Designation: ~~E1588 – 16~~ E1588 – 16a

Standard ~~Guide~~Practice for Gunshot Residue Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry[1]

This standard is issued under the fixed designation E1588; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

~~1. Scope~~

~~1.1 This guide covers the analysis of gunshot residue (GSR) by scanning electron microscopy/energy-dispersive X-ray spectrometry (SEM/EDS) by manual and automated methods. The analysis may be performed manually, with the operator manipulating the microscope controls and the EDS system software, or in an automated fashion, where some amount of the analysis is controlled by pre-set software functions.~~

~~1.2 Since software and hardware formats vary among commercial systems, guidelines will be offered in the most general terms possible. For proper terminology and operation, consult the SEM/EDS system manuals for each system.~~

~~1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.~~

~~1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*~~

~~2. Referenced Documents~~

~~2.1 *ASTM Standards:*[2]~~

~~E1492 Practice for Receiving, Documenting, Storing, and Retrieving Evidence in a Forensic Science Laboratory~~

~~3. Terminology~~

~~3.1 *Definitions of Terms Specific to This Standard:*~~

~~3.1.1 *major, adj*—element whose main peak height is greater than ⅓ of the peak height of the strongest peak in the spectrum. **Wallace 1984 (15)**[3]~~

~~3.1.2 *minor, adj*—element whose main peak height is between 1⁄10 and ⅓ of the peak height of the strongest peak in the spectrum. **Wallace 1984 (15)**~~

~~3.1.3 *trace, adj*—element whose main peak height is less than 1⁄10 of the peak height of the strongest peak in the spectrum. **Wallace 1984 (15)**~~

~~4. Summary of Practice~~

~~4.1 From the total population of particles collected, those that are detected by SEM to be within the limits of certain parameters (for example, atomic number, size, or shape) are analyzed by EDS (**1-3**). Typically, particles composed of high mean atomic number elements are detected by their SEM backscattered electron signals and an EDS spectrum is obtained from each. The EDS spectrum is evaluated for constituent elements that may identify the particle as being consistent with or characteristic of GSR, or both.~~

~~5. Significance and Use~~

~~5.1 This document will be of use to forensic laboratory personnel who are involved in the analysis of GSR samples by SEM/EDS (**4**).~~

[1] This ~~guide~~practice is under the jurisdiction of ASTM Committee E30 on Forensic Sciences and is the direct responsibility of Subcommittee E30.01 on Criminalistics.
Current edition approved ~~March 1, 2016~~May 1, 2016. Published ~~March 2016~~July 2016. Originally approved in 1994. Last previous version approved in ~~2010~~2016 as ~~E1588 – 10~~E1588 – 16.~~ε1~~. DOI: ~~10.1520/E1588-16.~~10.1520/E1588-16A.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[2] The boldface numbers in parentheses refer to a list of references at the end of this standard.

5.2 SEM/EDS analysis of GSR is a non-destructive method that provides (**5**, **6**) both morphological information and the elemental profiles of individual particles.

5.3 Particle analysis contrasts with bulk sample methods, such as atomic absorption spectrophotometry (AAS) (**7**), neutron activation analysis (NAA) (**8**), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS), where the sampled material is dissolved or extracted prior to the determination of total element concentrations, thereby sacrificing morphological information and individual particle identification.

5.4 X-ray fluorescence spectrometry (XRF) is a technique that has been used to map the placement and distribution of GSR particles surrounding bullet holes in order to establish shooting distances (**9**). Unlike the solution-based bulk methods of analysis, XRF is non-destructive; however, XRF still does not provide morphological information and is incapable of individual GSR particle identification.

6. Sample Preparation

6.1 Once the evidence seal is broken, care should be taken so that no object touches the surface of the adhesive SEM/EDS sample collection stub and that the stub is not left uncovered any longer than is reasonable for transfer, mounting, or labeling.

6.2 Label the sample collection stub in such a manner that it is distinguishable from other sample collection stubs without compromising the sample; for example, label the bottom or side of the stub.

6.3 If a non-conductive adhesive was used in the sample collection stub, the sample will need to be coated to increase its electrical conductivity, unless an environmental SEM or variable-pressure/low-vacuum SEM is used for the analysis. Carbon is a common choice of coating material, since it will not interfere with X-ray lines of interest. For high-vacuum SEM, coat the sample sufficiently to eliminate charging of the sample.

6.4 Observe the appropriate procedures for handling and documentation of all submitted samples as described in Practice E1492.

7. Sample Area

7.1 Sample collection stubs for SEMs typically come in one of two diameters: 12.7 mm or 25.4 mm, which yield surface areas of 126.7 mm^2 and 506.7 mm^2 respectively.

7.2 Manual analysis of the total surface area of the stub is prohibitively time-consuming. Because the particles are collected onto an adhesive surface in a random manner and the particles do not tend to cluster, it is reasonable to analyze a portion of the stub surface by employing an appropriate sampling and analytical protocol (**6**, **10**).

7.3 Automated SEM/EDS analysis can enable data collection from nearly the entire surface area of the sample collection stub. Due to the disparity between the shape of the sample collection stub (round) and the SEM field of view search area (square or rectangular), analysis of 100 % of the sample collection area may not be possible in some systems.

7.3.1 Analysis of the maximum allowable surface area of the sample is recommended, however, many automated systems can be programmed to terminate the analysis of a stub or series of stubs once a pre-established number of particles with specified classification(s) have been detected. The decision as to how many particles satisfy the requirements of a particular case is a matter for the analyst to decide but should be subject to guidelines set out in the laboratory's standard operating procedures.

8. Instrument Requirements and Operation

8.1 *General:*

8.1.1 Most commercial-grade SEM/EDS systems should be adequate for GSR analysis.

8.1.2 Automated data collection of GSR involves some portion of the data collection being controlled by pre-set software functions. The extent to which the SEM and EDS systems communicate and are integrated varies according to the manufacturers involved and the capabilities of the hardware/software architecture.

8.2 *Scanning Electron Microscope (SEM):*

8.2.1 The SEM, operating in the backscattered electron imaging mode, must be capable of detecting particles down to at least 0.5 µm in diameter.

8.2.2 The SEM must be capable of an accelerating voltage of at least 20 kV.

8.2.3 Automated SEM/EDS systems include: communication and control between the SEM and EDS system, and a motorized stage with automated stage control. The system should have the ability to recall stage locations of particles for verification and software for particle recognition.

8.3 *Energy Dispersive Spectrometry (EDS):*

8.3.1 The detector's resolution should be better (less) than 150 eV, measured as the full width at half the maximum height of the Mn Ka peak.

8.3.2 At a minimum, the EDS spectrum should be acquired at 20 eV per channel.

8.3.3 Display of the EDS output must encompass the X-ray lines of analytical utility, with a minimum range of 0–15 keV.

8.3.4 Automated systems will also include software capable of acquiring X-ray spectra for a specified collection time or total X-ray counts.

8.3.5 It is desirable that the spectrum obtained from the analysis of each particle of interest be stored. At a minimum, an automated system must be capable of storing all of the particle location coordinates.

8.4 *Sample Placement:*

8.4.1 Record the positions of the stubs (sample and standard/reference stubs) on the SEM stage when the samples are inserted.

8.4.2 If it is anticipated or required that additional analyses will be needed, it is desirable that the stub can be returned to the same orientation as before its removal. This may consist of marking the side of each stub and aligning it with marks on the microscope stage or by having stubs that fit into the stage in only one position (for example, stubs with a pin that is a half-circle in cross section).

8.5 *Detection and Calibration:*

8.5.1 Particles of GSR are detected by their backscattered electron signal intensity. The absolute signal intensity that a particle produces is related to the electron beam current, mean atomic number, and size of the particle (for particle sizes on the order of the beam diameter). Particles whose mean atomic numbers are high will appear brighter than those of lower mean atomic number composition. As the beam current increases, the amount of signal each particle produces also increases (**11**).

8.5.2 The brightness and contrast settings (low and high thresholds) of the backscattered electron detector system determine the limits of detection and discrimination of particles whose mean atomic number exceed the minimum threshold setting but fall below the maximum threshold setting. Threshold settings for the backscattered electron signal should be done with a suitable reference sample of known origin (often supplied by the EDS manufacturer) or pure element standards at the same parameters that will be used for the sample analysis. This calibration sample should, if possible, be in the microscope chamber at the same time as the samples to be analyzed.

8.5.3 The backscattered electron detector's brightness and contrast should be set to include the high atomic number particles of interest and exclude low atomic number particles that are not of interest. Typically, high contrast and low brightness settings provide an adequate range between threshold limits for ease of detection. If the beam current is changed or drifts, the brightness and contrast threshold limits, which were based on the previous beam current, may no longer be compatible with the new conditions and should be readjusted. The beam current may be measured with a Faraday cup, a specimen current meter, or monitored by comparing the integrated counts within the same peak in sequentially collected spectra from a known standard.

8.6 *Quality Control:*

8.6.1 When conducting automated analysis of GSR, special measures have to be chosen in order to meet common quality management demands. Therefore, as minimum conditions:

8.6.1.1 Establish a protocol to confirm optimum instrument operation parameters on a routine basis.

8.6.1.2 Monitor the EDS X-ray energy calibration and SEM beam current stability regularly. This may be facilitated by the use of appropriate standards or reference samples, or both.

8.6.1.3 Analyze a reference sample (positive control) with particles of known size, range, and composition at regular intervals in order to test the accuracy of particle detection and identification, whether by automated or manual analysis. It is recommended that a reference sample has been prepared and mounted in a manner comparable to the collection method in use by the submitting agency. The reference sample may be a sample of GSR from a known source (caliber of weapon, ammunition manufacturer, number of rounds fired, collected area from shooter, or a synthetic GSR standard). Additional environmental particles may be added to ensure the inclusion or exclusion of particular classes of particles. Alternatively, a synthetic, simulated-GSR reference sample may be used for this purpose. The frequency of analysis of this sample is a matter for the analyst to decide and is subject to guidelines set out in the laboratory's standard operating procedures.

8.6.1.4 The incorporation of environmental or control samples into the analytical protocol is recommended in order to monitor the cleanliness of the sampling or analytical system, or both. An environmental sample may be prepared in a number of ways: for example, it may be an unused stub that has been prepared contemporaneously with the questioned samples or a sample taken from the sampling or analytical environment (exposed to the air or as a direct sampling of clean workspace), or both.

9. Data Analysis

9.1 *Definition and Classification:*

9.1.1 *Morphology:*

9.1.1.1 Particles identified as characteristic or consistent with GSR using this method are often spheroidal, noncrystalline particles between 0.5 µm and 5.0 µm in diameter; the remainder are irregular in shape or vary from 1 to 100+ µm in size, or both (**5**, **12**, **13**). In general, it is not consistent with the mechanisms of GSR formation to find particles with crystalline morphology. However, such particles have occasionally been observed in known primer GSR residues. Since morphology can vary greatly, it should never be considered as the only criterion for identification of GSR.

9.1.2 *Elemental Composition:*

9.1.2.1 The elemental composition is the most diagnostic property to determine if a particle may be GSR (**14**). When appropriate, the elemental composition of the recovered particulate can be compared with case-specific known source items, such as the recovered weapon, cartridge cases, or victim-related items.

9.1.2.2 Occasionally, GSR particles with apparent unusual elemental compositions may be encountered in case work. In this circumstance, the elemental compositions of these particles should be compared to case-specific sources, such as cartridges or ammunition/weapon test fire deposits.

9.1.3 Particles *characteristic* of GSR (that is, most likely associated with the discharge of a gun) will have the following elemental composition:

9.1.3.1 Lead, antimony, barium.

9.1.3.2 It is common for additional elements to become incorporated into particles containing these elements. Such particles may contain but not be limited to one or more of the elements: aluminum, silicon, phosphorus, sulfur (trace), chlorine, potassium, calcium, iron (trace), nickel, copper, zinc, zirconium, and tin.

9.1.4 Particles consistent with GSR (that is, may be associated with the discharge of a gun but could also originate from other sources unrelated to a gun discharge) will have one of the following elemental compositions:

9.1.4.1 Barium, calcium, silicon (with or without a trace of sulfur);

9.1.4.2 Antimony, barium (**15**) (with no more than a trace of either iron or sulfur (**16**));

9.1.4.3 Lead, antimony;

9.1.4.4 Barium, aluminum (with or without a trace of sulfur);

9.1.4.5 Lead, barium;

9.1.4.6 Lead (only in the presence of particles with compositions mentioned in 9.1.3 and 9.1.4);

9.1.4.7 Antimony (only in the presence of particles with compositions mentioned in 9.1.3 and 9.1.4);

9.1.4.8 Barium (with or without a trace of sulfur); or

9.1.4.9 Particles with the above compositions may also contain any one or several of the elements listed in 9.1.3.2.

9.1.5 The following compositions have been observed from different kinds of ammunition with "lead-free/non-toxic" primers (**17, 18**).

9.1.5.1 Particles that have a composition characteristic of GSR, will have one of the following elemental compositions:

(1) Gadolinium, titanium, zinc (**19**); or

(2) Gallium, copper, tin (**19**).

9.1.5.2 Particles with compositions consistent with GSR from different kinds of "lead-free or non-toxic" ammunitions will have one of the following elemental compositions:

(1) Titanium, zinc (**17, 20**); or

(2) Other elements that may occur include aluminum, silicon, calcium, copper, or tin (for example, from jacketing material).

9.1.5.3 Strontium (**20, 21**).

9.1.6 Additional classifications may be developed for specific types of primer compositions not included in the previous sections. Any new classification should aid in differentiating environmentally or occupationally produced particles that may be found in a sample from GSR. An assessment of the significance of these classifications must be made in consideration of appropriate research and documentation.

9.2 *X-Ray Analysis:*

9.2.1 *Manual Data Collection:*

9.2.1.1 Particles whose backscattered electron signal brightness exceeds the desired threshold setting, indicating high atomic number contrast, should be considered for analysis.

9.2.1.2 If appropriate, the operator should then collect an EDS spectrum from each detected particle by placing the electron beam in spot mode near the center of the particle or rastering an area completely within the particle's volume. Sufficient X-ray counts should be accumulated to provide a highly certain identification of all elements of interest. However, if a brief spectral acquisition indicates that the major elements are not characteristic of or consistent with GSR, acquisition may be stopped.

9.2.2 *Automated Data Collection:*

9.2.2.1 At a minimum, an automated SEM/EDS system should mimic the capabilities of a system being run manually. It should provide hard copy output and electronic storage of the data including, at minimum, stage *X* and *Y* coordinates, field of analysis *X* and *Y* coordinates, total number of particles detected, and total number of particles classified as GSR.

9.2.2.2 The composition of the particles identified (or classified) and reported as characteristic of GSR must be confirmed by manual relocation of the particle and re-acquisition of the X-ray spectrum.

9.2.2.3 The composition of the particles identified (or classified) as consistent with GSR should be verified by re-inspection of the stored X-ray spectra. Particle relocation and re-acquisition of X-ray spectra is optional but highly recommended if it is decided that the data adds to the probative value of the analysis.

10. Documentation

10.1 The following documentation is required for a representative number of particles identified and reported as characteristic of GSR by either manual or automated analyses:

10.1.1 Images of the particles showing their morphologies (as defined in 9.1.1).
10.1.2 X-ray spectra of the particles, with all relevant elements present clearly identified and labeled.
10.1.3 The operator/analyst should follow other intra-laboratory protocols for documentation as appropriate.

11. Keywords

11.1 energy dispersive X-ray spectrometry; forensic science; gunshot residue; scanning electron microscopy

1. Scope

1.1 This practice covers the analysis of gunshot residue (GSR) by scanning electron microscopy/energy-dispersive X-ray spectrometry (SEM/EDS) using manual and automated methods. The analysis may be performed manually, with the operator manipulating the microscope controls and the EDS system software, or in an automated fashion, where some amount of the analysis is controlled by pre-set software functions. This practice refers to the analysis of electron microscopy stubs and does not address sample collection (**1**).[2]

1.2 Since software and hardware formats vary among commercial systems, guidelines will be offered in the most general terms possible. For proper terminology and operation, consult the SEM/EDS system manuals for each instrument.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *This practice offers a set of instructions for performing one or more specific operations. This practice cannot replace knowledge, skill, or ability acquired through appropriate education, training, and experience and should be used in conjunction with sound professional judgment.*

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4. Summary of Practice

4.1 From the total population of particles collected, those that are detected by SEM to be within the limits of certain parameters (for example, atomic number, size, or shape) are analyzed by EDS (**3-5**). Typically, particles composed of high mean atomic number elements are detected by their SEM backscattered electron signals and an EDS spectrum is obtained from each. The EDS spectrum is evaluated for constituent elements that may identify the particle as being consistent with or characteristic of GSR, or both. See Section 9 for discussion on classification of particles.

5. Significance and Use

5.1 This document will be of use to forensic laboratory personnel who are involved in the analysis of GSR samples by SEM/EDS (**6**).

5.2 SEM/EDS analysis of GSR is a non-destructive method that provides (**7, 8**) both morphological information and the elemental profiles of individual particles.

5.3 Particle analysis contrasts with bulk sample methods, such as atomic absorption spectrophotometry (AAS) (**9**), neutron activation analysis (NAA) (**10**), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS), where the sampled material is dissolved or extracted prior to the determination of total element concentrations, thereby sacrificing morphological information and individual particle identification.

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