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Interlaboratory Comparison of Gunshot Residue Analysis

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Abstract – **The investigation of Gunshot Residue (GSR) in Forensic science was carried out by Scanning Electron Microscope (SEM) energy-dispersive x-ray spectroscopy (EDS) and Inductively Coupled Plasma - Optical Emission Spectrometer (ICP-OES). These techniques are not widely used in Thailand. However, the commercial laboratory (CL), Central Institute of Forensic Science (CIFS), and the Royal Thai Police (RTP) attempt to develop these techniques to promote novel ways for GSR analysis. Also, the standard measurement was a crucial requirement in ISO/IEC 17025 quality standard. As a part of quality assurance, proficiency testing and interlaboratory measurement are important criteria in technical requirements. Therefore, the preliminary design of GSR analysis for interlaboratory in Thailand was developed. The quality control sample (QC-sample) was prepared by collecting GSR from the right-hand's volunteer after shooting. The comparison of GSR analysis between the commercial laboratory and Royal Thai Police for SEM technique and between Central Institute of Forensic Science (CIFS) and the Royal Thai Police for ICP-OES technique. This research was then compared by a paired *t*-test at the 95% confidence level. The results indicated the efficiency to detect GSR under the same condition between the commercial laboratory and Royal Thai Police and Central Institute of Forensic Science (CIFS) and the Royal Thai Police are similar.**

Keywords – Gunshot Residue (GSR), Scanning Electron Microscope (SEM), Inductively Coupled Plasma - Optical Emission Spectrometer (ICP-OES), Quality control sample, ISO 17025, ISO 17043, Interlaboratory.

I. INTRODUCTION

Gunshot residue (GSR) is metallic particles produced after discharging. The major components of GSR consist of antimony (Sb), barium (Ba), and lead (Pb) [1]. The GSR can be found on the hands and clothing of the shooter. The GSR is investigated by Inductively Couple Plasma (ICP) [2-5] and Scanning Electron Microscope (SEM) [6-8]. These instruments require expertise to carry out. Thus, the testing program to evaluate them is important in the standard measurements.

Recently, laboratories need to validate and verify the measurement process to guarantee the result. The evaluation procedure of standard measurement is based on ISO/IEC 17025 [9]. The technical requirement in quality assurance requires information about proficiency testing and interlaboratory measurements. this step is an important issue to achieve the certification of ISO/IEC 17025. Besides, laboratory proficiency testing or ISO/IEC 17043 is an essential element of laboratory quality assurance. With the increasing demands for independent proof of competence from regulatory bodies and customers, proficiency testing is relevant to all laboratory testing for quality and safety in every country. Perhaps as important for the technical performance of the laboratory, regular proficiency testing provides independent feedback on the quality of analytical results, enabling laboratories to monitor and improve performance over time [10, 11].

In this research, the Royal Thai Police, the commercial Laboratory, and Central Institute of Forensic Science (CIFS) in Thailand developed quality control sample and testing procedure of GSR analysis to determine heavy metals, antimony (Sb), barium (Ba), and lead (Pb) which are the main components of gunshot residue (GSR) and produced by 130 GR ammunition. This preliminary testing program is performed by SEM/EDS and ICP-OES and evaluated statistic results by a paired *t*-test.

II. EXPERIMENTAL SETUP

All chemicals used in the experiment were analytical reagent (AR) grade and solutions were prepared using high pure water with a resistance of 18 MΩ cm. Ultrapure 65% nitric acid (HNO_3) (Merck, Germany) was used for preparation and extraction of the samples. All reagents and solvents were used as received. A multi-element stock solution (2000 µg/L) of the Sb, Ba, and Pb standards (Sigma Aldrich, Switzerland) was prepared to build a calibration curve (5-800 µg/L for Sb and 10-800 µg/L for

Ba and Pb). All standard solutions were acidified with 5% HNO_3 (v/v). All glassware was thoroughly cleaned with freshly prepared 1:1 HCl/HNO_3.

The GSR particles as a quality control sample (QC-sample) were obtained from shooting (from 1 and 3 for the 130 Gr ammunition). In research, we divided the experiment into 2 parts. Firstly, SEM technique was tested at Glock 9 (AUSTRIA 9x19 pistol) was fired and bullets were 130 Gr. IDPA (B00362). After a 1-round shooting, the GSR as a quality control sample was collected from the right-hand's volunteers. The sample from the 3-round shooting was selected by the same procedure. The GSR kit with a tape lifting method was used for GSR collection. The GSR kit is 12 mm-aluminum stubs covered by double-sided adhesive carbon tape. The skin between thumb to forefinger (sampling area) as shown in Fig. 1 was collected by pressing GSR kit onto this area. The scanning electron microscope (SEM) was performed at the Royal Thai Police Cadet Academy (RPCA, Thailand) using Hitachi FlexSEM 1000. This SEM contained EDAX Element EDS detector with TEAM software. For interlaboratory comparison, SEM from a commercial laboratory (ABSOTEC CO., LTD., Thailand) was performed using TESCAN VEGA3 XM. The SEM contained OXFORD INSTRUMENTS XMax N 80 EDS detector with INCA software. The QC-sample was directly transferred to SEM chamber. Both SEM instruments were validated by EDS reference sample and synthetic GSR particle. SEM was operated based in ASTM E1588-17 [12]. The accelerating potential at 20 kV with backscattered electron (BSE) mode was carried out for finding GSR particles. In this work, the same QC-sample was used for both instruments. As an appropriated sampling method, an evenly distributed 9-area on the stub surface was selected for manual analysis as shown in Fig 2.

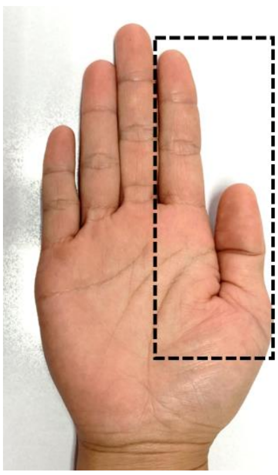

Fig. 1. The image of the sampling area on the right- hand palm (RHP) after shooting.

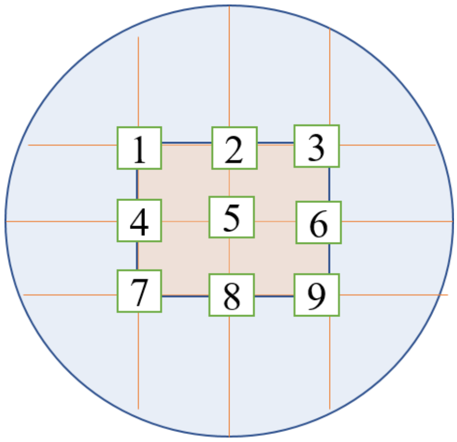

Fig. 2. The sampling area on the stub surface

Next, An ICP-OES (Analytikjena, Plasma Quant PQ 9000, Germany) was used for the quantification of Sb, Ba, and Pb. A Meinhard concentric nebulizer and cyclonic spray chamber with peristaltic pumping were used for introducing the samples into the plasma torch. The operating parameters were optimized using a central composite design. 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 Sb, Ba, and Pb, are shown in Table 1. 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 (*t*-test paired samples).

Table 1 ICP-OES parameters

Analytik Jena – PlasmaQuant PQ 9000	
Operating Condition	
RF power	1200 W
Argon Gas flow	12.00 L/min
Auxiliary Gas flow	0.50 L/min
Nebulizer Gas flow	0.50 L/min
Sample flow rates	1.00 mL/min
Torch	Quartz tube
Parameter	
Number of Replicate	3
Detector mode	Dual

III. RESULTS AND DISCUSSION

Energy dispersive x-ray spectroscope (EDS) displayed the characteristic x-ray of GSR particles, which contained elements of Sb, Ba and Pb as shown Fig.3.

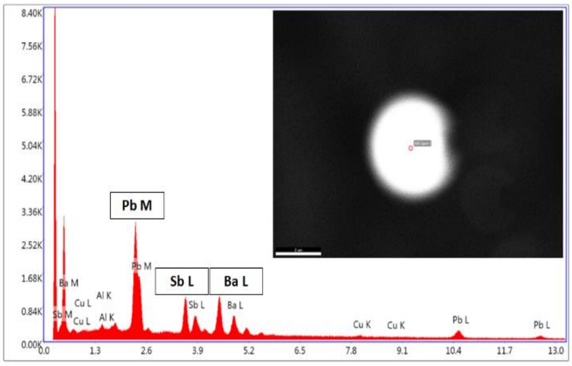

Fig.3. EDS spectra and the morphology of GSR (Inset image).

The dominant x-ray peak was obtained from the center of GSR particle. One of the spheroid GSR particles from a 1-round shooting was approximate 3 µm in diameter. The surface of adhesive carbon tape in presence of GSR particle showed the bright area and dark area in backscattered electron (BSE) mode. The brightness of GSR particle illustrated the elements with a high atomic number, while the dark area presented element with low atomic number (Inset image Fig.3.). The results showed the distribution of GSR on 9-point area as shown in Table 2. The information from the Royal Thai Police and the commercial laboratory illustrated an amount of GSR is highest at the fifth point. The data were then compared by a paired t-test. The results of the distribution of GSR on 9-point area between the Royal Thai Police and the commercial laboratory were not statistically significantly different from the standard methods at the 95% confidence level (*t*-test value = 0.89, *t* critical value = 2.13). Therefore, the proposed method can be used for the investigation of GSR for interlaboratory comparison.

Table 2 Area distribution of GSR on stub surface

Sample of 1-round shooting	Position of 9-area sampling area								
	1	2	3	4	5	6	7	8	9
RTP	0	0	1	0	2	2	0	1	0
CL	0	0	0	0	2	0	0	1	0

RTP = The Royal Thai Police, CL = The commercial laboratory

The comparison between 1- and 3-round shooting indicated that an amount of GSR particles increase with increasing in the number of shooting as shown in Fig. 4.

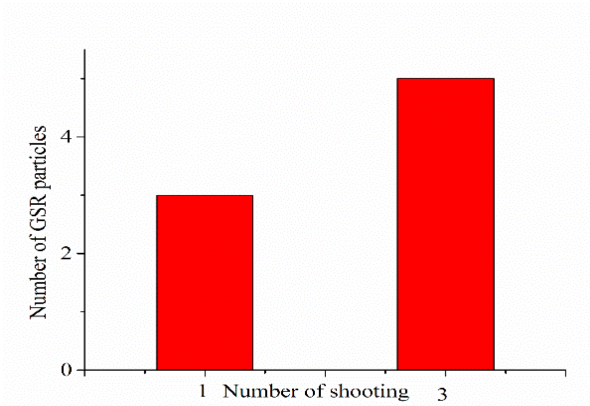

Fig.4. An amount of GSR from 1-and 3-round shooting

The measurement of Sb, Ba, and Pb by ICP-OES depended on the concentration of elements as shown in Fig. 5-7. These show that the intensity changed with the addition of different concentrations of Sb, Ba, and Pb. The maximal absorption at 206.833 nm of Sb, 455.403 nm of Ba, and 220.353 nm of Pb, which indicated the linearity of Sb, Ba, and Pb have a relationship with the concentration. The linear correlation existed between the intensity and the concentration of Sb ranging from 10 – 800 µg L^{-1} ($R^2 = 0.99982$) as shown in Fig. 5, Ba, and Pb ranging from 5 – 800 µg L^{-1} ($R^2 = 0.99985$ of Ba and $R^2 = 0.99990$ of Pb). The detection limit (LOD) of Sb, Ba, and Pb were found at 5, 4, and 1 µg L^{-1}, respectively, and the detection limit of quantity (LOQ) of Sb, Ba, and Pb were found at 10, 4 and 3 µg L^{-1}, respectively.

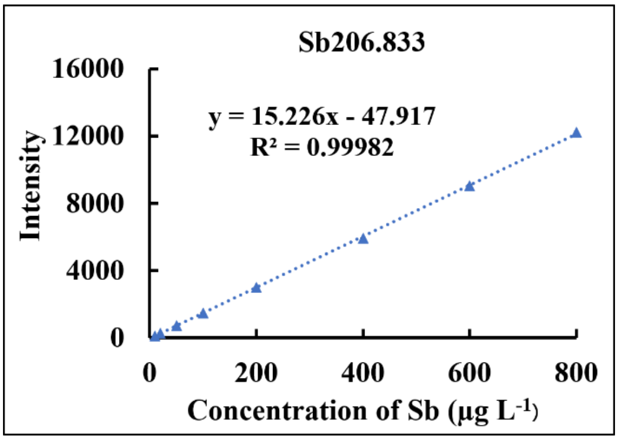

Fig.5. Calibration of Sb in the range from 10-800 µg L^{-1}

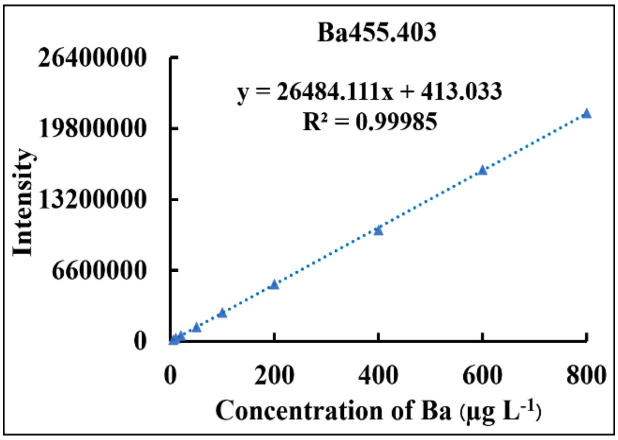

Fig.6. Calibration of Ba in the range from 5-800 μg L^{-1}

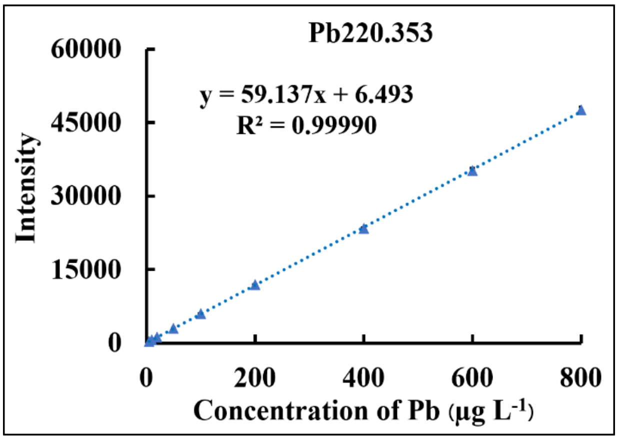

Fig.7. Calibration of Pb in the range from 5-800 μg L^{-1}

To validate the efficiency of the proposed for the determination of Sb, Ba, and Pb in GSR samples were analyzed to detect gunshot residues (GSR) from 130 Gr ammunition. A study to determine the best shooter hand region for collection right-hand back (RHB), right-hand palm (RHP), left-hand back (LHB), and left-hand palm (LHP) were analyzed by ICP-OES of the Royal Thai Police compared with ICP-MS of Central Institute of Forensic Science (CIFS) as shown in Table 3-5. The data were then compared by a paired *t*-test. The results were not statistically significant different from the standard methods at the 95% confidence level (*t*-test value = 0.81, *t* critical value = 2.36 of Sb, *t*-test value = 2.35, *t* critical value = 2.36 of Ba and *t*-test value = 2.21, *t* critical value = 2.36 of Pb). When the sensitivity was analyzed as a function of the hand region, the highest concentrations of the three elements (Sb, Ba, and Pb) were found mainly in the left-hand back (LHB), but the other three regions also found Sb, Ba, and Pb in GSR depends on the aptitude of the shooter. This difference may be explained by the presence of a larger number of folds in these hand regions, which can store greater amounts of GSR. This result indicates that the proposed method is sensitive for most cases because it can detect GSR produced by 5 shots.

Table 3 Sb concentration for 5 shots. The values in table are based on three measurements.

Sample	The concentration of Sb (μg L^{-1})	
	Central Institute of Forensic Science (CIFS)	The Royal Thai Police (RTP)
RHB_001	361.40	360.35
RHP_001	1152.00	1125.50
LHB_002	2053.00	2098.00
LHP_002	230.10	225.00
RHB_003	272.60	278.45
RHP_003	613.20	619.50
LHB_004	599.70	618.00
LHP_004	1319.00	1323

Table 4 Ba concentration for 5 shots. The values in table are based on three measurements.

Sample	The concentration of Ba (μg L^{-1})	
	Central Institute of Forensic Science (CIFS)	The Royal Thai Police (RTP)
RHB_001	6971.00	7140.00
RHP_001	14550.00	14780.00
LHB_002	27110.00	28230.00
LHP_002	4322.00	4275.00
RHB_003	3381.00	3399.00
RHP_003	6821.00	6950.00
LHB_004	4468.00	4640.00
LHP_004	14780.00	15420.00

Table 5 Pb concentration for 5 shots. The values in table are based on three measurements.

Sample	The concentration of Pb (μg L^{-1})	
	Central Institute of Forensic Science (CIFS)	The Royal Thai Police (RTP)
RHB_001	439.00	435.00
RHP_001	1222.00	1233.50
LHB_002	2641.00	2694.00
LHP_002	250.40	245.20
RHB_003	428.90	434.40
RHP_003	790.00	801.00
LHB_004	744.50	777.50
LHP_004	1225.00	1254.00

ANOVA results are showed in Table 6-8 corresponding to fit results expressed in Table 3-5. These data presented *F* value of Sb, Ba, and Pb: 0.000333, 0.001869, and

0.005355, respectively as F critical = 4.6001, thus indicating a regression form. Additionally, the determination of the maximum and minimum Sb, Ba, and Pb concentrations is important in the crime scene to identify as a gun shooter.

Table 6 ANOVA of Sb in GSR samples

Anova: Single Factor
SUMMARY (Sb)

Groups	*Count*	*Sum*	*Average*	*Variance*
Central Institute Forensic Science (CIFS)	8	6601	825.125	404077.8
The Royal Thai Police	8	6648	830.975	416891.9

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	136.89	1	136.89	0.000333	0.9857	4.6001
Within Groups	5746788.4	14	410485			
Total	5746925.2	15				

Table 7 ANOVA of Ba in GSR samples.

Anova: Single Factor
SUMMARY (Ba)

Groups	*Count*	*Sum*	*Average*	*Variance*
Central Institute Forensic Science (CIFS)	8	7741	967.6	585221.2
The Royal Thai Police	8	7875	984.325	612095.3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1118.9025	1	1118.9	0.001869	0.9661	4.6001
Within Groups	8381215.8	14	598658			
Total	8382334.7	15				

Table 8 ANOVA of Pb in GSR samples

Anova: Single Factor
SUMMARY (Pb)

Groups	*Count*	*Sum*	*Average*	*Variance*
Central Institute Forensic Science (CIFS)	8	82403	10300.4	65930850
The Royal Thai Police	8	84834	10604.3	72028183

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	369360.06	1	369360	0.005355	0.9427	4.6001
Within Groups	965713231	14	6.9E+07			
Total	966082591	15				

IV. CONCLUSION

The interlaboratory comparison between the Royal Thai Police and commercial laboratory for SEM technique and the Royal Thai Police and Central Institute of Forensic Science (CIFS) for ICP-OES technique presented similar results. The development of QC-sample and protocol for GSR investigation by SEM/EDS and ICP-OES was done. The statistical results from interlaboratory were not statistically significantly different from the standard methods. Thus, our research will be developed to international standards in the part of proficiency testing ISO/IEC 17043 is an essential element of laboratory quality assurance.

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