Title: Performance Monitoring Protocol (QA/QC) for the Scanning Electron Microscope (SEM) Instrument

A. Purpose

Conducting elemental analysis on samples, whether for the presence of primer-gunshot residue (GSR or pGSR) particles, for paint composition, or general 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 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 amble 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.

B. Scope

This procedure serves as a general guideline for evaluating SEM instruments for paint, GSR-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.

C. Responsibility

Scientists who use the SEM/EDS instrument(s)

D. 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.

1. Scanning Electron Microscope with Backscattered Electron (BSE) and secondary electron imaging (SEI) detectors (Hitachi, or equivalent)
2. Energy Dispersive X-ray Analyzer/Detector (EDAX or equivalent)
3. Aluminum Stubs (Electron Microscopy Services or equivalent)

4. Adhesive-backed tabs or double-sided adhesive tape (Electron Microscopy Services or equivalent)
5. Copper reference standard, 99.9% or better (Sigma-Aldrich or equivalent)
6. Gunshot Residue Control Standard (PLANO ; Ted Pella, or equivalent)

E. Standards/Controls/Reagents

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. This stub reference standard needs no further preparation and is used repeatedly due to the non-destructive nature of the technique.
2. GSR Performance Standard (i.e., PLANO disk)
   A GSR reference (aka. PLANO) standard is received from the manufacturer in an already-prepared and mounted state on an SEM stub. This stub reference standard needs no further preparation and is used repeatedly due to the non-destructive nature of the instrumental technique.
3. Negative Control/Blank
   An aluminum SEM stub containing a carbon adhesive tab. This stub control does not contain any GSR-related particles and needs no further preparation. It can be used repeatedly due to the non-destructive nature of the instrumental technique. It is manipulated contemporaneously and in a manner similar to casework samples.

F. Calibration

Not applicable.

G. Sampling

Not applicable.

H. Procedure

1. Ensure instrument is ready for analysis. Check for error readings and/or error indicator lights.
2. Vent and open the sample chamber.
3. Insert sample(s) into the specimen holder. Never touch the surfaces of the discs with bare hands - use gloves appropriately. Use tweezers around step of stubs to manipulate discs.
4. Close chamber and evacuate.
5. 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.
6. 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 for the day prior to additional casework being analyzed.

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

b. Focus the SEM on a flat portion of the copper standard, preferable 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.

c. Set up the parameters according to the Instrumental Conditions 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).

d. 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).

e. 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).

f. 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).

g. 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.

7. Monthly QA/QC:
Examine the GSR Performance Standard (i.e., PLANO disk) and the Negative Control/Blank stubs on the first day of each month that the instrument is used for casework.

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

b. 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.

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

d. 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.

e. 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. Ensure appropriate images have been printed.
9. Ensure appropriate collection parameters have been captured, recorded, and/or noted.
10. 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.
11. The system should remain under vacuum with electronics off when not in use for an extended period of time.
12. 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).
13. 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.
14. 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.

I. Decision Criteria (Evaluation of Data)

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).
   a. Image quality will be evaluated but image printouts are optional.
   b. If an automatic identification application is used, peak identification must be confirmed by the analyst.
   c. 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-1/2-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.
   d. 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 be evaluated by the manufacturer's service personnel (either in person or via phone support).

2. Gunshot Residue Control Standard (PLANO) – Monthly
   Evaluate for GSR-related particles using the same methods for casework. Both backscatter electron and secondary electron (particle imaging) data will be used in the evaluation.
   a. The image printout of at least one (1) GSR-simulated particle that contains all three elements – Ba, Sb, and Pb will show expected morphology of a GSR particle.
   b. If an automatic identification application is used (i.e., GSR software), print out all necessary data as would be done for casework.
   c. 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.
   d. 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.

3. Negative Control/Blank – Monthly
   Evaluate for GSR-related particles using the same methods for casework. Appropriate detection (particle imaging) will be used in the evaluation.
   a. Evaluate for the presence of GSR-related particles using the same methods for casework.
   b. 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.
   c. All appropriate documentation will be kept in the corresponding instrument logbook.

4. Other Evaluation(s) – Annually (as needed)
   Preventive Maintenance: If a vendor annual system preventive maintenance agreement exists for either the SEM or any of its detectors, the vendors will perform 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.

J. Instrumental Conditions

General operating conditions can be:

Beam Voltage	25 kV
Spot Intensity	Adjusted to yield ~10,000 cps
Live counting time	100 seconds (Cu analysis ; Confirmatory Plano elements)
Working Distance	~10mm (optimal EDS X-ray collection)
Magnification	200x
Area of Cu Analyzed	Upper right quadrant
Analysis Mode Setting	Area Full Window

L. Uncertainty of Measurement

Not applicable.

M. Limitations

1. This technique is limited to solid samples.
2. X-ray fluorescence may excite characteristic X-rays from structures remote from the area visibly selected for analysis.
3. Elements present in trace concentrations, under average conditions, are generally not detected below 0.5%.

N. Safety

Use universal precautions when handling potentially biohazardous materials. Refer to the GL *Safety Procedure* and appropriate MSDS/SDS documents for additional information. Take standard precautions for the handling of chemicals and sharp cutting instruments.

O. References

1. Hitachi S-3700N operator's manual.
2. EDAX Genesis Manual(s)
3. Bearden, J.A. "X-ray Wavelengths," *Reviews of Modern Physics*, 39 (1), No. 1, 1967.
4. Goldstein, et al. *Scanning Electron Microscopy and X-ray Microanalysis*, second edition, Plenum Press, New York, 1992.
5. Ward, D.C. "A Small Sample Mounting Technique for Scanning Electron Microscopy and X-ray Analysis," *Forensic Science Communications*, 1(2), 1999

6. 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.