Analysis for Gunshot Primer Residue Using Scanning Electron Microscopy/Energy Dispersive Spectroscopy (SEM/EDS)

1. Scope

This method describes the analysis of microscopic particles to determine if they contain any combination of antimony, barium, and lead. These elements are the main components of gunshot primer residue (GSR). GSR can be deposited on items and subjects when a firearm is discharged. This analysis is performed using a Scanning Electron Microscope with Energy Dispersive Spectroscopy (SEM/EDS).

2. Terms and Definitions

Scanning electron microscope (SEM) – A form of microscopy using electrons rather than visible light to obtain high resolution images over a dynamic range of magnification.

Energy Dispersive X-ray Spectroscopy (EDS) – Makes use of the x-ray spectrum emitted by a solid sample bombarded with a focused beam of electrons to obtain a localized chemical analysis.

Sample stub – a specimen holder/collection device for examination using SEM/EDS usually made of aluminum. The stub consists of a wide, flat sample surface and a pin for mounting on the SEM stage.

Adhesive tab – round, carbon-based, double sided adhesive disc designed to be placed on a sample stub for GSR evidence collection.

Carbon tape – carbon-based, double sided adhesive tape that may be cut into short sections and placed on a sample stub for GSR evidence collection.

Peak overlaps – There are energy peak overlaps among different elements, particularly those corresponding to x-rays generated by emission from different energy-level shells (K, L, and M) in different elements. Particularly at lower energies, individual peaks may correspond to several different elements.

3. References

Guide for Primer Gunshot Residue Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry, SWGGSR

ASTM E1588 - Standard Practice for Gunshot Residue Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry

INCA Feature Instruction Manual, Oxford Instruments, Chapters 2 & 3.

The Principles and Practice of X-ray Microanalysis (compact disc) by Oxford Instruments

Aerospace Report No. ATR-77 (7915)-3 Equipment Systems Improvement Program, Final Report on Particle Analysis for Gunshot Residue Detection, Law Enforcement Development Group, Aerospace Corporation, September 1977.

4. Examination Procedures

4.1. Evidence Types

Commercially supplied SEM/EDS gunshot primer residue kits for subjects are the primary evidence type for this analysis. Other evidence for GSR SEM/EDS analysis may include, but is not limited to: subjects' outer clothing, vehicles, and items in the area of a firearm discharge. Generally, kits and clothing from an individual that has been wounded by a gunshot will not be processed, as this individual has already been associated with a firearm discharge.

4.2. Reagents and Chemicals

4.3. Procedural and Chemical Precautions

Refer to the TBI Safety Manual for general safety requirements.

When carbon coating samples, the carbon rods should only be observed using goggles rated for welders and cutters. The goggles conform to ANSIZ87.1.

Protective attire, including laboratory coat, mask, gloves, and eye protection should be worn when working with clothing or bloodstained items.

4.4. Quality Assurance Procedures

1. A positive standard for particles of gunshot primer residue will be created by firing ammunition with standard composition primers. A

sample stub with a sticky tab will be used to collect particles of GSR. This stub will be analyzed to ensure that gunshot residue particles one (1) micrometer and larger have been collected. Some particles smaller than one (1) micrometer may also be detected. These particles shall include those containing antimony, barium, and lead. This positive standard stub will be analyzed repeatedly, and a distribution map of the particles will be made to confirm reproducibility. Once this step is completed, this stub is the positive control for the SEM and will not be moved or removed from the SEM stage until it is necessary to create a new positive control.

2. Regular monitoring of the microanalysis laboratory environment can determine if the precautions taken and cleaning routines are effective. Suitable areas to be monitored include GSR sample collection areas, the SEM/EDS area, and exterior surfaces of the carbon coater. The monitoring of these areas shall be performed semi-annually. These results will be maintained in the Environmental Monitoring notebook. The stubs will not be kept.

3. Negative control samples will be analyzed with each batch of stub samples. These control samples monitor the sample coating process and the SEM interior chamber.

4. If contamination is indicated in the negative control sample:

    a. The incident will be diagnosed to determine the exact source.
    b. Case impact will be assessed.
    c. The areas indicated by the contamination will be thoroughly cleaned and tested for residual contamination.
    d. Case work can resume once the contamination procedures have been demonstrated to have been effective.
    e. A report may be issued stating the negative control was contaminated and no analysis was performed.
    f. If a systemic problem is found, a corrective action will be applied.

5. Appropriate measures will be taken to avoid contamination in the laboratory. Personal Protective Equipment (PPE), including fresh gloves and a clean lab coat, will be worn when processing GSR evidence. Prior to processing evidence, the work surface will be cleaned with a detergent solution followed by a 2% hydrochloric acid solution and then clean catch paper placed on this surface. This same cleaning shall be performed after processing an exhibit. Note:

After processing biohazardous evidence, the surface will also be cleaned with a bleach solution.

6. All sample and control stubs generated from casework will be maintained in a designated area that is secure and has limited access. These stubs will be properly sealed in boxes, uniquely identified with barcodes attached, and electronically transferred to Microanalysis Long Term Storage where they will be retained indefinitely.

4.5. Limitations

Antimony, barium, and lead must be identified using the EDS software. There are possible peak overlaps of:

Antimony (Sb) with Calcium (Ca) and Potassium (K)
Barium (Ba) with Titanium (Ti)
Lead (Pb) with Sulfur (S) and Molybdenum (Mo).

These do not preclude identification but must be taken into consideration when identifying specific peaks/patterns with specific elements.

4.6. Procedure

When both a kit and clothing from a subject are submitted to the laboratory for GSR analysis, the kit will be analyzed first. If two or more particles containing antimony, barium, and lead are confirmed on the kit, the clothing from the same subject need not be analyzed.

<u>Kits from Individuals</u>

A new sample stub with an adhesive tab (negative control) will be included with each batch to be analyzed. This stub is to be exposed to the same processes the evidence sample stubs experience.

Kits may be processed individually or as a batch. Kits from different individuals may be processed in the same batch.

Clean the work surface with a detergent solution followed by a 2% hydrochloric acid solution. Place clean, unused paper on the surface.

Put on a clean lab coat and fresh gloves.

Open the kit and document the contents on the GSR Analysis Worksheet. Place a copy of any paperwork present in the kit contents in case file.

Once all kits in the batch have been opened and documented, transfer stub vials to clean area at the carbon coater.

Open one vial at a time. Mark the bottom of the stub with a sample identifier. Place the stub in the coater chamber. Repeat for each vial in the kit. Change gloves after each kit. Repeat for each kit in the batch.

Items/Clothing

A new sample stub with an adhesive tab (negative control) will be included with each batch to be analyzed. This stub is to be exposed to the same processes the evidence sample stubs experience.

Items that have been recovered from different locations or persons shall be processed and analyzed separately.

Items that have been recovered from the same location or the same person may be processed successively without surface cleaning or changing lab coats and may be analyzed together. Exhibits that contain multiple items in one container may be processed as one item.

Clean the work surface with a detergent solution followed by a 2% hydrochloric acid solution. Place clean, unused paper on the surface.

Put on a clean lab coat and fresh gloves.

Remove the evidence from its original container. Evidence may be photographed for documentation. Large evidence items, such as doors, may be processed differently due to their size. Document changes in case notes.

Prepare new sample stubs with carbon tape or adhesive tabs.

Press the sample stub against the evidence item. This is repeated across the area of collection until the tape/tab is no longer sticky. If

the tape/tab is no longer sticky before the collection area is completed, multiple stubs may be used to completely collect the area.

Mark stub identifiers on the underside of the stub(s) and case identifiers on the container used for stub storage.

Transfer sample stubs in storage containers to clean area of carbon coater and place the stubs in the coater chamber.

Return evidence to its original container.

Remove lab coat and gloves.

Clean the work surface with a detergent solution followed by a 2% hydrochloric acid solution. Place clean, unused paper on the surface.

Process the next exhibit as above.

All Samples

Once all sample stubs and the negative control are placed into the carbon coater chamber, place the chamber under vacuum and coat with a thin film of carbon to increase electrical conductivity. This process is outlined in the carbon coater instructions. Return the chamber to atmospheric pressure and remove the stubs.

Bring the SEM/EDS chamber to atmospheric pressure and load stubs into the chamber. Document the location of the stubs in the sample holder. Return the chamber to high vacuum.

Optimize the SEM/EDS. This optimization will be run with each batch of samples. Optimization will be performed using the Cobalt from the MAC Reference Standard for X-Ray Microanalysis and the same SEM conditions as those used in casework. Using the *INCA* or *Aztec GSR* software, open a project to store data. Once the cobalt is displayed and in proper focus on the SEM, the acquisition can begin on the EDS software. The beam spot size should be adjusted to obtain an EDS detector dead time of approximately 25-40%.. Using the EDS software, compare two successive beam measurements. The value should be 100% +/- 3%. If not within the

3%, repeat the optimization. Generate a spectrum and record the measured value on the spectrum. These results are stored in each case file. The instrument parameters are printed and will be placed in each case file. These are the operating parameters for the Zeiss (EVO) SEM/EDS:

High Voltage: 20 KeV
Beam Current: 30 or 100 uA
Working Distance: 8.5 mm
Image Detector: Back Scatter Electron
Magnification: may vary from 300-500
Smallest feature: variable
Method used: GSR with Filters

Method Summary:

SbBaPb	Sb 0.0-100%	Ba 0.0-100%	Pb 0.0-100%
SbBa	0.0-100%	0.0-100%	------
SbPb	0.0-100%	-----	0.0-100%
BaPb	-----	0.0-100%	0.0-100%

The positive control must first be analyzed to prepare the SEM/EDS for the search of high atomic mass number particles. This same sample must also be run at the end of each batch.

The negative control stub prepared for each batch shall be run following the pre-analysis positive control and prior to the post-analysis positive control for that batch. If all sample stubs in the batch are from the same individual or location, then the negative control stub may be run only at the beginning of the batch.

If the analysis of the positive control sample does not consistently detect one micrometer particles or larger, the results from the batch of sample stubs will not be used and the samples will be reanalyzed.

For stubs that indicate the presence of particles of gunshot primer residue, the analyst will return to these particles on the stub and manually reacquire spectra and verify proper morphology to confirm their identity. It is not necessary to confirm every particle; however, multiple particles must be confirmed. The feature report containing the reacquired spectra is retained as part of the case notes.

When analysis is completed, the stubs may then be removed from the sample chamber of the SEM and placed into their designated containers which have been labeled with the laboratory case number, examiner's initials, and exhibit number. The negative controls will be placed in their own separate containers and labeled with case or batch identifiers. All samples and controls will be transferred to long term storage as described in section 4.4.

4.7. Interpretation

Particles that are identified as gunshot primer residue must contain the three elements antimony, barium, and lead. In the total population of suspected particles, multiple (two or more) particles with all elements of GSR must be present. Additionally, particles with two of the three elements of GSR may also be present in the total population. All of these particles shall have morphology consistent with a high temperature vapor condensing into solid droplets. The size of these particles ranges from approximately 0.5 to 100+ micrometers.

Particles considered to be consistent with gunshot primer residue must have morphology as described above and have one of the following elemental profiles:

- antimony, barium (with no more than a trace of iron or sulfur)
- lead, barium
- lead, antimony (with levels of antimony greater than trace amounts)

Additionally, multiple (two or more) particles must be present to be considered consistent with gunshot primer residue. This can include one three component particle and one two component particle. Particles that don't meet the above criteria will not be considered gunshot primer residue.

Instruments and Equipment

1. Vacuum Carbon Coater, carbon rods, and supplies

2. Scanning Electron Microscope with Energy Dispersive Spectrometer
3. SEM aluminum sampling stubs
4. Double-sided conductive tape or carbon adhesive tabs
5. Photographic equipment with accessories
6. SEM stub evidence containers

Measurement Traceability

The INCA software is optimized to detect one micrometer or larger GSR particles. This measurement is a detection threshold only. The size of the particle is irrelevant to the determination of GSR. Determination is based on the elemental profile and morphology of the particle.

Reference Materials

1. Nickel Grid
2. MAC Co/Rh/C/Au Reference Standard
3. GSR positive control

Reports

The following are possible results concluded from the examination:

NEGATIVE

(Individuals)
Electron microscopic examination and analysis of exhibit X did not reveal the presence of gunshot primer residue. The absence of gunshot primer residue is consistent with an individual who has not discharged a firearm or otherwise been exposed to a source of gunshot primer residue. A negative result could also occur when gunshot primer residue particles are lost due to washing, excessive time interval between firearm discharge and collection, or other routine activities.

(Items)
Electron microscopic examination and analysis of exhibit X did not reveal the presence of gunshot primer residue. The absence of gunshot primer residue is consistent with an item not being exposed to a source of gunshot primer residue. A negative result could also occur when gunshot primer residue particles are lost due to washing, excessive time interval between firearm discharge and collection, or other routine activities.

POSITIVE

Electron microscopic examination of exhibit X revealed the presence of particles identified as gunshot primer residue. These microscopic particles of gunshot primer residue have the appearance of being condensed from a vapor and contain the elements antimony, barium, and lead.
This result indicates that this individual could have discharged, handled, or been near a firearm when it was discharged.
OR
This result indicates that this exhibit was near a firearm when it was discharged, or came in contact with a recently discharged firearm or spent ammunition components.

CONSISTENT

Electron microscopic examination and analysis of exhibit X revealed the presence of particles consistent with gunshot primer residue. These consistent particles are microscopic and appear to have condensed from a vapor. However, they contain only two of the three elements (antimony, barium, and lead) required to identify them as gunshot primer residue. Such particles are found in gunshot primer residue, but may originate from other occupational or environmental sources.
This result cannot eliminate the possibility that this individual could have discharged, handled, or been near a firearm when it was discharged.
OR
This result cannot eliminate the possibility that this exhibit was near a firearm when it was discharged, or came in contact with a recently discharged firearm or spent ammunition components.

(Single 2-component)
Electron microscopic examination and analysis of exhibit X revealed the presence of a single particle consistent with gunshot primer residue. This consistent particle is microscopic and appears to have condensed from a vapor. However, it contains only two of the three elements (antimony, barium, and lead) required to identify it as gunshot primer residue. Such particles are found in gunshot primer residue, but may originate from other occupational or environmental sources.
This result is insufficient to determine if this individual could have discharged, handled, or been near a firearm when it was discharged.
OR

This result is insufficient to determine if this exhibit was near a firearm when it was discharged, or came in contact with a recently discharged firearm or spent ammunition components.

(Single 3-component)
Electron microscopic examination and analysis of exhibit X revealed the presence of a single particle identified as gunshot primer residue. This microscopic particle of gunshot primer residue appears to have condensed from a vapor and contains the elements antimony, barium, and lead.
This result is insufficient to determine if this individual could have discharged, handled, or been near a firearm when it was discharged.
OR
This result is insufficient to determine if this exhibit was near a firearm when it was discharged, or came in contact with a recently discharged firearm or spent ammunition components.

(One 2-component and one 3-component)
Electron microscopic examination and analysis of exhibit X revealed the presence of one three-component and one two-component particle consistent with gunshot primer residue. These particles are microscopic and appear to have condensed from a vapor. However, one of them contained only two of the three elements (antimony, barium, and lead) required to identify it as gunshot primer residue. Consistent particles are found in gunshot primer residue, but may originate from other occupational or environmental sources.
This result cannot eliminate the possibility that this individual could have discharged, handled, or been near a firearm when it was discharged.
OR
This result cannot eliminate the possibility that this exhibit was near a firearm when it was discharged, or came in contact with a recently discharged firearm or spent ammunition components.

(One 3-component & multiple 2-component)
Electron microscopic examination and analysis of exhibit X revealed the presence of one three-component and multiple two-component particles consistent with gunshot primer residue. These particles are microscopic and appear to have condensed from a vapor. However, all but one of them contained only two of the three elements (antimony, barium, and lead) required to identify it as gunshot primer residue. These consistent particles are found in gunshot primer residue, but may originate from other occupational or environmental sources.
This result indicates that this individual could have discharged, handled, or been near a firearm when it was discharged.
OR

This result indicates that this exhibit was near a firearm when it was discharged, or came in contact with a recently discharged firearm or spent ammunition components.

The wording of these results may vary.