Title: Performance Monitoring Protocol (QA/QC) for the Scanning Electron Microscope/Energy Dispersive X-Ray Spectroscopy (SEM/EDS) Instrument

1. Introduction

Conducting an elemental analysis examination on samples, whether for the presence of primer-gunshot residue (GSR or pGSR) particles or other forensic information using scanning electron microscopy (SEM) instrumentation, is a type of testing employed within the Chemistry Unit. The following procedure shall serve as a quality assurance/quality control (QA/QC) guideline when analysts use the SEM instrument and associated detectors (i.e., secondary electron imaging (SEI), backscatter electron (BSE), and energy dispersive X-ray spectroscopy (EDS)) for casework. These guidelines are used to ensure that the generated data are reproducible and accurate. A copper standard is used to demonstrate that the SEM instrument can produce ample electrons, so the SEI detector can produce a clear image, and so the EDS detector can appropriately detect correct X-ray spectra and peak energy intensities. The PLANO standard is used to demonstrate that the GSR software, in conjunction with the BSE detector, can reproducibly and consistently produce quality results, so the SEI detector can produce a clear image, and so the EDS detector can adequately produce X-ray spectra of GSR-simulated particles.

2. Scope

This procedure serves as a general guideline for evaluating SEM instruments for pGSR-related and other elemental-type analyses. Once particular milestones are achieved, instruments are considered appropriate and ready to use each day. The term 'daily' (or day) refers to a day wherein the instruments are used for casework. A copper reference standard is analyzed daily to demonstrate that the SEM, as well as the SEI and EDS detectors, are operating as expected. A simulated GSR particle-containing reference standard (i.e., PLANO disk) is analyzed [at least] monthly to demonstrate the instrument's ability to detect elements related to primer-gunshot residue (i.e., Ba, Sb, and Pb). The term 'monthly' refers to a month wherein the instruments are used for casework. Any scientist who operates the SEM/EDS instrument(s) will be responsible for following this procedure.

3. Principle

Instruments need to be routinely checked so that it is ensured that quality results will be produced. Such quality checks are accomplished through the evaluation of certain reference standards and comparing empirical data with expected results. For example, the Planotec GSR & Particle Analysis Calibration Kit® contains two test specimens: one with synthetic pGSR particles and one with synthetic particles for automated particle analyses using SEM/EDS. The Synthetic Particle Specimen (SPS) has been specifically designed for the adjustment and validation of analytical SEM/EDS systems used for automated analysis of GSR samples. It is especially useful for quick system validation checks and quality assurance procedures and not used for calibration purposes. The SPS-TM-A test specimen is manufactured on a glassy carbon substrate with a size of 8 x 8mm with various sizes of synthetic particles containing Pb, Sb and Ba. The

synthetic GSR particles with a size varying from 0.5 µm to 2.0µm are statistically distributed in an area of 6mm x 6mm. For improved conductivity, the SPS test specimens have been coated with a thin layer of carbon and are made with a defined number of Pb/Sb/Ba particles. However, some particles can be missing or could have been on a different location, so each SPS test specimen has been individually tested and comes with a certificate stating the exact number of Pb/Sb/Ba particles and their locations. To avoid contamination, the SPS-TM-A GSR test specimens are either stored in an SEM vacuum chamber or in a PELCO® SEM Sample Stub Vacuum Desiccator.

Even though the specimens within the calibration kit may have expiration dates associated with them, the metal particles are persistent, or inorganic, and analyses are non-destructive – thereby allowing them to be re-verified during each analysis. They are able to be used beyond their initial manufacturer-generated expiration date.

4. Specimens

This procedure primarily utilizes SEM stubs as the sample media. Aluminum stubs coated with a carbon conductive material which contain adhesive material can be used. Other sampling media can also be used if such materials are generally accepted within the relevant scientific community and/or by the instrument manufacturer.

5. Equipment/Materials/Reagents

Listed below are general supplies required for electron microscopy (EM). Additional supplies unique to a particular method are indicated within that topic.

5.1 General laboratory equipment/materials (e.g., tweezers, Kimwipes, gloves)

5.2 Scanning Electron Microscope with Backscattered Electron (BSE) and secondary electron imaging (SEI) detectors (Hitachi, or equivalent)

5.3 Energy Dispersive X-ray Analyzer/Detector (EDAX or equivalent)

5.4 Aluminum Stubs (Electron Microscopy Services or equivalent)

5.5 Adhesive-backed tabs or double-sided adhesive tape (Electron Microscopy Services or equivalent)

5.6 Copper reference standard, 99.9% or better (Sigma-Aldrich or equivalent)

5.7 Primer Gunshot Residue (pGSR) Control Standard (Planotec GSR & Particle Analysis Calibration Kit® ; Ted Pella, or equivalent)

5.8 Methanol (Reagent grade or equivalent)

6. Standards/Controls/Reagents

Performance Standards/Control Standards/Negative Control:
The SEM/EDS performance standard is a copper metal reference standard that has been purchased and embedded onto an SEM holder. The pGSR performance standard is a purchased reference material that contains simulated pGSR particles. Both performance standards have certificate of analysis (COA) paperwork. Negative controls can be either purchased (e.g., known negative proficiency test samples) or can be prepared in-house. They need to have been verified as free from containing pGSR-related particles. If necessary, other standards or controls can be purchased (e.g., past proficiency tests with known validated results) and used for quality purposes. When applicable, purchased materials should be stored as determined by their manufacturer (in-house prepared materials will be stored in a similar manner). When certain reference standards are not available then consult the appropriate FSE2 (or higher) for guidance.

The SEM/EDS performance standard, the pGSR control standard, and the negative control all need no further preparation more than how they are received (other than copper being mounted on a stub) and can be used repeatedly (without replacement) due to the non-destructive nature of the SEM/EDS technique. Because of the persistent nature of the copper reference standard, the pGSR-related particles on the Planotec kit stubs, and the negative control stub, any expiration dates associated with them can be extended. These materials are considered re-verified (and applicable expiration dates extended) each time they are analyzed, as long as each item's associated data conform to expected results.

6.1 SEM/EDS Performance Standard (i.e., copper reference standard)

A copper (Cu) pure element reference standard has already been mounted on an aluminum SEM stub using an carbon adhesive tab. Each re-verification will result in it being valid for one (1) year since its last documented verification.

6.2 Primer Gunshot Residue (pGSR) Control Standard (i.e., Planotec GSR & Particle Analysis Calibration Kit®)

A synthetic pGSR reference standard is received from the manufacturer in an already-prepared and mounted state on an SEM stub. Each re-verification will result in it being valid for one (1) year since its last documented verification.

6.3 Negative Control

An aluminum SEM stub containing a carbon adhesive tab and no pGSR-related particles. Each re-verification of this control will result in it being valid for one (1) year since its last documented verification.

7. Sampling

Not applicable.

8. Procedure

8.1 Ensure instrument is ready for analysis (including computer's file space (data backup may be needed)) and surrounding areas are clean.

8.1.1 Any unusual issues with instrument operability or overall setup (including any error messages) prior to start will be noted within the appropriate logbook and will be rectified (e.g., disk space, time/date information). If necessary the FSE2 (or higher) will be notified.

8.1.2 Potentially contaminated items and surfaces will be cleaned prior to use using methanol and disposable material (e.g., Kimwipes®, paper towels).

8.1.3 Analysts will change gloves in-between handling specimens.

8.2 Check for error readings and/or error indicator lights.

8.3 Vent and open the sample chamber.

8.4 Insert sample(s) into the specimen holder. Never touch the surfaces of the discs with bare hands - use gloves appropriately. Use pre-cleaned (e.g., rinsing with methanol and wiping dry) tweezers to manipulate discs.

8.5 Close chamber and evacuate.

8.6 After the chamber is properly evacuated (i.e., green light), turn on the filament (i.e., power at the main SEM console). Ensure that the filament, operating voltages, magnification, and other parameters are all at their correct settings and are operating properly.

8.7 Daily QA/QC:

Examine the SEM/EDS Performance Standard (i.e., copper reference standard) each day that the instrument is used for casework.

Note: If samples are analyzed overnight and the SEM sample chamber has not been opened, then the QA/QC procedure need not be performed prior to completing analyses.
However, once the SEM sample chamber has been opened, the Daily QA/QC must be performed and documented prior to additional casework being analyzed for that day.

8.7.1 Adjust brightness and contrast, as needed, in order to obtain a good overall view of copper.

8.7.2 Focus the SEM on a flat portion of the copper standard, preferably the same place each time (e.g., upper right hand corner). The stub should be oriented on the SEM platform in the same (or as close as possible) direction each time it is placed into the SEM chamber.

8.7.3 Set up the parameters according to the Instrumental Parameters section (e.g., collection time, magnification). Other parameters will be set as close to possible as previous QA/QC analyses (e.g., spot intensity, working distance).

8.7.4 The parameters will be electronically recorded within each EDS spectrum (e.g., sample info including lot #, working distance, acquisition magnification, live time (100 seconds), beam voltage (25 keV), instrument label (SEM-01, SEM-02), and initials of analyst).

8.7.5 Each filename will be of the format: Cu Std_SEM-##_XXX_YYYY-MM-DD (where ## is the instrument number, XXX are the analyst's initials, and YYYY-MM-DD are the Year-Month-Day, respectively).

8.7.6 Record/print the copper spectrum from 0 keV to 25 keV.
The energy values from each of copper's four (4) peaks will be labeled both by energy level (i.e., keV) and intensity (unit-less), at the apices of the peaks (i.e., Cu L_l, Cu L_α, Cu K_α, and Cu K_β).

8.7.7 Any significant peaks (other than C, O) will be labeled (if element is known) according to the energy value at peak apices. Peak intensities are not required to be documented, however.

8.8 Monthly QA/QC:

Examine the Primer Gunshot Residue (pGSR) Control Standard (i.e., PLANO disk) and the Negative Control stubs on the first day of each month that the instrument is used for casework.

8.8.1 The same method and parameters will be used as would be done when analyzing pGSR particles within casework.

8.8.2 The parameters and other important information (e.g., sample info including lot #, magnification, beam voltage (25 keV), instrument label (SEM-01, SEM-02), initials of analyst) will be electronically recorded within each data file, if possible. Any parameters not able to be electronically stored will be written on paperwork.

8.8.3 An image of at least one (1) pGSR-related particle within the PLANO disk (using the SEI detector) will be included in the pGSR Performance Standard paperwork.

8.8.4 Confirmation elemental data for the image in the form of an EDS spectrum (showing the presence of barium, antimony, and lead) will be acquired with at least 100 live time seconds, be printed, and be included in the QA/QC binder with the rest of the data for that day.

8.8.5 Images and data will be saved and printed. Filenames will be in the format: PLANO_SEM-##_XXX_YYYY_MM_DD (where ## is the instrument number, XXX are the analyst's initials, and YYYY-MM-DD are the Year-Month-Day, respectively).

8.9 Ensure appropriate images have been printed.

8.10 Ensure appropriate collection parameters have been captured, recorded, and/or noted.

8.11 Ensure spectra have been saved using appropriate filenames. Generic filenames will not be used – this is done to prevent spectra from being overwritten and data being lost.

8.12 The system should remain under vacuum with electronics off when not in use for an extended period of time.

8.13 Ensure the logbook contains all QA/QC activity and information, along with date and operator's initials, and a final determination has been made regarding operability of the instrument (i.e., pass/fail).

8.14 The analyst performing this procedure each day (or month) will ensure all QA/QC printouts are properly secured in the appropriate QA/QC binder prior to leaving for the day, and prior to allowing another analyst to perform casework.

Any significant environmental impacts on the instrument (e.g., power interruption, service to instrument) will require that both the Daily and Monthly QA/QC be performed prior to casework.

9. Instrumental Parameters

The following are the typical operating parameters for the instrument used in this procedure. With documented approval from the Lead Examiner and appropriate management, the instrument conditions may be modified to adjust or improve the procedure. Documentation of such changes must be included with casework so that any instrumental parameter change can be associated with data and until the procedure has been updated. For more specific parameters, see method printout(s) either attached to this procedure or that are contained within appropriate instrument binders.

Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM/EDS):

Beam Voltage	25 kV
Spot Intensity	Yielding: ~10,000 cps on Cu for SEM/EDS Performance Standard analysis ; Yielding: ~100,000 cps on Cu for all other analyses
Live counting time	100 seconds (Cu analysis ; Confirmatory elements on Primer Gunshot Residue (pGSR) Control Standard)
Working Distance	~10mm (optimal EDS X-ray collection)
Magnification	200x
Area of Cu Analyzed	Upper right quadrant
Analysis Mode Setting	Area Full Window

10. Decision Criteria (Evaluation of Data)

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. In most cases all of the criteria below should be met in order to identify the appropriate particle(s)/element(s).

10.1 SEM/EDS Performance Standard – Daily

Evaluate the Cu standard's image and X-ray spectra for elemental information using the parameters listed within this document. Final elemental peak determination will be based on EDS energy values appropriate to copper peak's theoretical value(s).

10.1.1 Image quality will be evaluated but image printouts are optional.

10.1.2 If an automatic identification application is used, peak identification must be confirmed by the analyst.

10.1.3 Compare the SEM/EDS Performance Standard's X-ray spectrum with the spectrum from the previous day (or other spectra). Determine if a significant energy value or signal intensity values have changed. Overlay and print the two spectra using the same scale, if possible. The daily SEM/EDS Performance Standard's X-ray spectrum must appear generally similar to the previous day's spectrum, including high-to-low energy peak ratios, Gaussian peak shapes, peak-width-at-half-maximum (PWHM) distance, and absence of any spectral artifacts. Changes in the low-to-high peak intensity ratio may indicate problems in the detector. A peak intensity change > 20% of the previous day's value(s) will be considered significant. A peak energy change > 30eV (+/- 0.03 keV) of the previous day's value(s) will be considered significant.

10.1.4 If significant energy value changes are found, or if additional unwanted peaks are discovered, corrective measures must be taken. If issues can't be resolved it will be documented in the appropriate QA/QC logbook, the Lead Examiner (or higher) will be notified, and a determination on instrument operability and acceptance will be made. Changes in any of the observed performance criteria may indicate that the instrument needs to be evaluated by the manufacturer's service personnel.

10.2 Primer Gunshot Residue (pGSR) Control Standard (Planotec) – Monthly
Evaluate for pGSR-related particles using the same methods for casework. Both backscatter electron and secondary electron (particle imaging) data will be used in the evaluation.

10.2.1 The image printout of at least one (1) GSR-simulated particle that contains all three (3) elements – Ba, Sb, and Pb will show expected morphology of a GSR particle.

10.2.2 If an automatic identification application is used (i.e., GSR software), print out all necessary data as would be done for casework.

10.2.3 Compare resulting data with the previous month's (or other) data to determine if significant changes have occurred. A significant drop is considered (>20% loss in the number of GSR particles detected). In cases of significant particle detection drop, the Lead Examiner (or higher) will be notified. A drop of >20% may indicate the need for vendor service.

10.2.4 If significant changes are noted, corrections will be instituted to resolve the problem(s). If problems can't be resolved, the unit's Lead Examiner (or above) will be notified and a determination on instrument operability acceptance will be made collectively.

10.3 Negative Control – Monthly

Evaluate for pGSR-related particles using the same methods for casework. Appropriate detection (particle imaging) will be used in the evaluation.

10.3.1 Analyze the negative control stub for the presence of GSR-related particles using the same methods for casework.

10.3.2 If particles contain any combination of the three elements – Ba, Sb, and Pb then the SEM instrument, as well as the surrounding sample preparation area(s), will be thoroughly cleaned. A new Negative Control/Blank sample will be prepared, re-analyzed, and evaluated. Continued detection of GSR-related particles will result in the Lead Examiner (or above) being notified. Further actions will be decided and documented in the appropriate instrument logbook.

10.3.3 All appropriate documentation will be kept in the corresponding instrument logbook.

10.4 Other Evaluation(s) – Periodically (or as needed)

10.4.1 Preventive Maintenance:
If a vendor-system preventive maintenance agreement exists for either the SEM or any of its detectors, the vendors will perform work as per the agreement. This will usually include cleaning and calibration of the instrument and/or its components – both mechanically and electronically. All maintenance (as well as problems) will be recorded within the appropriate instrument log book. Documentation from the service company will also be kept in the appropriate instrument book. Instrument software upgrades and/or changes will be included in the appropriate logbook. All aspects of the SEM/EDS system must be demonstrated to be operational and at the same (or better) detection/identification ability prior to the instrument being placed back into service and used for casework. Any negative-impacting situations after vendors perform service on an instrument will be brought to the attention of both the FSE2 and management.

10.4.2 Contamination Evaluation (minimally in six (6) month intervals):

10.4.2.1 Surface Evaluation

10.4.2.1.1 Verify that a stub is negative and has no pGSR-related particles (i.e., Ba, Sb, Pb).

10.4.2.1.2 Using the same stub dab general areas where evidence has been known to have been placed near the instruments (this does not include evidence lockers).

10.4.2.1.3 Examine for pGSR-related particles.

10.4.2.1.4 Any pGSR-related 2-component or 3-component particles that would normally be reported as found within casework will result in the following:

10.4.2.1.4.1 Appropriate FSE2 and managers will be notified.

10.4.2.1.4.2 Cleaning of all instrument surface areas as well as the room in general will occur and be documented in the appropriate logbooks.

10.4.2.1.4.3 Repeat of the surface evaluation.

10.4.2.2 Analyst Evaluation

10.4.2.2.1 Verify that a stub is negative and has no pGSR-related particles (i.e., Ba, Sb, Pb).

10.4.2.2.2 Using the same stub dab both hands (back/front).

10.4.2.2.3 Examine for pGSR-related particles.

10.4.2.2.4 Any pGSR-related 2-component or 3-component particles that would normally be reported as found within casework will result in the following:

10.4.2.2.4.1 Appropriate FSE2 and managers will be notified.

10.4.2.2.4.2 Analyst will change to a clean lab coat and wash hands outside of instrument room.

10.4.2.2.4.3 Cleaning of all instrument surface areas as well as the room in general will occur and be documented in the appropriate logbooks.

10.4.2.2.4.4 Repeat of the analyst evaluation.

11. Calibration

Not applicable.

12. Uncertainty of Measurement

Not applicable.

13. Limitations

13.1 This technique is limited to solid samples.

13.2 X-ray fluorescence may excite characteristic X-rays from structures remote from the area visibly selected for analysis.

13.3 Elements present in trace concentrations, under average conditions, are generally not detected below 0.5%.

14. Safety

This procedure is carried out in a laboratory environment and standard safety procedures appropriate for such an environment will be utilized, including gloves, safety glasses, and protective clothing (e.g., lab coat). When casework samples are being processed/analyzed then brown paper (or other similar barrier) will be placed in between surfaces and specimens. Refer to the GL *Safety Procedure* and appropriate MSDS/SDS documents for additional information on chemicals or other materials. Take standard precautions for the handling of chemicals and sharp cutting instruments.

15. References

Hitachi S-3700N operator's manual.

EDAX Genesis Manual(s)

Bearden, J.A. "X-ray Wavelengths," *Reviews of Modern Physics*, 39 (1), No. 1, 1967.

Goldstein, et al. *Scanning Electron Microscopy and X-ray Microanalysis*, second edition, Plenum Press, New York, 1992.

Ward, D.C. "A Small Sample Mounting Technique for Scanning Electron Microscopy and X-ray Analysis," *Forensic Science Communications*, 1(2), 1999

Ward, D.C. and Sibert, R.W., "The Use of Vacuum Evaporation of Metals for Surface Feature Enhancement," *AFTE Journal*, 18(4), 1986.

Revision #	History
3	Significant format and verbiage changes throughout document. Added more specification to the 'Procedure.' Added other sections (e.g., Decision Criteria, Instrumental Conditions, and Limitations. Added a Revision/History section.
4	Added clarification that PLANO standard's expiration data can be extended to one (1) year past last verification. Updated title to include CAS document designation.. Significant format changes throughout document. Replaced GSR with pGSR throughout document. Updated Section 2 (Scope) to include responsibility. Added computer check within Procedure section. Added section 3 (Principle section) and section 4 (Specimen section). Added methanol and other descriptions (e.g., Planotec® kit) within equipment section. Updated section 6 (Standards/Controls/Reagents). Replaced positive control term with pGSR control standard. Updated instrumental parameter section. Added cleaning step and general evaluation to procedure (Section 8). Updated format and some description within Instrumental parameter section. Added to the Decision Criteria section both guidance on post-vendor instrument work and contamination evaluation. Updated the safety section. Updated header within Qualtrax from FLIN SOP-04, "QC and Calibration Procedure" to CHEM-09, "Performance Monitoring Protocol (QA/QC) for the Scanning Electron Microscope/Energy Dispersive X-Ray Spectroscopy (SEM/EDS) Instrument."