Performance Monitoring Protocol (QA/QC) for the Raman Spectrometers

1 Scope

This document addresses the performance monitoring (QA/QC) of the Raman Spectrometers (sample compartment and/or microscope). This document applies to personnel using the associated instrument(s)/equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, paint, explosives (chemistry), and Chemistry Unit general physical and chemical analysis.

2 Principle

A Raman spectrometer can be used to analyze samples in larger quantities in the sample compartment, if available, or in smaller quantities on the microscope, utilizing one or more objectives (e.g., 10X, 50X, 100X). In general, the signal for opaque samples can be maximized with a high numerical objective utilizing a microscope, while the signal for transparent samples can be maximized using a macro lens (i.e., sample compartment) or small magnification objective. The Raman spectrometer, either Fourier transform or dispersive, may utilize one or more excitation lasers (e.g., 1064 nm, 785 nm, 780 nm, 532 nm). In general, the signal will be more intense with a shorter wavelength excitation source; however, there is a trade off as samples may fluoresce and/or overheat with higher energy. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

a. Thermo Almega XR Dispersive Raman Spectrometer System with sample compartment and microscope, Omnic Software (or equivalent)

b. Thermo DXR Dispersive Raman Spectrometer System with Omnic Software (or equivalent)

c. Thermo NXR FT-Raman Module with Omnic Software (or equivalent)

d. Horiba Xplora Raman Microscope with LabSpec6 Software (or equivalent) and Bio-Rad Know-It-All (Horiba Edition) Software

e. Polystyrene slide, rod, or disk, such as FT-Raman standard (Thermo or equivalent)

f. Silicon and Polystyrene Slide (Horiba or equivalent)

g. Potassium Bromide (KBr) Raman Test Standard (Thermo or equivalent)

h. Alignment tool containing calibration slide with pinhole and white light (Thermo or

equivalent)

4 Standards and Controls

4.1 Performance Verification Standard

Polystyrene is used to assess daily operating performance and continued integrity of the Thermo Raman systems. Polystyrene requires no preparation and does not expire.

Silicon is used to assess daily operating performance and continued integrity of the Horiba Raman systems. Silicon requires no preparation and does not expire.

4.2 Alignment Tool (Thermo Only)

The alignment tool is used as needed to verify that the sample compartment and microscope are aligned and functioning properly. There is no sample preparation involved. The tool does not expire.

5 Sampling or Sample Selection

Not applicable.

6 Procedures

6.1 Daily Checks

6.1.1 Microscope (Thermo Almega/DXR Only)

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

a. Start Omnic and turn on the desired laser (if applicable) under 'Experiment Setup.' Allow time for the laser(s) to warm up and for the detector/ccd to cool. If the detector/ccd is not cool, a message will be displayed when data collection is attempted. The current temperature readout can also be viewed under the Advanced Tab in Experiment Setup. When the detector/ccd temperature setpoint has been reached, data collection can begin.

b. Turn the microscope illuminator on.

c. Set the operating parameters as listed in the 'Instrumental Conditions' section of this protocol.

d. Place the polystyrene slide on the stage and focus. Ensure that the illuminator is off, then collect the sample spectrum. Perform a peak analysis by using the 'Find Peaks' option under the 'Analyze' menu. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the labeled spectrum.

e. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.1.2 Sample Compartment (Thermo Almega Only)

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

a. Start Omnic and turn on the desired laser (if applicable) under 'Experiment Setup'. Allow time for the laser(s) to warm up and for the detector/ccd to cool. If the detector/ccd is not cool, a message will be displayed when data collection is attempted. The current temperature readout can also be viewed under the Advanced Tab in Experiment Setup. When the detector/ccd temperature setpoint has been reached, data collection can begin.

b. Set Operating Parameters as listed in the 'Instrumental Conditions' section of this protocol.

c. Place the polystyrene rod in the sample compartment. Collect the sample spectrum. Perform a peak analysis by using the 'Find Peaks' option under the 'Analyze' menu. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the labeled spectrum.

d. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.1.3 Sample Compartment (Thermo NXR Module Only)

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

a. Replace the standard KBr beamsplitter with the CaF_2 beamsplitter. Start Omnic and verify that the 'Use Raman Accessory' is checked. Prepare to collect the Reference spectrum by setting the Laser to OFF, and the White Light to either Medium or Low, to coincide with sample analysis parameters. Set to collect for 256 scans (in the Experiment Setup/Collect Tab). Place the NMR tube holder containing the KBr standard in the sample compartment. Adjust the focus, laser power, and/or aperture to maximize the signal within the acceptable range (0-9). Close Experiment Setup, and select Collect Reference (under the Collect tab).

b. Set Operating Parameters as listed in the 'Instrumental Conditions' section of this protocol.

c. Analyze the polystyrene standard. Place the polystyrene in the sample compartment and focus in the Experiment Setup/Bench Tab by using the focus arrows to maximize the signal within the acceptable range (0-9). Collect the sample spectrum. Perform a peak analysis by using the 'Find Peaks' option under the 'Analyze' menu. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the labeled spectrum.

d. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.1.4 Microscope (Horiba Only)

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

a. Start the LabSpec6 software and verify the lasers are on.

b. Place the Silicon sample on the stage and focus using the short working distance 100x objective. Perform the 'Full Autocalibration' procedure, and verify a 'Pass' result for each laser and grating combination.

c. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

6.2 As Needed Checks (Thermo Only)

The following procedure will be performed as needed based on performance. Enter the appropriate information in the QA/QC log to indicate completion.

a. Alignment of the Spectrometer:
Perform the appropriate alignment for the instrument in use. See appropriate instrument personnel for assistance if needed.

b. System tuning (Almega and DXR Only):
Focus the alignment tool on the white light. Verify calibration by choosing 'Calibrate Instrument' under the 'Collect' pulldown and, if applicable, check the boxes for:
- Laser frequency calibration
- Wavelength calibration

c. If all requirements are within specification or a successful result is returned, prepare

the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

7 Instrumental Conditions

7.1 Microscope (Thermo Almega/DXR Only)

Collect Tab
Set exposure time = 20 seconds
Set number of exposures = 2
Set final format - shifted spectrum (cm^{-1})
Cosmic ray rejection, to coincide with analysis set-up
Set correction to white light, if applicable
Set number of background exposures = 2

Bench Tab
Select microscope as 'Beam path/Accessory'
Select laser, to coincide with analysis set-up
Set laser power level to 100%
Select aperture, to coincide with analysis set-up
Select objective, to coincide with analysis set-up
Set resolution to same conditions as sample analysis
Set Grating Positions = multiple
Set maximum range limit to 3300 cm^{-1}, and minimum range limit to 200 cm^{-1}

7.2 Sample Compartment (Thermo Almega Only)

Collect Tab
Set exposure time = 20 seconds
Set number of exposures = 2
Set final format - shifted spectrum (cm^{-1})
Check 'Cosmic ray rejection'
Set correction to white light
Set number of background exposures = 2

Bench Tab
Select '180-degree' as 'Beam path/Accessory'
Select laser, to coincide with analysis set-up
Set Focus, 'Side to side', and 'Up/down' parameters to achieve maximum signal
Set laser power level to 100%
Select aperture, to coincide with analysis set-up
Set resolution to same conditions as sample analysis
Set Grating Positions = multiple
Set maximum range limit to 3300 cm^{-1}, and minimum range limit to 200 cm^{-1}

7.3 Sample Compartment (Thermo NXR Only)

Set laser power level to coincide with analysis set-up
Set Focus, 'Side to side', and 'Up/down' parameters to achieve maximum signal
Set final format - corrected spectrum (cm^{-1})
Set cosmic ray rejection, to coincide with analysis set-up
Set white light to OFF
Set maximum range limit to 3701 cm^{-1}, and minimum range limit to 100 cm^{-1}
Set number of scans = 64
Detector = InGaAs
Beamsplitter = CaF_2
Optical Velocity = 0.3165

7.4 Microscope (Horiba Only)

Autocalibration parameters set by the system.

8 Decision Criteria

- The Polystyrene spectrum is acceptable if all peaks are within ±5 cm^{-1} of the expected values, listed below (in cm^{-1}):

  621 796 1001 1032 1155 1451 1583 1602 2852 2905 3054

- The Autocalibration for the Horiba Raman system should result in a Pass result for all lasers and gratings.

9 Calculations

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

12 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. Refer to the *FBI Laboratory Safety Manual* for the proper handling and disposal of all chemicals. Personal protective equipment should be used when handling any chemical and when performing any type of analysis.

13 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual.*

Almega Users Guide.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	11/12/14	New document to replace "Performance Monitoring Protocol (QA/QC) for the Thermo Almega Raman Spectrometer" (Inst 503) *Instrument Operation and Support Subunit SOP Manual.*
1	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Updated heading in Section 5. Added 'appropriate instrument support personnel' to Sections 6.1.1 e, 6.1.2 a & d, 6.1.3 d, 6.1.4 c and 6.2 c. Added illuminator to Section 6.1.1 d. Updated 'Instrument Operation and Systems Support' in Section 13 and header.

Approval Redacted - Signatures on File

Drug Chemistry/
General Chemistry
Technical Leader: Date: 09/28/2018

Paints and Polymers
Technical Leader: Date: 09/28/2018

Explosives (Chemistry)
Technical Leader: Date: 09/28/2018

IOSS Manager: Date: 09/28/2018

Chemistry Unit Chief: Date: 09/28/2018

QA Approval

Quality Manager: Date: 09/28/2018