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Original Article

External Quality Assessment Scheme for Biological Monitoring of Occupational Exposure to Toxic Chemicals

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Objectives: In this study, we summarized the External Quality Assessment Scheme (EQAS) for the biological monitoring of occupational exposure to toxic chemicals which started in 1995 and continued until a 31st round robin in the spring of 2010. The program was performed twice per year until 2009, and this was changed to once a year since 2010. The objective of the program is to ensure the reliability of the data related to biological monitoring from analytical laboratories.
Methods: One hundred and eighteen laboratories participated in the 31st round robin. The program offers 5 items for inorganic analysis: lead in blood, cadmium in blood, manganese in blood, cadmium in urine, and mercury in urine. It also offers 10 items for organic analysis, including hippuric acid, methylhippuric acid, mandelic acid, phenylglyoxylic acid, N-methylformamide, N-methylacetamide, trichloroacetic acid, total trichloro-compounds, trans,trans-muconic acid, and 2,5-hexanedione in urine. Target values were determined by statistical analysis using consensus values. All the data, such as chromatograms and calibration curves, were reviewed by the committee.
Results: The proficiency rate was below 70% prior to the first round robin and improved to over 90% for common items, such as PbB and HA, while those for other items still remained in the range of 60-90% and need to be improved up to 90%.
Conclusion: The EQAS has taken a primary role in improving the reliability of analytical data. A total quality assurance scheme is suggested, including the validation of technical documentation for the whole analytical procedure.

Key Words: Quality assessment, Biological monitoring, Occupational exposure, Toxic chemicals

Introduction

Biological monitoring of chemical exposure in the workplace is a helpful tool for evaluating individual exposure and intake amounts in the field of occupational health. It can provide useful information for making a decision on the diagnosis of occupational disease caused by hazardous chemicals. Biological monitoring can be used for the evaluation of the magnitude of exposure against reference values or to compare the levels and trends of exposure in different regions or countries [1]. In the early 1980s, there were some case reports of cadmium and lead poisoning in Korea. But the workers and the factory owners argued on differences of analytical data from different laboratories. Experts agreed on the need of an analytical quality assurance program for biological monitoring to assure the reliability of analytical data in the field of occupational medicine [2]. In 1995, the Industrial Safety and Health Law was revised so that laboratories in clinics or hospitals that perform biological monitoring for workers should participate in the External Quality Assessment Scheme (EQAS) and qualify their analytical reliability.

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Some external quality assurance programs of biological monitoring in occupational and environmental health have been successfully performed in various countries [3-5]. Biological samples, such as blood and urine samples, are unstable and not homogeneous. It is hard to maintain homogeneity and stability of reference samples. Commercially available certified reference samples for biological monitoring of chemical exposure in the workplace were limited and expensive [6]. We found that "home made" samples prepared from human blood and urine in liquid form in a relatively simple manner could serve as the reference sample of the program after stability and homogeneity testing [7,8]. After successfully developing the reference samples for the program, the Korea Occupational Safety and Health Agency (KOSHA) was able to initiate the EQAS in 1995 [9].

In this study, the EQAS for biological monitoring of occupational exposure to toxic chemicals is summarized. The EQAS has been operated since 1995 by the KOSHA. In the 31st round robin that was performed in the spring of 2010, 118 laboratories in Korea participated in inorganic and/or organic analyses. The purpose of the program is to upgrade the capability of the analysis of biological samples and to ensure the quality of analytical data with respect to biological monitoring in the area of occupational medicine. The program is based on the Industrial Safety and Health Law, Chapter 43 by the Ministry of Employment and Labor (MOEL) [10]. By this law, laboratories that performed biological monitoring should enroll in the program and need to be qualified with the criteria designated by the program committee.

Materials and Methods

Operation of the program

The EQAS has been performed twice per year until 2009. The frequency of this program changed to once a year since 2010, which was mentioned in an amended regulation [10]. The operation schedule of the program was posted at http://oshri.kosha.or.kr/main. The general operation scheme of the program was reviewed and approved by the specialist committee. New participants could apply for the education program if they desired to do so. After receiving the samples, the participants should report the result within three weeks. Notification was sent to the participants within three weeks after the committee's review.

Table 1. Current set of analytical items and concentration ranges of reference samples

Item	Symbol	Exposure	Unit	Range	Analytical method
Lead in blood	PbB	Lead	μg/dL	10-80	AAS, ICP-MS
Cadmium in blood	CdB	Cadmium	μg/L	1-10	AAS, ICP-MS
Manganese in blood	MnB	Manganese	μg/L	10-60	AAS, ICP-MS
Mercury in urine	HgU	Mercury	μg/L	50-400	CVG-AAS
Cadmium in urine	CdU	Cadmium	μg/L	1-10	AAS, ICP-MS
Hippuric acid in urine	HA	Toluene	g/L	0.5-3.0	GC-FID, HPLC-UV
Mandelic acid in urine	MA	Styrene	g/L	0.1-1.5	GC-FID, HPLC-UV
Phenylglyoxylic acid in urine	PGA	Styrene	g/L	0.05-0.80	GC-FID, HPLC-UV
Methylhippuric acid in urine	mHA	Xylene	g/L	0.3-2.5	GC-FID, HPLC-UV
Muconic acid in urine	ttMA	Benzene	mg/L	0.5-5.0	HPLC-UV
Trichloroacetic acid in urine	TCA	Trichloroethylene	mg/L	10-200	GC-ECD, UV
Total Trichloro compd in urine	TCC	Trichloroethylene	mg/L	10-500	GC-ECD, UV
N-methylformamide in urine	NMF	Dimethylformamide	mg/L	5-50	GC-NPD
N-methylacetamide in urine	NMAC	Dimethylacetamide	mg/L	5-60	GC-NPD
2,5-hexanedione in urine	HD	n-Hexane	mg/L	1.5-10	GC-FID

AAS: atomic absorption spectrometer, ICP-MS: inductive coupled plasma mass spectrometer, CVG-AAS: cold vapor generation AAS, GC-FID: gas chromatograph with flame ionization detector, HPLC-UV: high performance liquid chromatograph with ultra violet detector, GC-ECD: gas chromatograph with electron capture detector, UV: violet detector, GC-NPD: gas chromatograph with nitrogen selective detector.

Table 2. Comparison of consensus and reference values for some organic and inorganic items

	Item	Unit	M_{con} ± SD	n	M_{ref} ± SD	n	Ratio	p-value*
Organic analysis	mHA1	(g/L)	0.69 ± 0.06	56	0.70 ± 0.03	9	0.99	0.88
	mHA3	(g/L)	0.49 ± 0.01	15	0.49 ± 0.01	9	1.00	1.00
	mHA4	(g/L)	1.19 ± 0.04	15	1.20 ± 0.03	9	0.99	0.90
	MA1	(g/L)	0.63 ± 0.03	19	0.62 ± 0.02	9	1.02	0.87
	MA2	(g/L)	1.29 ± 0.06	18	1.31 ± 0.05	9	0.98	0.83
	MA3	(g/L)	1.58 ± 0.08	50	1.55 ± 0.04	9	1.02	0.70
	PGA1	(g/L)	0.34 ± 0.01	12	0.34 ± 0.01	9	1.00	1.00
	PGA2	(g/L)	0.22 ± 0.01	9	0.22 ± 0.01	9	1.00	1.00
	PGA3	(g/L)	0.75 ± 0.04	38	0.76 ± 0.01	9	0.99	0.83
	NMF1	(mg/L)	66.6 ± 5.5	18	64.6 ± 4.9	9	1.03	0.04
	NMF2	(mg/L)	49.0 ± 4.3	9	49.1 ± 4.0	9	1.00	1.00
	NMF3	(mg/L)	36.4 ± 5.7	18	34.2 ± 4.6	9	1.06	0.03
	TCA1	(mg/L)	20.6 ± 1.8	13	21.1 ± 1.6	3	0.98	0.58
	TCA2	(mg/L)	13.9 ± 0.9	3	13.9 ± 0.9	3	1.00	1.00
	TCA3	(mg/L)	31.9 ± 2.9	13	33.4 ± 1.3	3	0.96	0.10
	TTC1	(mg/L)	36.1 ± 3.5	10	35.9 ± 1.8	3	1.01	0.84
	TTC2	(mg/L)	64.1 ± 2.1	3	64.1 ± 2.0	3	1.00	1.00
	TTC3	(mg/L)	87.9 ± 6.5	10	85.4 ± 2.4	3	1.03	0.06
	ttMA1	(mg/L)	2.6 ± 0.2	10	2.6 ± 0.2	4	1.00	1.00
	ttMA2	(mg/L)	4.3 ± 0.2	4	4.3 ± 0.2	4	1.00	1.00
	ttMA3	(mg/L)	5.5 ± 0.6	10	5.6 ± 0.2	4	0.98	0.77
	HD1	(mg/L)	6.5 ± 0.6	14	6.7 ± 0.4	6	0.97	0.56
	HD2	(mg/L)	3.3 ± 0.5	9	3.3 ± 0.5	9	1.00	1.00
	HD3	(mg/L)	4.4 ± 0.2	14	4.5 ± 0.4	9	0.98	0.69
Inorganic analysis	MnB1	(μg/dL)	3.02 ± 0.2	71	3.07 ± 0.17	8	0.98	0.75
	MnB2	(μg/dL)	3.87 ± 0.23	56	3.93 ± 0.33	8	0.98	0.79
	MnB3	(μg/dL)	4.74 ± 0.52	71	4.82 ± 0.17	8	0.98	0.64
	CdB1	(μg/dL)	0.49 ± 0.05	57	0.50 ± 0.06	8	0.98	0.92
	CdB2	(μg/dL)	0.65 ± 0.06	21	0.66 ± 0.08	8	0.98	0.93
	CdB3	(μg/dL)	0.82 ± 0.07	57	0.82 ± 0.05	8	1.00	1.00
	HgU1	(μg/L)	27.8 ± 4.1	17	28.4 ± 4.2	9	0.98	0.49
	HgU2	(μg/L)	72.4 ± 12	17	74.8 ± 12.7	9	0.97	0.12
	CdU1	(μg/L)	5.4 ± 0.9	19	5.7 ± 1.0	8	0.95	0.48
	CdU2	(μg/L)	11.4 ± 2	19	11.8 ± 2.3	8	0.97	0.54

SD: standard deviation, mHA: methylhippuric acid in urine, MA: mandelic acid in urine, PGA: phenylglyoxylic acid in urine, NMF: N-methylformamide in urine, TCA: trichloroacetic acid in urine, TCC: total trichloro-compounds in urine, ttMA: muconic acid in urine, HD: 2,5-hexanedione in urine, MnB: manganese in blood, CdB: cadmium in blood, HgU: mercury in urine, CdU: cadmium in urine, M_{con}: consensus value, mean value reported from participants, M_{ref}: mean value reported from reference laboratories.
*95% confidence level.

Preparation of reference samples

The program offers 5 items for heavy metals: lead in blood (PbB), cadmium in blood (CdB), manganese in blood (MnB), cadmium in urine (CdU), and mercury in urine (HgU). It also offers 10 items for organic metabolites, including hippuric acid (HA), methylhippuric acid (mHA), mandelic acid (MA), phenylglyoxylic acid (PGA), N-methylformamide (NMF), N-methylacetamide (NMAC), trichloroacetic acid (TCA), total trichloro-compounds (TTC), trans,trans-muconic acid (ttMA), and 2,5-hexanedione (HD) in urine. The concentration range of the reference samples was set between normal and biological threshold limit values. The current set of analytical items and concentration range is summarized in Table 1.

Donor blood was used to make the reference sample for metals in blood. A blood pool was prepared by repeating a cycle of freezing at -50°C and thawing three times. The reference samples were prepared by spiking a standard stock solution into the sample pools made from human blood or urine. The sample was divided into 2 mL or 5 mL cryogenic vials and stored in a deep freezer until further use. Homogeneity and stability testing was performed before adding new items to the program.

Determination of "target values" and tolerance range

The consensus values, which were determined by the statistical analysis of data from all participants, were used for the target value and compared with the average values from the reference laboratories. The outliers that were attributed to variances of over 10% from the mean value were eliminated until the variance reached below 10%. We operated 3-9 reference laboratories to check the consensus values, except for the values for PbB and HA, which maintained proficiency levels of over 90% with data and maintained coefficients of variance of less than 3%. So there was no issue with using consensus values as the target values for these two items. The reference laboratories were nominated by the committee among the laboratories that were previously proficient for four subsequent round robins for specific items.

Table 2 is an example of a comparison for the consensus and reference values. Most of the items such, as HA and PbB, used consensus values of participants as target values. These values were compared with the mean values from reference laboratories. In the case of NMF, which was not familiar to the participants and we did not have enough participants to calculate consensus values, we used the mean values from reference laboratories or the target values calculated from the added amount.

The accuracy was calculated as a numeric tool, such as mean % variance of the participants from the target values, for the comparison of analytical performance. The tolerance range was set to the mean value ± 15% for HA and PbB, and it was set to the mean value ± 2SD for the other items until the 20th round robin; the mean value ± 2SD or 3SD was used until the 30th round robin, and this was changed to the mean value ± 2SD or mean value ± 15%, whichever is wider, since the 31st round robin. In the case of the concentration of PbB being below 40 μg/dL, we applied the range of mean ±6 μg/dL from the 1st to 28th round robin.

Reviewing the chromatogram and calibration curves

All data, such as chromatograms and calibration curves, used to explain the analytical results were requested to be submitted and reviewed by the expert committee. Adequate advice from the committee after reviewing the data was very helpful for achieving the purpose of the program. We checked the documents to review the analytical conditions, calibration, processing of data, and reliability of the data. The importance of checking accuracy using known samples was emphasized.

Education for participants

The program included education for the participants. KOSHA provided the basic course of education for new participants. The course included the basic concept of the EQAS and the recommended analytical method for each item. All the education materials could be downloaded from the KOSHA website (http://oshri.kosha.or.kr). Participants can request on-site education.

Statistical analyses

The statistical analysis was performed by using SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA). Independent t-test or χ^2 test was performed to compare the mean concentrations between groups.

Results

Fig. 1 shows the number of participants participating in organic analysis. The program started with HA, which was the most popular and basic item for biological monitoring of organic solvents. From the questionnaire that was given out in 1998 to the analysts in occupational medicine, the most frequently analyzed items related with organic solvents were HA, MA, mHA, PGA, TTC, TCA, ttMA, NMF, HD, and so on. The program included these items in the program, and nine organic metabolites were analyzed in the program starting in 2007. The

number of participants has increased gradually for each newly added item. However, the number of participants for ttMA, NMF, and NMAC is still below 20. Fig. 2 shows the number of participants for inorganic analysis. Five items for four heavy metals were provided, but most of the participants selected PbB, CdB, and MnB, which were familiar to them. The number of participants analyzing HgU and CdU are still below 20, and labs need to be encouraged to analyze these items.

In case of the 31st round robin performed in the spring of 2010, the number of participants for the most common items, such as PbB and HA, was over one-hundred and fourteen and also the proficiency rates were over 90%. CdB, MnB, mHA, MA, and PGA have proficiency rates of over 80%. But, with respect to HgU, CdU, ttMA, TCA, TTC, NMF, NMAC, and HD, we still need to improve both of the number of participants and proficiency rates. Recently, some organic solvents, such as n-hexane, trichloroethylene, benzene, and N,N-dimethylformamide caused toxic health effect in some workers in Korea. Some cases of occupational disease caused by these solvents were reported in the field of occupational medicine [11]. For biological monitoring of these organic solvents, accurate analysis is essential. In the case of NMF, which is a major biological determinant of N,N-dimethylformamide, gas chromatography with nitrogen selective detection is applied to detect NMF sensitively and selectively. However, most labs still analyzed NMF by gas chromatography with flame ionization detection, which is universally installed in laboratories even though this is not selective for the detection of NMF.

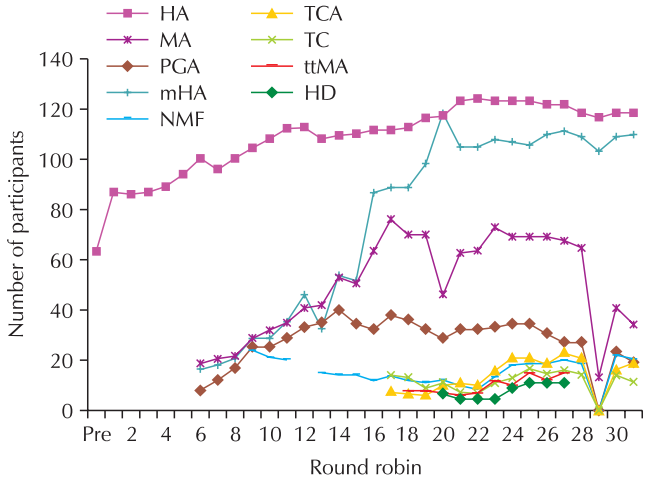

Fig. 1. Number of participants for organic analysis. HA: hippuric acid, MA: mandelic acid, PGA: phenylglyoxylic acid, mHA: methylhippuric acid, NMF: N-methylformamide, TCA: trichloroacetic acid, TC: trichloro-compounds, ttMA: t,t-muconic acid, HD: 2,5-hexanedione in urine.

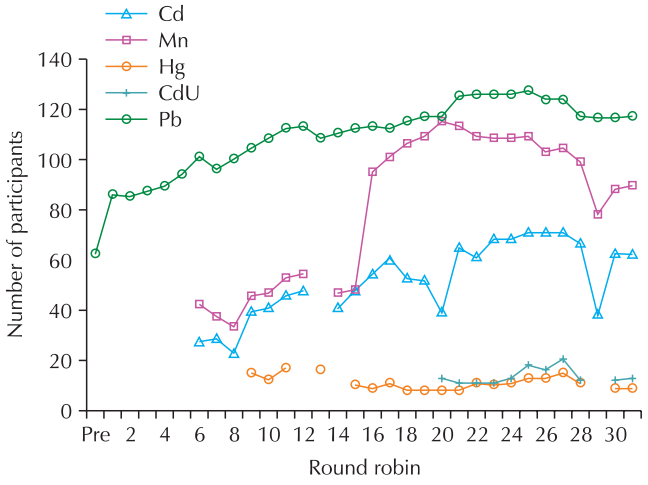

Fig. 2. Number of participants for inorganic analysis. Cd: cadmium in blood, Mn: manganese in blood, Hg: mercury in urine, CdU: cadmium in urine, Pb: lead in blood.

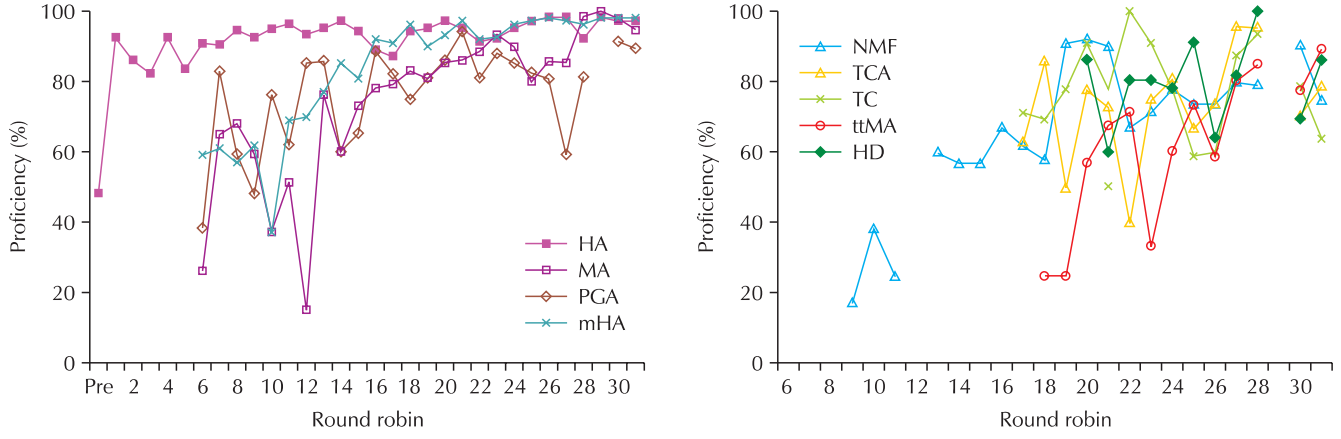

Fig. 3. Proficiency rate for organic analysis. HA: hippuric acid, MA: mandelic acid, PGA: phenylglyoxylic acid, mHA: methylhippuric acid, NMF: N-methylformamide, TCA: trichloroacetic acid, TC: trichloro-compounds, ttMA: t,t-muconic acid, HD: 2,5-hexanedione in urine.

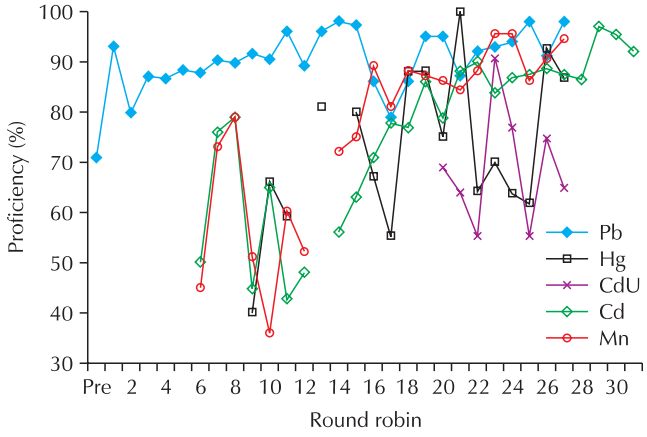

Fig. 4. Proficiency rate for inorganic analysis. Pb: lead in blood, Hg: mercury in urine, CdU: cadmium in urine, Cd: cadmium in blood, Mn: manganese in blood.

Fig. 3 and 4 shows the proficiency rates until the 31st round robin. It increased dramatically after the first round robin and slowly continued to increase up to 90% for HA and PbB. Other items indicate the tendency to increase, but some items, such as NMF, TCA, TTC, ttMA, and HD for organic analysis as well as CdU and HgU for inorganic analysis, indicate fluctuations. As the cases of toxicity related with these chemicals are increasing, improvement with respect to the accuracy of these analysis techniques is further required [11]. Except for the obligatory items and main organic acids, the proficiency rates of the optional items are low compared with the numbers of laboratories which are involved in the analysis of those items. So it is necessary to encourage the laboratories that actually perform the analyses of these specific items to participate in the program. Also we need to add more items to our program, especially the items that have been increasingly analyzed in the field of occupational medicine, like organic solvents in urine or blood.

Reviewing the documents that explain the analytical data, such as chromatograms and calibration curves, requires much time and effort. But it was very useful with respect to finding mistakes during calculations or experimental procedures. The review process helped the analysts to be better trained to make validated documentation with respect to laboratory records. During the first stage of the program, problems in documents were found in 10 to 15% of the participants in their documents. Now, the rate has decreased to 2 to 3%.

Discussion

The EQAS in the biological monitoring of occupational exposure to toxic chemicals started in 1995 with 2 items and now it has been expanded to 15 items for inorganic and organic analysis. The participants should pass the program for the mandatory items which were designated by the program committee. The two basic items, HA and PbB, which were most frequently analyzed in biological monitoring, have been chosen by KOSHA as mandatory items since 1995. Also, the participants can select at least one item for each inorganic and/or organic analysis. The participating organizations should pass the proficiency test for both mandatory and selective items to be qualified and to maintain their license for the health examination of workers [10]. This means if a laboratory failed in either mandatory or selective items, their analytical job would be waived for the fields that they failed until they pass the test at the next round robin. The participants were encouraged to apply for other items which were free from restrictions of law.

Most participants were designated for the health examination of workers by the Occupational Safety and Health Act by the MOEL. The participants belonged to regional clinics and hospitals (50%), non-profit organizations (34%) or research institutes (11%). Eighty percent of participant analysts had more than 2 years of job experience. Twenty percent of them had more than 10 years of experience.

In the early stage of the scheme, the percentage of participants that used UV spectrometer to measure hippuric acid in urine was 61%. Now in the 31st round robin, all of participants used gas chromatography (35%) or a liquid chromatography (65%) to measure hippuric acid. There has been no significant difference between the results of the two analytical methods. Hippuric acid can be quantified accurately by UV spectrometer if there are no metabolites from other organic solvents in urine samples [12]. However, chromatographic separation of the other metabolites is necessary if they co-exist in the sample in the form of organic acids. Gas chromatography or liquid chromatography can be used to analyze the mixture of metabolites of organic solvents with higher accuracy and precision than UV [13]. Now only the chromatographic method is used and there is no laboratory utilizing the UV method for analysis of hippuric acid. For inorganic analysis, 22% of laboratories changed their analytical method from the flame atomic absorption method, which required more than 10ml of blood, to the graphite furnace atomic absorption method. All of the participants except one that used ICP-MS analyzed heavy metals with atomic absorption spectrometers.

Now we suggest a total quality assurance system for the future direction of the external quality assurance scheme. The total quality assurance scheme will concentrate on the control of total quality of participants, including inter-laboratory qual-

ity assurance program and also validation of technical documentation for the whole analytical procedure. The protocol for the total quality assurance in compliance with the GLP guideline can be applied commonly in the analytical laboratories in occupational medicine. The total quality assurance program covers every detail relating to certifying the objective data, including the result of the inter-laboratory quality assurance program, personnel training, and the use of a validated analytical method. A laboratory accredited by this program must have a general reliability above a certain level, and admission to the program requires the organization to have a special ability to analyze specified items.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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References

1. Jakubowski M, Trzcinka-Ochocka M. Biological monitoring of exposure: trends and key developments. J Occup Health 2005;47:22-48.
2. Kang SK, Yang JS, Lee MY, Park IJ, Chung HK. Analysis of Korean analytical quality assurance program for the special health examination from 1995 to 1999. Korean J Occup Environ Med 2000;12:139-47.
3. Menditto A. Quality assurance in biological monitoring of environmental and occupational exposure to chemicals. Microchemical J 1996;54:252-61.
4. Valkonen S, Kallio A. Finnish quality assurance programme for biological monitoring of organic solvents. J Chromatogr B Analyt Technol Biomed Life Sci 2002;778:419-27.
5. Schaller KH, Angerer J, Drexler H. Quality assurance of biological monitoring in occupational and environmental medicine. J Chromatogr B Analyt Technol Biomed Life Sci 2002;778:403-17.
6. Menditto A, Palleschi S, Minoprio A, Rossi B, Calibotti A, Chiodo F, Patriarca M. Quality assurance in biological monitoring of environmental exposure to pollutants: from reference materials to external quality assessment schemes. Microchem J 2000;67:313-31.
7. Yang JS, Lee MY, Kang SK, Sohn DH, inventors; Korean Industrial Property Office, assignee. Preparation method of standard reference samples for the analyses of organic acid metabolites in urine. KR patent No. 165653. 1998.
8. Yang JS, Park IJ, Kang SK, Sohn DH, inventors; Korean Industrial Property Office, assignee. Preparation method of standard reference samples for the analyses of heavy metals in blood. KR patent No. 165621. 1998.
9. Yang JS, Lee MY, Park IJ, Moon YH, Kang SK. Korean analytical quality assurance (KAQUA) program for biological monitoring. Int Arch Occup Environ Health 1997;69:361-6.
10. Occupational Safety and Health Law Chapter 43 Section 9, Notice 2010-37. Ministry of Employment and Labor (Korea). 2010.
11. Kang SK, Kim EA. Occupational diseases in Korea. J Korean Med Sci 2010;25(Suppl):S4-12.
12. Inoue O, Kanno E, Kasai K, Ukai H, Okamoto S, Ikeda M. Benzylmercapturic acid is superior to hippuric acid and o-cresol as a urinary marker of occupational exposure to toluene. Toxicol Lett 2004;147:177-86.
13. Angerer J, Schaller KH. Analyses of hazardous substances in biological materials. Vol. 1. Weinheim (Germany): VCH; 1992. p. 165-75.