Technical Procedure for Infrared Spectroscopy

1.0 Purpose - This procedure specifies the required elements for the performance verifications, quality control checks, and use of Fourier Transform Infrared Spectrophotometers with Universal Attenuated Total Reflectance (ATR) Sampling Accessories.

2.0 Scope - This procedure applies to all infrared spectrophotometers used in the Drug Chemistry Sections of the State Crime Laboratory.

3.0 Definitions

- **Performance verification -** The initial confirmation of the reliability of a previously or externally validated method or instrument.
- **Quality control (QC) check -** Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
- **Reference Material** – Material sufficiently homogeneous and stable, with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.

4.0 Equipment, Materials, and Reagents

4.1 Equipment

- Fourier Transform Infrared Spectrophotometer with Universal Attenuated Total Reflectance (ATR) Sampling Accessory

4.2 Materials and Reagents

- Traceable Reference Material (TRM) Polystyrene film
- TRM Polystyrene film standard spectra (see Forensic Advantage (FA) Resource Manager)
- Print2PDF capability with instrument network
- Spatula
- Methanol or other suitable organic solvent
- Water

5.0 Procedure

5.1 Standards and Controls

5.1.1 Naming of Instrument Files (*Perkin Elmer* ".sp" and *Nicolet* ".spa" files)

5.1.1.1 Instrument files used for documenting performance verifications and monthly/yearly QC checks shall be named with at least the instrument identifier, year/month/day and a description (abbreviations are acceptable).

5.1.1.2 Instrument files published to a casefile for blanks, sample scans, and spectral subtractions shall be named with at least the case number, item number and a description (abbreviations are acceptable).

5.1.1.3 If multiple spectral subtractions are performed on the same sample and published to the case record, each resulting scan shall be named as above, with a unique identifier for each subtraction.

5.1.2 Saving of Instrument Files (*Perkin Elmer* ".sp" and *Nicolet* ".spa" files)

5.1.2.1 Instrument files described above (See **5.1.1**) shall be saved on the instrument computer hard drive, (or the specific instrument folder on the instrument network).

5.1.2.2 Instrument files described above (See **5.1.1**) shall be placed in a compressed (.zip) file named with the instrument identifier, year, and month in which they were collected.

5.1.2.3 The compressed (.zip) files containing at least the instrument files described above shall be archived in the FA object repository ("Manage Files") associated with the FTIR instrument on which they were collected.

5.1.3 Operating parameters – The following operating parameters shall be used in the Drug Chemistry Section:

- Resolution 4.00 cm^{-1}
- Range 4000.00 to 550.00 cm^{-1}
- CO_2/H_2O Correction "On"
- Diamond/Zinc Selenide crystal – one bounce

5.1.4 Negative control

5.1.4.1 Perform a background spectrum upon instrument start up, and at the beginning of each day the instrument is in use. Additional background spectrums may be obtained as needed.

5.1.4.2 Perform a blank (clean sample path) in the same manner as the sample to be analyzed.

5.1.4.3 An acceptable blank spectrum does not exhibit extraneous peaks indicative of contamination.

5.1.4.4 If the blank is contaminated, clean the crystal again and repeat the blank until no contamination is present.

5.1.4.5 An acceptable blank (clean sample path) shall be obtained between each new sample scan obtained.

5.1.5 Positive control - Monthly QC Check

5.1.5.1 A Forensic Scientist shall obtain a polystyrene scan monthly for each instrument to ensure proper functioning, and record the completion of the Monthly QC Check.

5.1.5.2 The internal polystyrene (applicable only to *Perkin-Elmer* FTIRs) or the external TRM polystyrene (applicable to all model FTIRs) may be used for the Monthly QC Check.

5.1.5.2.1 Name the instrument file according to section **5.1.1**, and label the resulting peaks utilizing instrument software.

5.1.5.2.2 Label the scan with notations for internal or external (TRM) polystyrene (include serial number if TRM polystyrene was used) and Forensic Scientist's initials and date.

5.1.5.2.3 Print the labeled scan as a .pdf file and save on the instrument hard drive (or the specific instrument folder on the instrument network).

5.1.5.2.4 Copy the .pdf file to the FA object repository ("Manage Files") associated with the FTIR instrument on which it was collected.

5.1.5.2.5 Reset the FA Resource Manager expiration date for one month to signal the next Monthly QC Check.

5.1.5.2.6 After evaluation of data (Section **5.1.5.3**) record completion in the instrument log for QC checks.

5.1.5.3 Evaluate the designated wave numbers from the Monthly QC check data and compare to the Traceable Reference Material polystyrene film. Refer to the chart below for parameters.

Traceable Polystyrene Reference Material Parameters
******Perkin Elmer* **and** *Nicolet* **380 FTIR**
3082.XX cm^{-1} (+/- 4 cm^{-1})
3060.XX cm^{-1} (+/- 4 cm^{-1})
1601.XX cm^{-1} (+/- 4 cm^{-1})
1583.XX cm^{-1} (+/- 4 cm^{-1})
1028.XX cm^{-1} (+/- 4 cm^{-1})
***Refer to current certificate for Polystyrene Standard Reference Material for full peak wave numbers.**

5.1.5.4 The allowable variance from the certified values shall be within the resolution of the instrument (+/- 4 cm^{-1}). If the results are outside these specifications, the instrument shall be removed from casework immediately and the following shall be done:

- Place an "Out of service" sign on the front of the instrument and enter the Out of Service status in the logbook.
- Notify the Section IR Coordinator so he/she can call the service engineer to schedule an on-site assessment and document by entering an "Out of Service" Action History.

5.1.6 Yearly Internal Polystyrene QC Check (Applicable only to *Perkin Elmer* FTIRs)

5.1.6.1 A scan of a Traceable Reference Material polystyrene film shall be collected yearly for each instrument with the KBR accessory in place, followed by the collection of a scan of the internal polystyrene with the ATR attachment in place. This shall be performed by the IR Coordinator or designee.

5.1.6.2 The instrument file and .pdf scans generated for the yearly QC Check shall be handled according to the specifications listed above for the monthly QC check, except for resetting the instrument expiration date, which has been designated for use as a monthly reminder.

5.1.7 Initial Performance Verification for New Instrument Set Up (Applicable to all models)

5.1.7.1 New FTIR instruments shall be installed by a certified engineer according to the manufacturer's guidelines, and procedure specifications (See **5.1.3**).

5.1.7.2 External (and internal, applicable only to *Perkin-Elmer* FTIRs) polystyrene scans shall be obtained according to the procedure for Monthly QC Check listed above.

5.1.7.3 Scans from at least three controlled substance primary standards shall be obtained (e.g., methamphetamine, phentermine, and cocaine base). Other controlled substances may be used depending on the availability of standards. The data obtained shall be reviewed by the IR Coordinator and found to be substantially the same as the library standard for that compound.

5.1.7.4 The .pdf files generated during the performance verification shall be filed in the FA object repository ("Manage Files") associated with the instrument by the IR Coordinator or designee to document set up of the new instrument.

5.1.7.5 If the polystyrene checks are acceptable, and the controlled substance standard spectra match the respective library entries, the instrument shall be released for casework. A new entry for the instrument shall be made in the Resource Manager section of FA prior to use in casework. The new entry shall include:

- The manufacturer's serial number.
- The unique section identifier for the new instrument. Infrared instruments are numbered in numerical order with the notation "FTIR" in front of the number.
- An "Initial Validation" Action History, stating that the instrument has been released for casework.
- A notation under "Verification Date" to reflect the date the initial performance validation was completed.

5.2 Suggested Maintenance Schedule

5.2.1 Yearly preventive maintenance shall be performed by an approved outside vendor.

5.2.2 Desiccants shall be changed at approximately six month intervals, or sooner when needed if external indicators begin to change color.

5.2.3 Record completion of maintenance and repairs, the date and identity of the person performing the work in the instrument log for Maintenance/Repairs. The instrument log shall be kept in a notebook near the instrument.

5.2.4 Document the maintenance or repair by entering a "Maintenance" Action History, listing the name of the vendor/person performing the work, and the work performed on the instrument. Any documentation associated with a service call may be placed in the associated FA resource.

5.3 Shutdown/Startup

- The power switch to the infrared instrument shall be left ON at all times to ensure the optics stay warm and excess moisture does not build up in the instrument.
- The software and computer may be shut down at the end of each business day.
- Each time the software is restarted, a background and an acceptable blank (clean sample path) shall be performed.
- When an IR has been placed out of service for an extended period of time, document the status by entering an "Out of Service" Action History in FA.
- A successful QC Check (as outlined in section **5.1.5** above) is required following an "Out of Service" status, and routine maintenance when the instrument has been out of direct control of the laboratory. Document the QC Check by entering an Action History in FA when the instrument is returned to casework.
- Laboratory personnel shall examine the effect(s), if any, of a malfunction on analysis results and implement the Procedure for Corrective Action as required.

5.4 Application of Procedure on Evidence

5.4.1 Solid samples using the ATR Method

5.4.1.1 Clean the ATR sampling accessory crystal using water or an organic solvent. Ensure the crystal is completely dry.

5.4.1.2 Perform a background scan at least daily and additional backgrounds as needed (e.g., when atmospheric conditions warrant.

5.4.1.3 Perform the negative control check as described above in **5.1.4.**

5.4.1.4 Print the results of the blank (clean sample path) for the FA case record. The blank scan may be ATR and baseline corrected before it is printed for the FA case record.

5.4.1.5 Place approximately 1 milligram of sample evenly onto the ATR crystal.

5.4.1.6 Apply force using the ATR force arm to ensure good contact between the sample and the surface of the crystal.

5.4.1.7 Scan to acquire data.

5.4.1.8 Data can now be processed.

5.4.1.8.1 A macro may be used to perform the following functions (only applicable to *Perkin-Elmer* FTIRs):

- Delete
- Scan sample (4 times)
- ATR Correction, 0.00
- Auto baseline correction, 4000.00-550.00

5.4.1.9 Print the properly labeled data generated by the FTIR/ATR instrument to a .pdf file and import to the FA case record.

5.4.1.10 Compare the completed scan to a known reference standard.

5.4.1.11 The reference standard used for identification, and any reference standards used for spectral subtraction(s) shall also be imported to the FA case record if a positive identification of a controlled substance is made.

5.4.1.11.1 If the reference standard is from a Laboratory generated collection, the unique identifier shall be included in the case file.

5.4.1.11.2 If the reference standard is from a published source, a citation for the source shall be included in the case file.

5.4.2 Liquid samples using the ATR Method

5.4.2.1 Clean the ATR sampling accessory crystal using water or an organic solvent. Ensure that the crystal is completely dry.

5.4.2.2 Perform the negative control check as described above in **5.1.4.**

5.4.2.3 Apply enough liquid sample to cover the ATR crystal.

5.4.2.4 Scan to acquire data.

5.4.2.5 Data can now be processed in the same manner as solid samples. (See **5.4.1.8 – 5.4.1.11**)

5.4.3 Identification: If the Forensic Scientist, based on his/her training and experience, determines that the spectrum of the controlled substance does not compare favorably to the reference standard due to the presence of other substances in the mixture, the controlled substance shall be separated from the mixture and an IR spectrum obtained of the isolated controlled substance.

5.4.3.1 A known impurity within a mixture containing a controlled substance may also be subtracted from the IR spectrum by using the "difference" function of

the FTIR. A printout of the straight material before any spectral subtractions are performed shall be required for the FA case record.

5.4.3.2 An IR spectrum of a controlled substance shall compare favorably to the IR spectrum of a known reference standard before an identification is confirmed.

5.4.3.3 When using FTIR as the primary structural elucidation technique, the sample spectrum shall compare favorably with a spectrum of a known standard in both its overall appearance and in the presence and location of major peaks.

5.4.3.4 When using FTIR to differentiate cocaine base from cocaine hydrochloride or another salt form, the areas of the spectrum which are different between cocaine base and cocaine hydrochloride shall be clear. Other areas may have interfering peaks present that do not mask the "salt form" identity.

5.5 Sampling - See Drug Chemistry Section Administrative Procedure for Sampling.

5.6 Calculations - N/A

5.7 Uncertainty of Measurement - N/A

6.0 Limitations

6.1 Generally, infrared spectra cannot distinguish between optical isomers.

6.2 Compounds may exist in different crystal forms which may produce unique spectra. (Mannitol is an example of one compound that exhibits these polymorphic characteristics.)

6.3 Due caution shall be exercised when using the similarity index generated by the library search algorithm. The Forensic Scientist shall evaluate the data and not singularly rely on the computer software index.

7.0 Safety - Do not over tighten the force gauge.

8.0 References

Moffat, A.C., et al., ed. *Clarke's Isolation and Identification of Drugs*. 2nd Edition. London: Pharmaceutical Press, 1986.

Mills, III, Terry and J. Conrad Roberson. *Instrumental Data for Drug Analysis*. 2nd Edition. CRC Press,Inc.: Volumes 1-5, 1993.

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Sliverstein, Robert M., et al. *Spectrometric Identification of Organic Compounds*. 5th Edition. New York Wiley, 1991.

Keller, Roger. *The Sigma Library of FT-IR Spectra*. 1st Edition. Missouri: Sigma Chemical Company, Volumes 1 and 2, 1986.

Pouchert, Charles J. *The Aldrich Library of Infrared Spectra*. Aldrich Chemical Company: 1981.

ASTM Standard E-1252, 2002, "Standard Practice for General Techniques for Obtaining Infrared Spectra for Qualitative Analysis." ASTM International: West Conshohocken PA, 2002, www.astm.org.

9.0 Records

- FA Resource Manager for Initial Performance Verification
- FA Resource Manager for Traceable Polystyrene Film Infrared Spectrum
- FA Resource Manager/Instrument log for maintenance and QC Checks
- FA Resource Manager for instrument files
- FA Case Record for .pdf data imported to case records

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original Document Technical Procedure F-01 converted to ISO standards. Instrument log added for monthly/yearly QC checks. Print2PDF new option for storage of polystyrene data. Instructions added for collection of polystyrene data and for desiccant changes. KBr Methods rescinded. They may be reinstated by Drug Chemistry Section FSM.
02/15/2013	2	Scope – changed to include Raleigh, Triad and Western Laboratories. **5.1.2.2** Removed operating instructions for Monthly QC check. **5.1.2.2.1** Add date to scan. Remove operating instructions for software. **5.1.2.4** List allowable variance for each instrument type. **5.1.3** Only applies to Perkin Elmer FTIRs. Nicolet FTIRs have no internal polystyrene. **5.1.4.2** Internal Polystyrene only applicable to Perkin Elmer FTIRs. **5.2** Remove instructions for desiccant change. Corrected typo on "desiccant." **5.5.2.9.1** Formatting error corrected on quotation marks. **5.5.2.10** Removed the word "major."
03/08/2013	3	Purpose – Remove reference to Perkin-Elmer to accommodate equipment in all three laboratories. **5.1.2.4** – Removed reference to +/- 0.50 cm^{-1} for Perkin-Elmer instruments so allowable variance is +/- 1.0 cm^{-1} for all instruments. **5.1.3.3** – Changed reference to +/- 1.0 cm^{-1} instead of 0.5 cm^{-1}

		for yearly polystyrene checks.
05/03/2013	4	**5.5.2.9** - Changed wording for substantially **5.5.2.9.2** - Changed wording for substantially comparable.
05/10/2013	5	**5.1.2.3, 5.1.2.4, 5.1.3.3** – Revised parameters for Monthly QC check to match resolution of the instruments. **5.5.1.7, 5.5.2.5** – Amended instrument resolution to match instrument parameters. **5.6** – Revised name of Sampling Plan from Technical to Administrative Procedure.
11/15/2013	6	Added issuing authority to header.
12/18/2013	7	**5.2.2** - Reworded **5.4** – Fourth bullet point - Removed unneeded reference to performance verification and referred to Line **5.1.2** **5.5.1.9.1** - Removed extra period **5.5.1.11.1**, **5.5.1.11.2, 5.5.2.8.1, and 5.5.2.8.2**- Add clarification for addition of reference standard identifier/source.
11/14/2016	8	**Header –** updated issuing authority **Entire document** - Replaced "Key Operator" with "Coordinator," replaced "contamination check" with "blank," and replaced "analyst" with "Forensic Scientist." **1.0** – Clarified purpose and added ATR Accessories. **4.2** – Changed location of TRM Polystyrene standard spectra, removed hard copy printer reference. **5.1.1 - 5.1.2** – Added new sections reference naming and saving of instrument files. **5.1.3 –** Moved original section **5.5.1.7** operating parameters **5.1.4** – Added background scan to negative control and stated when one is required. Defined acceptable blank. **5.1.5** – Added "Positive Control" **5.1.5.1** – Added record completion of QC check **5.1.5.2** – Clarified internal polystyrene checks applicable only to *Perkin-Elmer* instruments. Clarified monthly QC check scan instructions. **5.1.5.4** – Clarified resolution of the instrument and out of service instructions. **5.1.6** – Changed procedure for documentation of yearly QC check. **5.1.7** - Added procedure specifications to new instrument setup, clarified yearly internal/external polystyrene scans be obtained according to monthly QC check instructions, stated where files will be saved. Added FA Action History documentation. **5.2** – Changed procedure for documentation of maintenance. **5.3** – Clarified procedure for "Out of Service" and added FA Action History documentation.

		5.4.1.2 - 5.4.1.3 – Reworded **5.4.1.4** – Added requirement to print the blank for the FA case record. **5.4.1.8.1** – Clarified macro applicable only to *Perkin-Elmer* instruments. **5.4.1.9 -** Reworded **5.4.1.11** – Added requirement for standards used in spectral subtractions to be included in the FA case record if positive identification is made. **5.4.2.5** – Removed **Original 5.5.2.5 thru 5.5.2.8.2** duplication and referred to earlier corresponding section. **5.4.3.1** – Modified requirement for straight scan to be published to the FA case record. **5.4.3.3** – Added "major" **Records** – Clarified FA Resource Manager or Instrument log for records location