Standard Practice for Gunshot Residue Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry

Gunshot Residue Subcommittee
Chemistry Scientific Area Committee
Organization of Scientific Area Committees (OSAC) for Forensic Science

OSAC Proposed

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Prepared by
Gunshot Residue Subcommittee
Version: 1.0 March 2020

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Date: 3/12/2020
To: Main Committee E30 members and Subcommittee E30.01 members (Concurrent Ballot)
Tech Contact: David Freehling, dfreehling@ncdoj.gov, 919-582-8920
Work Item #: WK70036
Ballot Action: Revision of E1588-17
Rationale: This document was reviewed by the OSAC Gunshot Residue Subcommittee. This is the second time this standard has been balloted. Negatives were found to be persuasive and comments from the previous ballot have been addressed. Only the tracked changes are up for comment. Any extra text is provided for context.

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

1. Scope

1.1 This practice covers the analysis of gunshot residue (GSR) by scanning electron microscopy/energy-dispersive X-ray spectrometry (SEM/EDS). The analysis is performed using automated software control of both the SEM and EDS systems, to screen the sample for candidate particles that could be associated with GSR. Manual control of the instrument is then used to perform confirmatory analysis and classification of the candidate particles. This practice refers solely to the analysis of electron microscopy stubs (**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 document cannot replace education or experience and should be used in conjunction with professional judgment. Not all aspects of this practice may be applicable in all circumstances. This ASTM standard is not intended to represent or replace the standard of care by which the adequacy of a given professional service must be judged, nor should this document be applied without consideration of a project's many unique aspects. The word "Standard" in the title means only that the document has been approved through the ASTM consensus process.*

1.5 *This practice does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user when applying this practice to establish appropriate safety and health practices and deter- mine the applicability of regulatory limitations prior to use.*

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[1] This 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 Feb. 1, 2017. Published February 2017. Originally approved in 1994. Last previous version approved in 2016 as E1588 – 16a. DOI: 10.1520/E1588-17.

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

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

1. 2. Referenced Documents

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

2.2 E2917 Practice for Forensic Science Practitioner Training, Continuing Education, and Professional Development Programs.

3. Terminology

3.1 Definitions of Terms Specific to This Standard that are accepted based on consensus of the forensic GSR community (1):

3.1.1 stub, n—sample device with an adhesive surface used to collect materials for SEM/EDS analysis.

3.1.2 characteristic particles, n—particles that have compositions rarely found in particles from any source other than GSR.

3.1.3 consistent particles, n—particles that have compositions that are found in GSR and also arise from other non-firearm sources.

3.1.3.1 Discussion- Particles within this group are produced through the operation of a variety of processes, equipment, or devices and can be found in the environment with varying levels of frequency.

3.1.4 commonly associated particles, n—particles have compositions that are also commonly found in environmental particles from numerous sources.

3.1.4.1 Discussion - When present in addition to particles that are characteristic of, and/or consistent with, GSR these particles can be of use in the interpretation of a population of particles and, consequently, the likelihood that that population is GSR. In isolation, however, such particles have little use in examinations for GSR.

3.1.5 morphology, n; morphological, adj—referring to size, shape, structure, and texture.

4. Summary of Practice

4.1 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 could identify the particle as being consistent with or characteristic of GSR (**2-4**). 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 (**5**).

5.2 SEM/EDS analysis of GSR is a non-destructive method that provides (**6, 7**) both morphological information and the constituent elements detected in individual particles.

5.3 Particle analysis contrasts with bulk sample methods, such as atomic absorption spectrophotometry (AAS) (**8**), neutron activation analysis (NAA) (**9**), 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 size, shape, and individual 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 The sample collection stub shall be labeled 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, for example 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 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 is not always possible in some systems.

7.3 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 have been detected. The decision as to how many particles satisfy the requirements of a particular case should be 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 given that the criteria set forth in 8.2 and 8.3 are met.

8.1.2 Automated data collection of GSR involves some portion of the data collection being controlled by instrument automation software. The extent to which the SEM and EDS systems communicate and are integrated varies according to the manufacturers and the capabilities of the hardware/software. The system shall have the ability to recall stage locations of particles for verification and software for particle recognition.

8.2 Scanning Electron Microscope (SEM):

8.2.1 The SEM, operating in the backscattered electron imaging mode, shall be configured to detect particles down to at least 1.0 µm in diameter.

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

8.2. SEM/ systems shall include: a motorized stage with automated stage control.

8.3 Energy Dispersive X-ray Spectrometry (EDS):

8.3.1 The detector shall be configured to produce a resolution of better (less) than 150 eV during analysis, measured or extrapolated as the full width at half the maximum height of the Mn K_a peak (**1**).

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

8.3.3 Display of the EDS output shall 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 The instrument shall be capable of recording spectra obtained from the analysis of candidate particles. At a minimum, an automated system shall 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 could 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 (**10**).

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 based on their mean atomic number. 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 elemental standards at the same instrumental settings used for sample analyses. This reference 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, could no longer be compatible with the new conditions and should be readjusted. Beam current measurements can be taken with (but are not limited to) 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. The use of control charts and/or tracking of instrument parameters and quality control metrics is recommended. Therefore, as minimum conditions:

8.6.1.1 Establish a protocol to confirm appropriate instrument operation parameters prior to sample analysis.

8.6.1.2 Monitor the EDS X-ray energy calibration and SEM beam current stability at least once per batch of samples using appropriate reference materials of known elemental composition such as copper, cobalt, etc.

8.6.1.3 Analyze a reference material with particles of known size range and composition to test the accuracy of particle detection and identification. It is recommended that the reference material has been prepared and mounted in a manner comparable to the collection method in use by the submitting agency. The reference material can be a sample of GSR from a known source. Additional environmental particles may be added to ensure the inclusion or exclusion of particular classes of particles. Alternatively, a synthetic, GSR standard containing particles comprised of the primary elements of interest may be used for this purpose. The frequency of analysis of this sample shall be 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 sample collection or analytical system, or both. An environmental sample can 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 sample collection or analytical environments (exposed to the air or as a direct sample collection from clean workspace), or both.

8.6.1.5 Care should be taken when transferring samples to and from the instrument to prevent cross contamination. Tools should be cleaned using a solvent and lint free tissue between samples from different sources.

9. Data Analysis

9.1 Definition and Classification:

9.1.1 Morphology:

9.1.1.1 Particles identified as characteristic of or consistent with GSR using this method are often spheroid particles, typically between 0.5 µm and 5.0 µm in diameter; the remainder are irregular in shape or vary from 0.5 µm to 100+ µm in size, or both (**6, 11, 12**). In general, it is not consistent with the mechanisms of GSR formation to find particles by SEM- displaying 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 could be GSR (**13**). In some instances, it could provide additional forensic value to compare the elemental composition of the recovered particulate with case-specific known source items, such as the recovered firearm, cartridge cases, or victim-related items.

9.1.2.2 Particles with unusual elemental compositions could 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/firearm test fire deposits.

9.1.3 Particles classified as characteristic of GSR will have the following elemental composition:

9.1.3.1 Lead, antimony, barium (**7**).

9.1.4 Particles classified as consistent with GSR will have one of the following elemental compositions:

9.1.4.1 Lead, barium, calcium, silicon.

9.1.4.2 Barium, calcium, silicon.

9.1.4.3 Antimony, barium (**15**).

9.1.4.4 Lead, antimony.

9.1.4.5 Barium, aluminum.

9.1.4.6 Lead, barium.

9.1.5 Particles classified as commonly associated with GSR will have one of the following elemental compositions:

9.1.5.1 Lead.

9.1.5.2 Antimony.

9.1.5.3 Barium.

9.1.6 The following compositions have been observed from different kinds of ammunition with “lead-free/non-toxic” primers (**16, 17**).

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

(1) Gadolinium, titanium, zinc (**18**)

(2) Gallium, copper, tin (**18**).

9.1.6.2 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 (**16, 19**).

(2) Strontium (**19, 20**).

9.1.7 It is common for additional elements to become incorporated into particles of these classes. Such particles can contain but not be limited to one or more of the elements: aluminum, silicon, phosphorus, sulfur, chlorine, potassium, calcium, iron, nickel, copper, zinc, zirconium, and tin (**1, 7**). The presence of sulfur in classified particles that contain barium without lead should be viewed with caution as barium sulfate is used in many applications unrelated to the discharge of a firearm. Although rare, the presence of iron should be viewed with caution as these particles can be associated with brake dust (**21**). The presence of zirconium should be viewed with caution as these particles can be associated with vehicle air bags (**22**).

9.1.8 Additional classifications can 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 could be found in a sample from GSR. An assessment of the significance of these classifications shall be made in consideration of appropriate research and documentation (14).

9.2 X-Ray Analysis - Data Collection and Confirmation:

9.2.1 An automated SEM/EDS system should collect elemental data from particles whose backscattered electron signal brightness exceeds the desired threshold setting. It should be able to 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 The candidate particles meeting the above criteria (**9.1**) shall only be considered confirmed after manual relocation of the particles and re-acquisition of the X-ray spectra. The operator shall collect an EDS spectrum from candidate particles by placing the electron beam in spot mode near the center of the particle or raster an area completely within the particle's volume. X-ray counts should be collected to permit robust manual identification of all elements of interest. If a brief spectral acquisition indicates that the elements are not characteristic of or consistent with GSR acquisition may be stopped. The number of candidate particles to be confirmed shall be subject to guidelines set out in the laboratory's standard operating procedures.

10. Documentation

10.1 The following documentation is required for each particle confirmed in section 9.2.2:

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

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RELATED MATERIAL

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