Quick Guide to Classify Qualitative and Quantitative Performance of SEM/EDS and ICP-OES for Gunshot Residue Analysis

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ABTRACT The investigation of inorganic gunshot residues (iGSR) is aimed to detect particles containing antimony, barium, and/or lead on a sample. The iGSR can be determined via either destructive or non-destructive techniques. The laboratory competence on iGSR analysis is assessed via successful participation in proficiency tests (PT). According to ISO 13528 Section 11.1, PT schemes can be identified into three types, e.g., classification of the sample with or without considering its magnitude and investigation of its properties. At present, two types of PT for iGSR analysis are carried out, both based on ISO 13528. In the first type (I-iGSR PT), participants are requested to submit the results of quantitative information on GSR particles. For the second type (II-iGSR PT), participants shall report the presence or absence of characteristic iGSR particles on the PT sample. However, to avoid wasting budget and unnecessary efforts, selection of the type of PT scheme should consider the actual performance of individual instrument available to each laboratory. In this study, the Royal Thai Police cooperated with laboratory network to propose a quick guideline for performance assessment of scanning electron microscope with energy dispersive x-ray spectrometer (SEM/EDS) and inductively coupled plasma-optical emission spectrometry (ICP-OES), based on preliminary studies of the standard deviation index (SDI). By using SDI, the difference between the iGSR sample mean and the mean of inter-laboratory were compared. The results revealed that when the SDI values were nearly zero, the performance of SEM/EDS or ICP-OES was suitable for participating in both types of iGSR PT programs. The SDI value which is more than 1 pointed out that the operated instrument was only sufficient for qualitative testing. Thus, the observed SDI values can be used as a preliminary tool for instrument performance assessment of iGSR analysis prior to the decision of PT subscription for the first or consecutive rounds.

INTRODUCTION

Gunshot Residue (GSR) is considered to be important evidence in firearm-related incidents because, after firing, gunshot residue particles are always present, and the sharp increase in pressure changes after the combustion of gunpowder. It was therefore found that gunshot residue was spreading through the gun gap in all directions[1], causing the accumulation of gunshot residue particles in the environment and the people involved in the incident. It was found that gunshot residue particles accumulated on the skin, hands, faces, and necks of those who used guns. It was also found accumulating on clothes, stuff, and surrounding environments[2]. The type of gunshot residue that is recognized and can be used as evidence in litigation both nationally and internationally is that produced by combustion in the primer mixture. An analysis of the metal components of the primer mixture reveals antimony (Sb), barium (Ba), and lead (Pb), which are the products produced every time the gun is shot[3]. If these elements are detected, they can be confirmed to be gunshot residue so they can be recognized and used as important evidence in firearm-related investigations[4]. The information obtained is classified as important forensic evidence in firearm-related cases, presenting that a person has been through a shooting or had been in contact with a gun, and can link the suspect to the firearm, the victim, and the crime scene, which is useful for the case and investigation[5]. Moreover, the detection of gunshot residue can provide information on the gun type, bullet type, and at the same time estimate the firing range and direction of shooting from the material evidence[5,6]. The collection of various material pieces of evidence at the crime scene for analysis to determine the amount of gunshot residue is a necessary and important step in proving the facts. Those involved in the collection of gunshot residue in crime should understand the importance of gunshot residue analysis to provide the most reliable and effective standardized analysis. Accounting to ISO 13528 sections 11.1, PT schemes are identified for 3 types as follows. Type 1, the PT scheme aimed to report the classification of the sample (no magnitude) as called nominal. Type 2, the PT scheme aimed to report the magnitude of the sample as called ordinal. Type 3, this PT scheme was designed to investigate the existent or extinct PT sample's property [7,8]. In the case of the GSR PT program followed ISO 13528, the PT provider designed PT schemes, which were divided into 2 types. For example, type 1 of the GSR PT scheme (I-GSR PT) required the results of both ~~of~~ characteristic classification and the amount of GSR particles in the PT report[9]. Next, type 2 of GSR PT scheme (II-GSR PT) required the report of the presence or absence of characteristic GSR particles on the PT sample. Especially, SEM/EDS must have been validated accounting to ASTM 1588 standard before testing the GSR PT sample[10]. The PT sample was monitored via only the optimized performance of SEM/EDS and ICP-OES. Although participation in the PT scheme was crucial for every forensic science laboratory, many laboratories underwent suffering to subscribe PT scheme because of affordability, expensive PT samples, closing a testing round, etc[11]. Thus, the subscription of the GSR PT scheme must select the correct types of PT based on evaluating proper SEM/EDS and ICP-OES performance in each laboratory. In the GSR analysis, there are a group of experts in firearms and gunshot residue in the European Union to actively and jointly develop the quality system of such analyzes and proficiency testing (PT). In this work, we aimed to propose a quick guide to reusing old PT samples or old reference materials for interlaboratory testing. The quick guide provided the procedure that can reveal the SEM/EDS and ICP-OES performance with cost-effectiveness. By interpreting the standard deviation index (SDI)[12], a newly established laboratory might employ a quick guide to observe the actual performance of SEM/EDS and ICP-OES. The quick guide might be useful to decide on selecting types of GSR PT programs. To enhance the efficiency of police officers in the laboratory of forensic science, The Royal Thai Police has foreseen this problem. Therefore, the Forensic Science Police Cadet Academy has continuously tried to carry out work related to laboratory proficiency testing (PT). To demonstrate the testing capabilities of laboratories and to ensure that they can develop and optimize standard analytical methods to meet international standards.

MATERIALS AND EXPERIMENTAL SETUP

Scanning Electron Microscope and Energy Dispersive X-ray Spectrometer (SEM/EDS)

- Proficiency Testing Sample from the European Network of Forensic Science Institutes (ENFSI)

The sample of proficiency testing from the company Quodata GmbH which distributes a PT sample of expert working group Firearm/Gunshot residue of the European Forensic Science Institute (ENFSI) was utilized as evaluating the performance of SEM/EDS. Briefly, the used PT-sample, code B-07-13, was chosen in this study and the preparation of the PT sample is the same procedure as gunshot residue reference materials. The determination of position and elemental analysis of GSR particles were operated on a glassy carbon chip with a diameter of 6x6 mm^2 following ASTM E1588-20 [10] standard as shown in Figure1. The GSR analytical condition of SEM/EDS was performed in back-scattering electron mode (BSE-COM) using a faraday cup to adjust spot size. The particle-size, brightness, and contrast calibration of gunshot particles with a diameter of 0.5 µm uses synthetic gunshot particles from PLANO (Sample SPS-C6-A). The parameters of SEM/EDS in this work were as follows:

Table 1 SEM/EDS condition of GSR analysis follow ASTM E1588-20

Parameters used in the test	Royal Police Cadet Academy	Absotech Company Limited
Specimen Current (nA)	1.25	1.2
Accelerating Voltage: Vacc (kV)	20	20
Working Distance (mm)	9.950	15
Magnification	2100	1029
Amp Time	3.84	20

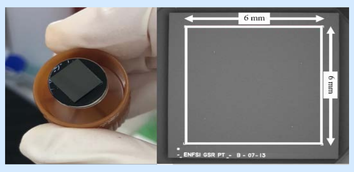

Figure 1 shows the photograph of PT sample (Left) and the GSR analysis area in 6x6 mm^2 (Right) by using SEM.

For SEM/EDS performance test, the scanning electron microscope (SEM) was performed at the Royal Thai Police Cadet Academy (RPCA, Thailand) by using Hitachi FlexSEM 1000. This SEM contained an EDAX Element EDS detector with Genesis GSR Particle Analysis software version 6.332. Next, SEM from a commercial laboratory (ABSOTEC CO., LTD., Thailand) was performed using TESCAN VEGA3 XM. The SEM contained OXFORD INSTRUMENTS XMax N80 EDS detector with INCA software. The qualitative and quantitative performance of SEM/EDS was estimated by the calculation of the standard deviation index (SDI).

Inductively Coupled Plasma - Optical Emission Spectrometer (ICP-OES) [13]

All chemicals used in the experiment were analytical reagent (AR) grade and solutions were prepared using high-purity water with a resistance of 18 MΩ cm. Ultrapure 65% nitric acid (HNO_3) (Merck, Germany) was used for the preparation and extraction of the samples. All reagents and solvents were used as received. The mixture stock solution of the Sb, Ba, and Pb standards (Sigma Aldrich, Switzerland) was prepared to a calibration curve ranging from 40-600 µg/L. All standard solutions were acidified with 5% HNO_3 (v/v)[14]. All glassware was thoroughly cleaned with freshly prepared 1:1 HCl/HNO_3. The sample was prepared from the mixture stock standard of NIST as a certified reference material (CRM). The optimized operating parameters, as well as the values of the limit of detection (LOD), limit of quantification (LOQ), and correlation factor of the linear curve for the analytes Pb, Ba, and Sb, are shown in Table 2. After the collection step, the samples were digested in an ultrasonic bath (DKSH, Elmasonic S 30 H, Germany) and a microwave (Analytikjena, TOPwave, Germany). The interlaboratory comparison results use the 95% confidence level (F-test and t-test paired samples).

Table 2 ICP-OES Conditions for Gunshot Residue Analysis

Analytik Jena PQ9000 ICP-OES Series	
Machine Working Conditions	
Radio Frequency Generator	1200 W
Argon Gas Flow Rate	12.00 L/min
Gas-Assisted Flow Rate	0.50 L/min
Spray Air Flow Rate	0.50 L/min
Sample Substance Flow Rate	1.00 mL/min
Plasma Torch	Torch Tube
Parameters	
Number of Reanalysis	3
Measurement Mode	2 Views

Standard Deviation Index (SDI) [12]

SDI is the standard deviation interval or index, and SD_{group} is the standard deviation (SD) of the group. The difference between the individual laboratory's test result and the average of the group is interpreted via SDI. An SDI of > 2 is unsatisfactory with the performance testing program, while an SDI of < 2 is satisfactory with the performance testing program. Especially, an SDI of zero implies that the laboratory had perfect efficiency with the performance testing program as shown in equation (1).

$$SDI = \frac{\bar{X}_{individual} - \bar{X}_{group}}{SD_{group}} \quad (1)$$

RESULTS AND DISCUSSION

The SEM was used to observe the spherical shapes of GSR particles and the possibility of detecting contamination from the environment as GSR particle was minimized. By using the GSR calibration condition of SEM, the possibility of obtaining false results originating from the background is eliminated because the elements such as Pb, Ba, and Sb cannot form a spherical particle in the environment. The particle was detected using the backscattered electron signal (BES). Typical GSR particles appeared as bright particles in the image. The software recognized and found the particles by applying a threshold to the signal. The samples whose spectrum is presented in Figure 2 were analyzed using SEM-EDS (Figure 3). The scanning was carried out on the sample surface and the chemical composition of the particles, showing a consistent morphology with GSR, was determined. The spectra were displayed and analyzed by using Genesis GSR particle analysis software version 6.332 and INCA software. The GSR spectra of the key 3 elements (Pb, Ba and Sb) in a single residual particle were observed. The results were found in accordance with the ASTM E1588-20 standard.

The GSR analysis results of the PT samples from both the Royal Thai Police's laboratory (RTP) and commercial laboratory (CL) were operated via SEM/EDS accounting to ASTM 1588 standard. For particle analysis of PT sample code B-07-13 according to GSR particle analysis software, the result of the distribution of particle analysis from RTP's laboratory depicted that the particle within the area of 6x6 mm^2 regularly spread on a silicon substrate, as shown in figure 4(A), while an amount of monitoring particle distribution in PT sample from the commercial laboratory was higher than the result of RTP's laboratory, as shown in figure 4(B). The benchmark of those results was compared via standard deviation index (SDI) with a particle size distribution of PT sample code B-07-13 as type 1 of GSR PT sample. The GSR particle size distribution of the PT sample with the manufacturer's information served as reference data. Regarding the manufacturer's information on the PT sample as shown in figure 4(C), the PT sample contained 142 GSR particles with various size diameters, such as 12 µm (4 particles), 2 µm (15 particles), 1.5 µm (20 particles), 1.25 µm (27 particles), 1 µm (25 particles), 0.75 µm (27 particles) and 0.5 µm (24 particles). Analysis time of PT sample showed in figure 4(D). The time consumption of RTP's laboratory for the PT sample investigation were 7,421 minutes while CL were 284.5 minutes, indicating the difference in GSR particle detection capacity of SEM/EDS in each laboratory. In this study, the actual performance of SEM/EDS was determined by using the standard deviation index (SDI). The SDI is considered as a comparison between the mean of individual laboratory results and the networking group mean, which is divided by the overall standard deviation of the networking group [15].

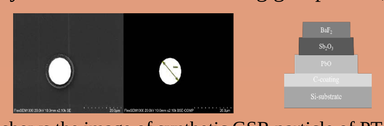

Figure 2 shows the image of synthetic GSR particle of PT sample by using SEM in secondary electron and backscattered electron mode (Left) and the schematic cross-section of PT sample (Right).

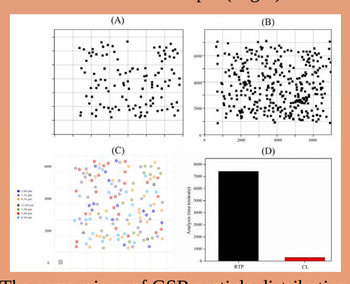

Figure 4 The comparison of GSR particle distribution from the Royal Thai Police's laboratory, RTP (A), commercial laboratory, CL (B), and PT manufacturer's information (C). The analysis time between RTP and CL (D).

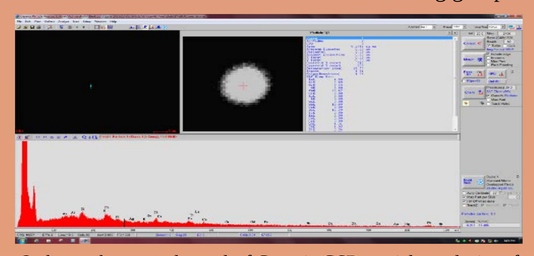

Figure 3 shows the control panel of Genesis GSR particle analysis software version 6.332 during SEM/EDS is working.

Table 3 SDI evaluation of GSR particles analysis.

Particle diameter (µm)	QuoDATA	Commercial laboratory (CL)	Royal Thai Police's laboratory (RTP)	Group-SD	Group-mean	SDI-CL	SDI-RTP
12.00	4	4	3	0.57	3.66	0.58	-1.15
2.00	15	13	12	1.52	13.33	-0.21	-0.87
1.50	20	19	16	2.08	18.33	0.32	-1.12
1.25	27	25	19	4.16	23.66	0.32	-1.12
1.00	25	25	20	2.88	23.33	0.57	-1.15
0.75	27	26	23	2.08	25.33	0.32	-1.12
0.50	24	22	17	3.60	21.00	0.27	-1.10

The repetition of GSR analysis by using SEM/EDS in this study was neglected in this study owning to time consumption[16]. Regarding SDI determination with the quick procedure in this study, the individual laboratory's results mean was replaced by GSR analysis data from one operation of each laboratory. Thus, the GSR analysis data from one operation and GSR analysis data from reference data were calculated for the networking group mean and standard deviation of the networking group to estimate in this study.

The measurement of Sb, Ba, and Pb by ICP-OES showed GSR particles with diameters 0.5, 0.75, 1, 1.25, 1.5, 2, and 12 µm as shown in Table 3. The value of SDI from RTP was -1.15 for a group of particles 0.5 and 1.5 µm, -1.12 for a group of particles 1, 1.25, 2 µm, -1.10 for a group of particles 12 µm and -0.87 for a group of particles 0.75 µm. In the case of CL, the value of SDI was -0.21 for a group of particles 2 µm, 0.27 for a group of particles 0.5 µm, 0.32 for a group of particles 0.75,1.25 and 1.5 µm and 0.57 for a group of particles 1 and 12 µm. For interpreting SDI value, RTP's SDI values of >1 indicated that there is a problem on SEM/EDS performance, which might be incomplete synchronization between SEM/EDS and particle recognition software or changed beam current during the test[10]. RTP's SDI value pointed that the participation in I-GSR PT might be changed into II-GSR PT due to the limitation of quantitative data collection. Thus, the report that inform the existent or extinct of GSR particle of II-GSR PT was appropriate for RTP. In contrast, all CL's SDI values tended to become 0, which indicated that actual SEM/EDS performance for investigating GSR particles was satisfactory.

The measurements of Sb, Ba, and Pb by ICP-OES depended on the concentration of elements. These show that the linearity between the concentration and the intensity of Sb, Ba, and Pb ranging from 40 to 600 µg/L ($y = 16.135x + 86.007$, $R^2 = 0.9999$, $y = 23635x + 18473$, $R^2 = 1$ and $y = 61.625x + 92.362$, $R^2 = 0.9997$), respectively. The limit of detection (LOD) of Sb, Ba, and Pb were found at 4, 4, and 1 µg/L, respectively, and the detection limit of quantity (LOQ) of Sb, Ba, and Pb were found at 6, 4 and 2 µg/L, respectively. To validate the efficiency of the proposed determination of Sb, Ba, and Pb in cotton swabs were analyzed to detect by ICP-OES of the Royal Thai Police compared with ICP-MS of the Central Institute of Forensic Science (CIFS) as shown in Table 4. The data were then compared by F-test and t-test. These data presented F values of Sb, Ba, and Pb as 1.05, 1.06, and 1.04, respectively (F critical = 5.05), indicating a regression form and t-test results of Sb, Ba, and Pb as 1.02, 010 and 1.03, respectively (t critical = 2.57). Additionally, the determination of the concentration of Sb, Ba, and Pb is important for crime scene investigation to identify the gun shooter. Results were not significantly different from the standard methods at the 95% confidence level. This indicates that the proposed method is sensitive enough and applicable for general cases of GSR analysis.

For the analysis of antimony (Sb), barium (Ba), and lead (Pb) at concentrations of 25, 100, and 250 µg/L, the Standard Deviation Index (SDI) and HORRAT (Horwitz's ratio) were calculated from the tested 10 repetitions, as shown in table 5. It was found that repeatability of the method was with accuracy at concentrations of 25, 100, and 250 µg/L of Sb, Ba, and Pb with the SDI in the ranges from -5.7E-15 to -0.18%. Moreover, the HORRAT (Horwitz's Ratio) was within the specified criteria (Horwitz's Ratio ≤ 2). In addition, the F-test results presented F values of Sb, Ba, and Pb: 1.05, 1.06, and 1.04, respectively (F critical 5.05). Hence, no significant difference was observed. For t-test, t values of Sb, Ba, and Pb represented 1.02, 010, and 1.03, respectively, while t critical was 2.57. This indicated that the difference in efficiency between ICP-OES and ICP-MS instruments in this experiment did not affect the quantitative analysis of Sb, Ba, and Pb. That is crucially benefit to the identification of a gun shooter involving in the crime scene.

Table 4 The analysis comparison results of antimony (Sb), barium (Ba) and lead (Pb) in gunshot residue samples between the Central Institute of Forensic Science, Ministry of Justice and the Faculty of Forensic Science, Royal Police Cadet Academy

Volume of CRM	Sample	The Central Institute of Forensic Science			Faculty of Forensic Science The Royal Police Cadet Academy		
		Sb	Ba	Pb	Sb	Ba	Pb
Dropped 20 µL	1	12.09±4.99	89.70±5.78	101.73±0.59	9.69±0.18	93.22±4.93	105.09±1.50
	2	10.37±0.18	95.22±2.06	105.47±2.25	10.84±0.16	95.53±0.81	108.23±6.75
	3	11.68±0.74	91.23±3.60	108.30±2.64	11.52±0.65	95.07±1.20	104.18±1.16
Dropped 50 µL	1	25.33±4.04	222.32±11.52	303.82±11.53	26.07±0.69	229.75±10.88	307.67±13.84
	2	27.29±1.50	219.63±17.71	221.60±4.78	25.78±0.99	211.12±1.37	222.87±10.52
	3	26.01±0.87	244.00±3.61	207.180±8.67	25.83±1.29	240.00±6.08	210.90±3.73

Table 5 Stadard Deviation Index (SDI) and HORRAT of Sd, Ba and Pb

Elements	Standard Deviation Index (SDI)	HORRAT (Horwitz's ratio)		
		25 µg/L	100 µg/L	250 µg/L
Sb	-0.1791038	0.48	0.20	0.14
Ba	-5.272E-15	0.40	0.20	0.24
Pb	-0.0497679	0.52	0.17	0.25

CONCLUSION

The interlaboratory comparison for the SEM between the Royal Thai Police (RTP) and commercial laboratory and the RTP and the Central Institute of Forensic Science (CIFS) for the ICP-OES presented similar results. The development of QC-sample and protocol for GSR analysis by SEM and ICP-OES was done. The statistical data from interlaboratory were not significantly different from the standard methods. Thus, our research can be developed to international standards in terms of proficiency testing ISO/IEC 17043 and ISO 17034 which are the essential elements of laboratory quality assurance. The performance estimation of SEM/EDS and ICP-OES was monitored by using SDI. For this study, the SDI provides not only information on the actual performance of instruments but also the guideline for selecting proficient testing (PT) schemes for gunshot residue (GSR) analysis. In the case of GSR analysis by SEM/EDS, the SDI of >1 compared to the peer groups can imply that these laboratories should select the qualitative type of GSR-PT scheme. On the other hand, the laboratory can perform both qualitative and quantitative types of GSR-PT scheme while SDI of <1. In the case of GSR analysis under ICP-OES, the result with SDI of <1 is consistent with Horwitz's Ratio of <1, indicating that trace analysis of GSR elements is acceptable. In conclusion, we expect that the SDI can be applied as a performance evaluation tool for SEM/EDS and ICP-OES prior to the official PT subscription.

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