15. Proceed to the section **Printing sample spectra and standard PDMS spectra** to print the subtracted spectrum.

c. Library search

Perform a library search on those spectra that appear to contain only trace amounts of PDMS or do not resemble PDMS (no library search is required for spectra that resemble the positive PDMS control spectra):

- 1. Make sure that the display limits are set for 1300-750cm⁻¹. If not, change the display limits by selecting Display Limits. in the View menu, and set the X-Axis Limits from a Start: value of 1300 to an Exo. value of 750. Click OK.
- 2. Click the Full Scale button

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3. Select the Library Setup... option in the Analyze menu. A Library Setup window appears.

In the Search Libraries folder, select the Sample Libraries from the list of Available search libraries and groups: and then click the Add button.

NOTE: Ensure that the PDMS Standards Library is searched.

In the Extended Search folder, the Use extended search box should NOT be checked.

In the Search Results folder, the Configure search results button should be checked and the Search type: box, Correlation should be selected.

In the QC Libraries folder, no libraries should appear under the QC in raries and groups:

In the Search Regions folder, the Use spectral regions batton should be checked. Input the values 1300 under the Start column and 750 under the End column. A shaded area should appear in the displayed spectrum corresponding to the selected area.

- 4. Click OK.
- 5. Click the Search button. A display of search esults in the 1300-750cm⁻¹ range will appear. The display will include a list of the top ten matches based on the match value for each compound and a spectrum for the top three matches.

NOTE: Search compares the selected library spectra to that of the unknown spectrum and displays the spectra that most closely match the unknown.

6. Compare every spectrum (search result) whose match value is ≥80 to the sample spectrum. If a PDMS spectrum (search result) is listed with a match value of ≥80, the sample may contain PDMS. (Make a note of any PDMS spectra that yield a match value of ≥80 to the sample. Do we print the search results.

NOTE: Library search results may assist in preliminarily identifying trace amounts of PDMS or other unknown compounds. If a preliminary identification is made, a known standard must be run and compared directly with the unknown spectrum. No definite conclusions can be drawn from a library search alone.

7. Click the Close button.

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d. Printing sample spectra and standard PDMS spectra

All casework sample spectra MUST be printed for every case file. Additionally, a standard PDMS spectrum (positive control) and extraction negative control MUST be printed for every case file.

- 1. Press the % Transmittance button to convert the sample spectrum to a transmittance scale.
- 2. Place the cursor in the title bar and remove the asterisk in front of the sample title by deleting it (an asterisk indicates that the sample has been baseline corrected).
- 3. From the Report menu, select Preview/Print Report....
- 4. Press the Print button and click OK...
- 5. From the Analyze menu, select Library Manager....
- 6. Click on Search Libraries. Click on PDMS standards.
- 7. Select the standard spectrum (previously noted standard spectrum with the highest match value) and click the Add to Window button
- 8. In the Add to Window window, select Add to a new window and click the Add button.
- 9. A New Window window appears which reads:
 Enter a title for the window:
- 10. For the title of the new window, type the title of the selected standard spectrum exactly as it appears in the Library Wanager window. Click OK.
- 11. The spectrum will appear in a new window. Press the % Transmittance button to convert the standard PDMS spectrum to a transmittance scale.
- 12. From the Report menu, select Print/Preview Report....
- 13. Press the Print button and click OK...
- 14. Return to Window 1 and analyze the next spectrum.
- When finished with all samples, fill in the *Time Ended* column of the *FT-IR Instrument Usage Log*.

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4. Interpretation of spectra

a. General guidelines

- 1. Each peak represents absorption of infrared energy by a molecular bond or functional group (see table of Characteristic Infrared Absorptions of Various Functional Groups).
- 2. The peak area is related to the concentration of the IR active species.
- 3. The degree of peak broadening is determined by a combination of the following factors:
 - instrument resolution
 - different vibrational energy states of a molecular bond
 - intermolecular and intramolecular interactions
- 4. The extraction negative spectrum should appear similar to Figures 1a and 1b. If additional peaks are present, the methylene chloride has been contaminated and the extracted samples must be discarded. Each sample must be re-cut and taken through the extraction process again using fresh quality control checked methylene chloride.
- 5. The positive control spectrum must be compared to the spectra in the PDMS Standards Library and it should appear similar to ope of the archived PDMS standards (and Figures 2a and 2b). The spectrum must possess the jour characteristic peaks in the fingerprint region that are around 1262, 1096, 1020, and 801cm⁻¹ and the rest of the spectrum must appear similar. If the positive control spectrum does not appear similar to any of the archived PDMS standards, the extracted samples must be discarded. Each sample must be re-cut and taken through the extraction process again using fresh quality control checked PDMS positive control.
- 6. Sample spectra that appear similar to the PDMS positive control spectrum (and Figures 2a and 2b) likely contail PDMS. The spectrum must possess the four characteristic peaks in the fingerprint region that are around 1262, 1096, 1020, and 801cm⁻¹ and the rest of the spectrum must appear similar.
- 7. Sample spectra that appear similar to the extraction negative spectrum (Figures 1a and 1b) can be considered negative for the presence of PDMS. However, the absence of characteristic peaks does not mean that the compound is not in the sample. An appropriate description of a negative result is "the compound was not detected."
- 8. Sample spectra that appear similar to the PDMS positive control spectrum (with the four characteristic absorption peaks) yet possess additional absorption peaks are indicative of a mixture. Mixtures MUST be interpreted with caution.

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9. Sample spectra that do not resemble the PDMS positive control spectrum nor the extraction negative spectrum yet possess absorption peaks indicate that some chemical compound(s) is (are) present in the sample. The library search may assist in a preliminary identification of the compound(s).

5. **Troubleshooting**

For simple troubleshooting of the spectrometer and software, follow the instructions outlined below.

- 1. Click on Help and select Spectrometer Help Topics.
- 2. Double click on Troubleshooting.
- 3. Search this section for the problem and solution.

For problems that are beyond the scope of the analyst, contact thermo Nicolet and schedule an appointment with a Field Service representative.

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Characteristic Infrared Absorptions of Various Functional Groups					
Functional Group	Frequency Range (cm ⁻¹)	Intensity			
C-H (alkyl)	2853-2962	medium-strong			
=C-H (alkenyl)	3010-3095	medium			
≡C-H (alkynyl)	~3300	strong			
C=C	1620-1680	variable			
C≡C	2100-2260	variable			
-CH(CH ₃) ₂	1365-1370, 1380-1385	strong, strong			
-C(CH ₃) ₃	~1365, 1385-1395	stiong, medium			
R-CH=CH ₂	905-920, 985-1000	strong, strong			
R ₂ C=CH ₂	880-900	strong			
cis-RCH=CHR	675-730	strong			
trans-RCH=CHR	960-975	strong			
O-H (alcohols and phenols, dilute solutions)	3590-6610	variable and sharp			
O-H (alcohols and phenols, H bonded)	(200-3550	strong and broad			
O-H (carboxylic acids, H bonded)	2500-3000	variable and broad			
C=O (aldehydes) C=O (ketones) C=O (esters)	1690-1780	strong			
C=O (ketones)	1680-1750	strong			
C=O (esters)	1735-1750	strong			
C=O (carboxylic acids	1710-1780	strong			
C=O (amides)	1630-1690	strong			
Aromatic-H	~3030	variable			
-N-H	3300-3500 (one peak)	medium			
- N-H ₂	3300-3500 (two peaks)	medium			
C≡N	2220-2260	medium			
Si-H	2100-2360	strong			
Si-OR	1000-1100	strong and broad			

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Si-CH ₃	1250±10	strong and sharp

C. Laboratory reports

Refer to the Case Management Manual for the general guidelines for writing laboratory reports. The template reports contain many pre-written sentences to guide explanations and interpretations of results.

- 1. For the majority of cases, the following type of summary is sufficient:
 - a. Polydimethylsiloxane (PDMS), a chemical used in many domestic condom lubricant preparations, was (not) detected.
- 2. Complicated or unusual cases involving additional absorption peaks can be difficult to write. The template reports are a place to start and many valuable insights can be gained by reading previous reports covering similar cases. It is a good idea for each analyst to maintain a file of his or her complicated reports for future reference.

For cases involving mixtures:

Additional peaks were present in the sample. PDMS may be a component of this mixture.

For cases involving identifiable organic Impounds:

Polydimethylsiloxane (PDMS), a chemical used in many domestic condom lubricant preparations, was (not) detected. However, another chemical compound(s) was detected which is consistent with (pro chemical name here). Confirmation of this compound cannot be completed at this time.

For cases involving unidentifiable organic compounds:

Polydimethyl Toxane (PDMS), a chemical used in many domestic condom lubricant preparations, was (not) detected. However, another chemical compound(s) was detected which cannot be identified at this time.

- 3. Qualitative statements may be part of the summary. They are used for probative samples when:
 - a. The sample is apparently unmixed.
 - b. The sample could be a mixture of two or more components, including PDMS.

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D. References

Blackledge, R. D. and Cabiness, L. R., "Examination for Petroleum Based Lubricants in Evidence from Rapes and Sodomies," J. of Forensic Sciences, Vol. 28, No. 2, April 1983, pp. 451-462.

Blackledge, R. D. and Vincenti, M., "Identification of Polydimethylsiloxane Lubricant Traces from Latex Condoms in Cases of Sexual Assault," J. of the Forensic Science Society, Vol. 34, 1994, pp. 245-256.

Blackledge, R.D., "Collection and Identification Guidelines for Traces from Latex Condoms in Sexual Assault Cases," Crime Laboratory Digest, Vol. 21, No. 4, Opport 1994, pp. 57-60.

Blackledge, R. D., "Viscosity Comparisons of Polydimethylsionane Lubricants in Latex Condom Brands via Fourier Self-Deconvolution of their FT-IR Spectra," J. of Forensic Sciences, Vol. 40, No. 3, May 1995, pp. 467-469.

Bryson, C., Garlo, A., and Piner, S., "Vaginal Swabs: Endigenous and Postcoital Components," J. of Forensic Science Society, Vol. 29, No. 3, 1994, pp. 157-171.

Fux, P., "Fourier Transform Infrared Spectrometric Determination of Trace Amounts of Polydimethylsiloxane in Extracts of Plastics Additives," Analyst, Vol. 114, April 1989, pp. 445-449.

Griffiths, P., "Photometric Precision in Infrared Spectra Measured by Fourier Transform Spectroscopy," Applied Spectroscopy, Vol. 29, No. 1, 1975, pp. 11-14.

Horner, J., Weiler, J., and Angelotti, N., "Visible and Infrared Spectroscopic Determination of Trace Amounts of Silitones in Foods and Biological Materials," Analytical Chemistry, Vol. 32, No. 7, 1960, pp. 853-367.

Keating, S., "The Laboratory's Approach to Sexual Assault Cases. Part 1: Sources of Information and Acts of Intercourse," J. of Forensic Science Society, Vol. 28, No. 2, 1988, pp. 35-47.

Keating, S., "The Laboratory's Approach to Sexual Assault Cases. Part 2: Demonstration of the Possible Offender," J. of Forensic Science Society, Vol. 28, No. 2, 1988, pp. 99-100.

Lipp, E.D., "Application of Fourier Self-Deconvolution to the FT-IR Spectra of Polydimethylsiloxane Oligomers for Determining Chain Length," Applied Spectroscopy, Vol. 40, No. 7, 1986, pp. 1009-1011.

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12A. The three **body cavity swabs (oral, vaginal/penile, and anal)** are used to collect possible biological fluids from those areas; the smears are used for a sperm search. The body cavity swabs and/or dried secretions swabs may also be tested for the presence of condom lubricants.

Testing of body cavity swabs (oral, vaginal/penile, and anal):

Visually check the swabs for the presence of biological fluids. If the swabs are reddish brown in color, be sure to test with KM reagent; note results. The slides accompanying the body cavity swabs are stained using the Christmas Tree staining procedure (refer to Christmas Tree Stain for Spermatozoa in the Forensic Biochemistry Methods Manual, version 4.0) and examined briefly for the presence of sperm; this search need not be exhaustive and should take no longer than five minutes. A new Criminalist must have the sperm search witnessed for a period of six months and the reviewing Criminalist must add their initials and date to the kit inventory form.

If sperm is found on a slide, a cutting from the accompanying swabs car go directly for DNA extraction. The analyst at this point should be sure to make an additional cutting from the vaginal or penile swab to submit for amylase testing (be sure to always treat or nile swabs as an external area or "stain" for the purposes of P30 and/or amylase interpretations).

If no sperm is found on a slide, submit a cutting from one of each of the sperm (-) body cavity swabs for P30 confirmatory testing. Again, the pertinent swabs (vaginal/penile and dried secretions) will all automatically be tested for the presence of amylase by the P30 ELISA rotation upon completion of the P30 testing.

If the swabs are semen and amylase negative, there is no need to retain the swabs, even if KM+.

Upon completion of P30 testing, interpret results (refer to the Biochemistry Methods Manual) to determine further steps to take. If semen positive, make a second cutting from each P30 positive swab for DNA testing.

If semen negative but any lase positive, check to see if the case has a named suspect. If so, again, make a second cutting from one swab from each designated body cavity area that is amylase positive. Submit this cutting to one-step Y STR typing. If the case has no named suspect, retain the amylase politic items(s) for further testing if deemed necessary. See testing of dried secretions swabs above for the treatment of amylase positive dried secretions swabs.

- 12B. If the vaginal swabs are semen negative, submit a cutting for FTIR analysis for the presence of condom lubricants. Testing of any other items for the presence of condom lubricants will be at the discretion of the interpreting analyst.
- 13. The **control envelope** is a concept left over from the days of ABO testing. There is no need to examine the contents.
- The questionnaire, body diagram sheets, and instruction sheets are intended for the use of the medical personnel. If present, make a copy of the questionnaire and/or body diagram sheets for the left side of the case file; leave all originals in the kit. No item number is assigned if present.
- 15. Photographs and/or other paperwork are not supposed to be included in a kit. If present, make a note of it; leave them in the kit. No item number is assigned if present.

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Once all serology testing is completed on a kit, it should be promptly dealt with. Perform additional cutting and retaining of kit items all at once for time efficiency. Additionally, return the sexual assault kit to the Evidence Unit personnel. These tasks should be done within seven days of the date of testing for the last P30/amylase results that are obtained. Promptly type up finalized report.

If the kit is negative for semen, and there is no other evidence to examine, the case is finished and the report may be written.

If the kit is negative for semen, and there is other evidence to examine (see Scheduled Analysis), the case file must be replaced in the "to be examined" stack based on the P30 target date. See the evidence exam supervisor to ensure this gets done properly. The FB number is added to the "evidence to be assigned" sheet; the evidence sign-in supervisor modifies Paradox to reflect the change in the status of the case.

If the kit is positive for semen, and was examined by a Crim I, it needs to be transferred to an interpreting analyst (this process is not necessary if the kit was examined by a Crim II or higher; that criminalist will keep the case file for DNA testing and interpretations). Contact the next Crim IV on the Kit Transfer Worksheet to determine who will be the assigned IA for the file. Fill this information in on the "to be assigned" sheet. This needs to ledone prior to submission to Chelex so the assigned Criminalist II, III, or IV will receive subsequent paperwork. Upon the additional cutting, retaining and returning of the sexual assauthkit, it is at this time the Criminalist I should make a cutting of the victim's exemplar to submit for testing for DNA comparisons to any P30/amylase positive swabs submitted for DNA testing.

The person who examined a kit is responsible for returning the kit. It should be promptly returned to the Evidence Unit (voucher a kit) or transferred to retained storage (post mortem kits) upon completion of P30/amylase testing. There is no need to wait for completion of DNA testing to return a kit.

Prior to the return of a **vonchered kit** to the Evidence Unit, the items to be retained must be separated out and placed in an accommodating manila envelope. Ensure that the envelope is labeled with the appropriate identifying case information for the purpose of easy identification in retained storage. See below as a guide for which items should be retained:

any P30/sperm positive swabs and slides
any amylase positive swabs
any semen positive stains from underwear or other clothing items
substrate controls if applicable
exemplar from victim - e.g., buccal sample, dried blood control, saliva sample
(semen-free oral swab or semen-free/amylase-free vaginal swab as a last resort)

For **post-mortem kits**, all used envelopes containing post mortem items are retained; the kit box and any unused envelopes are discarded.

18. **Retained items** may remain in the possession of the interpreting analyst during any further testing. Once testing is complete, they must be properly sealed and transferred to retained storage.

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Mario, E. and Gerner, R., "Determination of Silicon in a Commercial Hand Lotion by Atomic Absorption Spectroscopy," J. of Pharmaceutical Sciences, Vol. 57, No. 7, July 1968, pp. 1243-1244.

Murthy, R. S., Leyden, D. E., and D'Alonzo, R. P., "Determination of Polydimethylsiloxane on Cotton Fabrics using Fourier Transform Attenuated Total Reflection Infrared Spectroscopy," *Applied Spectroscopy*, Vol. 39, No. 5, 1985, pp. 856-860.

Pozefsky, A. and Grenoble, M., "The Analytical Chemistry of Silicones in Cosmetics," *Drug and Cosmetic Industry*, Vol. 80, No. 6, June 1957, pp. 752-3, 826, 832-838.

Rihs, T., "Identification and Determination of Dimethylpolysiloxance in Pharmaceutical Preparations," Pharmaceutica Acta Helvetiae, Vol. 46, 1971, pp. 550-

Sabo, M., Gross, J., and Rosenberg, I., "Quantitation of Dimethicoge in Lotions using Fourier Transform Infrared Spectral Subtraction," J. of the Society of Cosmetic Chemists, Vol. 35, 1984, pp. 273-281.

Sinclair, A. and Hallam, T., "The Determination of Pimethylpolysiloxane in Beer and Yeast," *Analyst*, Vol. 96, 1971, pp. 149-154.

Smith, L. and McHard, J., "Spectroscopic Techniques for Identification of Organosilicone Compounds," Analytical Chemistry, Vol. 3. No. 7, July 1959, pp. 1174-1179.

Solomons, E. and Jones, J., "The petermination of Polydimethylsiloxane (Silicone Oil) in Biological Materials: A Case Report, J. of Forensic Sciences, Vol. 20, No. 1, 1975, pp. 191-199.

Standard Guide for Quality Assurance of Laboratories Using Molecular Spectroscopy, ASTM Designation: E924-84 (Reapproved 1994), American Society for Testing and Materials, Vol 03.06.

Standard Practice for Describing and Measuring Performance of Fourier Transform Infrared (FT-IR) Spectrometers: Level Zero and Level One Tests, ASTM Designation: E1421-94, American Society for Testing and Materials, Vol 03.06.

Standard Practice for General Techniques for Obtaining Infrared Spectra for Qualitative Analysis, ASTM Designation: E1252-98, American Society for Testing and Materials, Vol 03.06.

Standard Terminology Relating to Molecular Spectroscopy, ASTM Designation: E131-98, American Society for Testing and Materials, Vol 03.06.