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 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. The 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 adequately produce imaging for screening and confirmation, and the EDS software, in conjunction with the EDS detector, can adequately produce X-ray spectra of elements from GSR-simulated particles.

2. Scope

This procedure serves as a general guideline for evaluating SEM/EDS instruments for GSR-related elements and other elemental analyses. The term ‘daily’ (or day) refers to a day wherein the instruments are used for casework. The Cu standard is analyzed daily to demonstrate that the SEM, as well as the BSE and EDS detectors, are operating as expected. The GSR standard is analyzed after any significant changes or service to the system to demonstrate the instrument’s ability to detect elements related to GSR (i.e., Pb, Sb, and Ba). Any analyst who operates the SEM/EDS instrument will be responsible for following this procedure. The QA/QC logbook associated with this procedure may be physical and/or electronic.

3. Principle

Instruments need to be routinely checked to ensure quality results will be produced. Such 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. 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 PLANO standard may have an expiration, or retest, date from the manufacturer, its metal particles are persistent, inorganic, and the analyses performed as part of this procedure are non-destructive,

thereby allowing the PLANO standard to be reverified during each analysis. As such, these standards are able to be used beyond its manufacturer listed expiration, or retest, dates.

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) detector (Hitachi or equivalent)

5.3 Energy dispersive X-ray spectroscopy system (EDS) with detector and analyzer (Oxford or equivalent)

5.4 Aluminum stubs (SEM/GSR stubs) (Electron Microscopy Sciences or equivalent)

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

5.6 Copper (Cu) reference standard (99.9% purity or higher) (Sigma or equivalent)

5.7 Imaging standard (C, Co, Au, and Rh) (MAC or equivalent)

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

5.9 Isopropanol (reagent grade or higher)

5.10 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 imaging standard is solely used to adjust brightness and contrast settings prior to automated analysis. The GSR reference standard is a purchased reference material that contains simulated GSR particles. Both standards have certificates of analysis (COA). 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, imaging, and GSR standards need no further preparation (other than a sample of the Cu standard being 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 materials on the Cu, imaging, and GSR standards, any expiration dates associated with them can be extended. The Cu and GSR standards are considered reverified (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:

A copper (Cu) pure element reference standard mounted on an aluminum SEM stub. Each reverification will result in it being valid for 1 year from the date of reverification.

6.2 GSR standard:

A synthetic GSR reference standard mounted on an SEM stub. Each reverification will result in it being valid for 1 year from the date of reverification.

7. Procedure

7.1 Ensure the instrument is ready for analysis.

7.1.1 Any issues with instrument operability or overall setup (including any error messages) will be noted within the QA/QC logbook and will be rectified. If necessary, the FSE 2 (or higher) will be notified.

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

7.1.3 Analysts will change gloves between handling specimens.

7.2 Vent and open the sample chamber.

7.3 Insert sample(s) into the specimen holder. Never touch SEM stubs with bare hands. Avoid all direct contact with the stub surface to be examined. Use tweezers pre-cleaned with DI water and isopropanol to manipulate stubs.

7.4 Close the sample chamber and pump-down.

7.5 After the chamber is properly evacuated, turn on the filament. Adjust the beam and operating parameters (e.g., magnification, spot size, working distance, etc.) as needed.

7.6 Daily QA/QC:

Examine the Cu standard each day that the instrument is used for casework.

Note: If samples have already 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.

7.6.1 Select the BSE mode (BSE-comp) in the SEM software and adjust instrument settings as needed.

7.6.2 Focus the SEM on the Cu standard (preferably on a flat portion of the sample).

7.6.3 Save the project, listing the sample type (e.g., Cu), date, and analyst's initials in the project name.

7.6.4 Collect an EDS spectrum from 0 keV to 20 keV using a 30 second live time acquisition.

7.6.5 The information and parameters used will be documented within the EDS spectrum or on worksheets (i.e., instrument, working distance, magnification, live time, accelerating voltage, spot size, resolution, and process time).

7.6.6 The energy values from each of the four Cu peaks (Cu L_l, Cu L_α, Cu K_α, and Cu K_β) will be labeled both by energy level (i.e., keV) and abundance (counts), at the apices of the peaks.

7.6.7 Any additional peaks (other than C and O) with abundancies greater than the least abundant Cu peak will be labeled with the element, energy level, and abundance at peak apices. Additionally, the FSE 2 (or higher) will be consulted.

7.6.8 Save and include the EDS spectrum within the QA/QC logbook.

7.7 Post-Service QA/QC:

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

7.7.1 Load the PLANO standard stub for analysis.

7.7.2 Select the BSE mode (BSE-comp) in the SEM software and adjust instrument settings as needed.

7.7.3 Load an analysis template/profile and add the locations of the PLANO standard stub.

7.7.4 Save the project, listing the sample type (e.g., PLANO), date, and analyst's initials in the project name.

7.7.5 The PLANO standard will be scanned and analyzed automatically by the software based on particle size, intensity measurements, and preliminary elemental determination. Particles will be initially classified by the software (e.g., characteristic, consistent, commonly associated, or no classification).

7.7.6 The maximum number of automatically classified characteristic (Pb bearing) particles for the stub will be set to 30 within the collection parameters.

7.7.7 A minimum of 75 fields at 200x magnification will be mapped for analysis on a square layout template for the PLANO standard.

7.7.8 Adjust the brightness and contrast thresholds as needed using the imaging standard and save the brightness and contrast calibration to the project.

7.7.9 Start the automated GSR analysis.

7.7.10 Particles will be re-examined manually after the automated analyses are complete.

7.7.11 Collection information (e.g., individual stub reports, particle lists, etc.) will be generated and saved within the QA/QC logbook. List the information and parameters used during automated analysis (i.e., instrument, accelerating voltage, magnification, working distance, spot size, resolution, and process time) within the QA/QC logbook.

7.7.12 When the automated GSR analysis is complete, review the particle data for the stub (i.e., classifications, spectra, particle images, etc.).

7.7.13 Collect a BSE image of at least 1 characteristic (Pb bearing) particle.

7.7.14 Collect an EDS spectrum of said particle(s) using a 30 second live time acquisition, confirmation the presence of Pb, Sb, and Ba.

7.7.15 Save and include any confirmed particle images and spectra within the QA/QC logbook. These confirmed particle reports will list the information and parameters used during image and spectral capturing (i.e., instrument, accelerating voltage, magnification, working distance, spot size, resolution, live time, and process time).

7.8 When not in use for an extended period of time, the system should remain off and under vacuum.

7.9 Ensure the QA/QC logbook contains all necessary 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.

8. Instrumental Parameters

The following are the typical operating parameters for the instrument used in this procedure. With documented approval from the FSE 2 (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	20 kV
Spot Intensity	Yielding ~100,000 output counts on the Cu standard
Live Time	30 live seconds for manual EDS spectral collections
Working Distance	~10 mm (optimal for EDS X-ray collection)
Magnification	200x

9. Decision Criteria (Evaluation of Data)

The following criteria are used as a guideline in determining the acceptability of the data produced in this assay.

9.1 Cu Standard – Daily QA/QC

Evaluate the Cu standard's EDS spectrum for elemental information. Final elemental peak determination will be based on the theoretical energy values of the X-ray peaks for Cu.

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

9.1.2 Evaluate the peak energy values of the 4 expected X-ray peaks for Cu in the Cu standard's EDS spectrum. A peak energy change > 30 eV (+/- 0.03 keV) of the expected values (0.81 keV, 0.93 keV, 8.05 keV, and 8.90 keV) will be considered significant.

9.1.3 Compare the peak abundancies (counts) of the 4 expected X-ray peaks for Cu in the Cu standard's EDS spectrum with the most recently accepted spectrum (i.e., previous day/analysis). A peak abundance change > 20% of the previous value will be considered significant.

9.1.4 If significant changes in peak abundance and/or energy are found, corrective measures must be taken. If issues can't be resolved, it will be documented in the QA/QC logbook, 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.

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

9.2.1 A BSE image and EDS spectrum of at least 1 particle that contains Pb, Sb, and Ba will be electronically saved and/or printed.

9.2.2 A summary report of the automated screening analysis and any particle lists will be electronically saved and/or printed.

9.2.3 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 characteristic (Pb bearing) particle (Ba/Sb/Pb) found to only have two, one, or none of those elements) and would not be selected for manual particle confirmation and/or accepted for QA/QC requirements.

9.3 Other Evaluation

9.3.1 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 QA/QC logbook along with any documentation from the service vendor. Instrument software upgrades and/or changes will also be included in the QA/QC logbook. After preventative maintenance, the SEM/EDS system must be fully operational with acceptable detection/identification performance 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 section management.

10. Limitations

10.1 This technique is limited to solid samples.

11. References

Hitachi SU3500/SU3800 SEM & software user manuals

Oxford Instruments Ultim Max 65 EDS & AZtec software user manuals

ASTM Standards E1588 and E3309