Title: Quality Assurance / Quality Control (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., 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 (Cu) standard is used to demonstrate that the SEM instrument can produce ample electrons, so the BSE detector can produce clear images, and so the EDS detector can appropriately detect correct X-ray spectra and peak energy intensities. The GSR standard (i.e. PLANO standard) is used to demonstrate that after any significant changes or service to the system the GSR software, in conjunction with the BSE detector, can still produce quality results and 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/EDS instruments for pGSR-related and other elemental-type analyses. Once particular milestones are achieved, instruments are considered acceptable and ready to use. 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 BSE and EDS detectors, are operating as expected. A simulated GSR reference standard (i.e., PLANO standard) is analyzed after any significant changes or service to the system to demonstrate the instrument’s ability to detect elements related to primer-gunshot residue (i.e., Ba, Sb, and Pb). Any analyst who operates the SEM/EDS instrument will be responsible for following this procedure.

3. Principle

Instruments need to be routinely checked to ensure quality results will be produced. Such quality checks are accomplished through the evaluation of certain reference standards and comparing empirical data with expected results. The PLANO standard has been specifically designed for SEM/EDS systems that are used for the automated analysis of GSR samples. It is used for quick system checks and quality assurance procedures and not used for calibration purposes. The PLANO standard is manufactured on a glassy carbon substrate with various sizes of synthetic particles containing Pb, Sb and Ba. These synthetic GSR particles are statistically distributed on the surface of the standard. For improved conductivity, the 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 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 PLANO standard is stored inside the SEM vacuum chamber.

Even though the test specimens may have expiration dates associated with them, the metal particles are persistent, 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, which are aluminum stubs covered with a carbon adhesive material. 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. Additional supplies unique to a particular method are indicated within that topic.

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

5.2 Scanning electron microscope (SEM) with backscattered electron (BSE) and secondary electron imaging (SE or SEI) detectors (Hitachi or equivalent)

5.3 Energy dispersive x-ray analyzer/detector (EDS) (EDAX or equivalent)

5.4 Aluminum stubs (Electron Microscopy Services or equivalent)

5.5 Carbon adhesive tabs (double-sided, suitable for GSR collection) (Electron Microscopy Services or equivalent)

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

5.7 Gunshot residue (GSR) reference standard (PLANO standard) (Planotec GSR kit or equivalent)

5.8 Isopropanol (reagent grade or better)

5.9 Deionized (DI) water (Millipore or equivalent)

6. Standards/Controls/Reagents

Reference Standards:
The Cu reference standard is a copper metal reference standard that has been purchased and embedded onto an SEM stub. The GSR reference standard is a purchased reference material that contains simulated GSR particles. Both standards have certificate of analysis (COA) paperwork. If necessary, other standards can be used for quality purposes. When applicable, purchased materials should be stored as determined by their manufacturer. When certain reference standards are not available, consult the FSE 2 (or higher) for guidance.

The Cu standard and the GSR standard need no further preparation (other than the copper needing to be mounted on a stub) and can be used repeatedly due to the non-destructive nature of the SEM/EDS technique. Because of the persistent nature of the copper reference standard and the GSR-related particles on the Planotec kit stubs, 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 Cu standard (i.e., copper reference standard)

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

6.2 Gunshot residue (pGSR) standard (i.e., PLANO standard)

A synthetic GSR reference standard mounted on an SEM stub. Each re-verification 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.

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 FSE 2 (or higher) will be notified.

8.1.2 Potentially contaminated items and surfaces will be cleaned prior to use using DI water and isopropanol with paper towels or wipes (e.g., Kimwipes).

8.1.3 Analysts will change gloves in-between handling specimens.

8.2 Vent and open the sample chamber.

8.3 Insert sample(s) into the specimen holder. Never touch SEM stubs with bare hands - use gloves appropriately. Use tweezers pre-cleaned with DI water and isopropanol to manipulate stubs.

8.4 Close the sample chamber and evacuate.

8.5 After the chamber is properly evacuated, turn on the filament. Ensure that the filament, operating voltages, magnification, and other parameters are all at their correct settings and are operating properly.

8.6 Daily QA/QC:

Examine the Cu standard (i.e., copper reference standard) each day that the instrument is used for casework.

Note: If samples have been analyzed and the SEM sample chamber has not yet been opened on the following day(s) (i.e. SEM chamber has remained under vacuum), then the daily 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 deemed acceptable prior to additional casework being analyzed for that day.

8.6.1 Adjust brightness and contrast, as needed.

8.6.2 Focus the SEM on the copper standard (preferably on a flat portion of the Cu).

8.6.3 Adjust settings to those listed within section 9 (Instrumental Parameters) (e.g., collection time, magnification, working distance, etc.).

8.6.4 The parameters used will be recorded within the EDS spectrum information field or on the printout (e.g., sample information including lot #, working distance, magnification, live time, beam voltage, instrument label (SEM-1, SEM-2, etc.), and initials of the analyst).

8.6.5 Each filename will indicate the sample type (e.g. Cu, etc.), the analyst's initials, and the date.

8.6.6 Print and/or electronically save 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).

8.6.7 Any significant peaks (other than C, O) will be labeled (if element is known) according to the energy value at peak apices..

8.7 Post-Service QA/QC:

Examine the gunshot residue (GSR) standard (i.e., PLANO standard) after any significant instrument changes/service (e.g., filament replacement, aperture adjustment, vendor service, etc.) prior to the instrument's use for casework.

8.7.1 Similar parameters to casework analysis, aside from those necessary to compensate for sample differences (e.g. stub height, stub layout, working distance, brightness and contrast, etc.), will be used.

8.7.2 The parameters used will be recorded within the EDS spectrum information field or on the printout (e.g., sample information including lot #, working distance, magnification, live time, beam voltage, instrument label (SEM-1, SEM-2, etc.), and initials of the analyst).

8.7.3 An image of at least one (1) 3-component particle (i.e. containing Pb, Ba, and Sb) within the PLANO standard (using the BSE detector) will be collected.

8.7.4 Confirmation of 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.

8.7.5 Images and data will be electronically saved and/or printed. Each filename will indicate the sample type (e.g. PLANO, etc.), the analyst's initials, and the date.

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

8.9 Ensure the logbook (physical and/or electronic) contains all necessary QA/QC activity, information, and documentation along with the operator's initials and date of the activity.

Any significant instrument changes/service will require that both the daily and post service QA/QC be successfully completed 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 FSE 2 and/or higher, 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.

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

Beam Voltage	25 kV
Spot Intensity	Yielding ~10,000 cps on Cu for the Cu reference standard analysis ; Yielding ~100,000 cps on Cu for all other analyses
Live Time	100 seconds minimum for all EDS spectral collections
Working Distance	~10mm (optimal EDS X-ray collection)
Magnification	200x

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 Cu Reference Standard – Daily QA/QC

Evaluate the Cu standard's X-ray spectra for elemental information. Final elemental peak determination will be based on EDS energy values appropriate to the copper peak's theoretical value(s).

10.1.1 BSE image quality will be evaluated, but image printouts are optional.

10.1.2 Compare the Cu standard's X-ray spectrum with the spectrum from the previous day (or other spectra).
The Cu 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, and with the absence of any spectral artifacts. Changes in the low-to-high peak intensity ratio may indicate problems with the detector. A peak intensity (height) change > 20% of the previous day's value(s) will be considered significant. A peak energy change > 30 eV (+/- 0.03 keV) of the previous day's value(s) will be considered significant.

10.1.3 If significant changes in peak intensity and/or energy are found, or if extraneous peaks are discovered, corrective measures must be taken. If issues can't be resolved it will be documented in the appropriate QA/QC logbook (physical or electronic), the FSE 2 (or higher) will be notified, and a determination regarding instrument operability and acceptance will be made. Changes in any of the assessed performance criteria may indicate that the instrument needs to be evaluated by the manufacturer's service personnel.

10.2 Gunshot Residue (GSR) Standard (PLANO standard) – Post Service QA/QC
Evaluate the PLANO standard's GSR-related particles and their associated X-ray spectra for elemental information.

10.2.1 A BSE image and EDS spectrum of at least one (1) GSR-simulated particle that contains all three (3) elements (Ba, Sb, and Pb) will be electronically saved and/or printed.

10.2.2 The summary of the automated screening analysis (e.g., job summary) and any particle lists will be electronically saved and/or printed. Any necessary parameters used or settings required for analysis will be recorded (electronically and/or physically).

10.2.3 Unless noted otherwise, rejected particles are those which, upon manual particle review, do not match the originally screened particle classification (e.g., a particle screened as a 3-component particle (Ba, Sb, and Pb) found to only have two, one, or none of those elements) and would not be selected for manual particle confirmation.

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

10.3.1 Preventive Maintenance:
If a 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. Dates and types of service, as well as any issues, will be recorded within the appropriate instrument logbook (physical and/or electronic) along with any documentation from the service vendor. Instrument software upgrades and/or changes will also be included in the appropriate logbook (physical and/or electronic). All aspects of the SEM/EDS system must 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 impacted instrument performance after vendors complete service on an instrument will be brought to the attention of both the FSE 2 and management.

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.

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 an appropriate barrier layer (e.g., craft paper) will be placed in between surfaces and specimens. Refer to GL-2 and appropriate SDS documents for additional information for additional safety related guidance.

15. References

Hitachi S-3700N / SU3500 SEM system manuals

EDAX EDS detector and Genesis software manuals

ASTM Standards E1588 and E3309