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Use of Liquid Chromatography-Tandem Mass Spectrometry for Clinical Testing in Korean Laboratories: a Questionnaire Survey

Hyojin Chae, M.D.[1], Sung-Eun Cho, M.D.[2], Hyung-Doo Park, M.D.[3], Sail Chun, M.D.[4], Yong-Wha Lee, M.D.[5], Yeo-Min Yun, M.D.[6], Sang-Hoon Song, M.D.[7], Sang-Guk Lee, M.D.[8], Kyunghoon Lee, M.D.[9], Junghan Song, M.D.[9], and Soo-Youn Lee, M.D.[3]; On behalf of the Clinical Mass Spectrometry Research Committee of Korean Society of Clinical Chemistry

[1]Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea; [2]LabGenomics Clinical Laboratories, Seongnam, Korea; [3]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [4]Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea; [5]Department of Laboratory Medicine and Genetics, Soonchunhyang University Bucheon Hospital, Soonchunhyang University College of Medicine, Bucheon, Korea; [6]Department of Laboratory Medicine, Konkuk University Medical Center, Konkuk University School of Medicine, Seoul, Korea; [7]Department of Laboratory Medicine, Seoul National University Hospital and College of Medicine, Seoul, Korea; [8]Department of Laboratory Medicine, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea; [9]Department of Laboratory Medicine, Seoul National University Bundang Hospital and College of Medicine, Seongnam, Korea

Background: The use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) has substantially increased in clinical laboratories worldwide. To assess the status of clinical LC-MS/MS testing in Korean laboratories, a questionnaire survey was performed by the Clinical Mass Spectrometry Research Committee of the Korean Society of Clinical Chemistry.

Methods: The questionnaire was distributed to 19 clinical laboratories performing clinical LC-MS/MS from April to May 2018. It asked about general characteristics of the laboratory and commonly utilized clinical LC-MS/MS tests: newborn screening, tacrolimus test, vitamin D test, and plasma metanephrine test. Frequency analysis and other statistical analyses were performed.

Results: A total of 17 laboratories responded. The median number of LC-MS/MS instruments, laboratory medicine physicians, and technicians in each laboratory was three, one, and two, respectively. Nine laboratory directors had >10 years of experience with clinical LC-MS/MS. For each LC-MS/MS test, at least two concentrations of QC materials were measured every 24 hours during clinical testing, and all laboratories used QC acceptability criteria based on their established QC means and SDs. All laboratories participated in an external quality assessment program. However, there was inter-laboratory variability in sample preparation methods, instruments, reagents, internal standards, and calibrators.

Conclusions: LC-MS/MS has been successfully introduced in Korean clinical laboratories and is used within a quality framework. Further efforts for harmonization on a nationwide basis could facilitate the widespread use of LC-MS/MS.

Key Words: Liquid chromatography-tandem mass spectrometry, Korea, Survey, Harmonization

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Corresponding author: Soo-Youn Lee, M.D.
Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea
Tel: +82-2-3410-1834
Fax: +82-2-3410-2719
E-mail: suddenbz@skku.edu

INTRODUCTION

Mass spectrometry (MS) represents a key technology in biomedical research areas, such as proteomics, pharmacology, and metabolomics [1]. With the advent of soft ionization techniques, such as electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI), the use of liquid chromatography-tandem MS (LC-MS/MS) has substantially increased in clinical laboratories [2, 3]. Current clinical applications of LC-MS/MS include screening for inherited metabolic disorders, measurement of numerous small-molecule biomarkers, and quantification of drugs and their metabolites [1]. The strengths of LC-MS/MS are its high selectivity and sensitivity, capability for multi-analyte analyses, and high throughput.

As an effort to popularize and improve the use of LC-MS/MS in clinical laboratories across Korea, a questionnaire survey was conducted by the Clinical Mass Spectrometry Research Committee (CMSRC) of the Korean Society of Clinical Chemistry (KSCC). It aimed to provide an accurate and updated overview of clinical LC-MS/MS testing in Korean clinical laboratories and shed light on the challenges of using LC-MS/MS in this setting.

METHODS

Survey population

The survey population consisted of all 19 clinical laboratories performing clinical LC-MS/MS tests in medical institutions (hospitals and medical centers) and referral medical laboratories accredited by the Korean Laboratory Accreditation Program (KOLAS) [4]. The survey was performed from April to May 2018. The questionnaire was e-mailed to the directors and laboratory physicians in charge of these 19 laboratories. This study was approved by the Institutional Review Board (IRB)/Ethics Committee of Seoul St. Mary's Hospital, Korea (IRB No. KC18QCDI0566).

Questionnaire

The questionnaire was in Korean, with multiple-choice questions, and consisted of two sections. The first comprised questions on the general characteristics of the LC-MS/MS laboratory: type of medical institution; number of LC-MS/MS instruments; number of LC-MS/MS laboratory physicians, technicians, and researchers; years of experience with clinical LC-MS/MS tests; clinical LC-MS/MS tests performed; laboratory area; and instrument management (nitrogen gas supply and preventive maintenance plans).

The second section comprised questions on four commonly utilized clinical diagnostic tests: newborn screening test (NST), tacrolimus test, vitamin D test, and plasma metanephrine test. The questionnaire asked about sample pretreatment and sample volume, test volume, turnaround time (TAT), testing frequency, calibrators, internal standards, reagents, internal quality control, test reporting, proficiency testing, and components of method validation.

Data analysis

An Excel spreadsheet was used to summarize the data. Categorical data were summarized as frequency and percentage,

Table 1. Clinical LC-MS/MS tests performed in 17 clinical laboratories in Korea

	Laboratories, N (%)
Newborn screening	
Amino acid/organic acid/fatty acid disorders	11 (65)
Galactosemia	2 (12)
Krabbe disease	1 (6)
Lysosomal storage disorders	3 (18)
Therapeutic drug monitoring	
Immunosuppressants	9 (53)
Anti-infective agents	6 (35)
Anticonvulsants	6 (35)
Antidepressants/antipsychotics	2 (12)
Anticancer agents	2 (12)
Amiodarone	1 (6)
Drugs of abuse	3 (18)
Metabolite/hormones	
5-HIAA	1 (6)
Metanephrines	7 (41)
Amino acids	3 (18)
Acylcarnitines	2 (12)
Carnitine	3 (18)
Homocysteine	4 (24)
Methylmalonic acid	1 (6)
Citrate	1 (6)
Oxalate	1 (6)
Steroid profile	1 (6)
Cortisol	1 (6)
Catecholamines	2 (12)
Aldosterone	2 (12)
Vitamin D	9 (53)

Abbreviations: LC-MS/MS, liquid chromatography-tandem mass spectrometry; HIAA, hydroxyindoleacetic acid.

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and continuous data were summarized as median and range. Normality was assessed using the D'Agostino and Pearson normality test. The association between the types of the clinical laboratory and TAT or test volume was evaluated using Fisher's exact tests. The correlation between the number of LC-MS/MS instruments and the number of laboratory medicine physicians, the number of laboratory technicians, and the number of clinical LC-MS/MS tests was assessed using Pearson's correlation coefficient. GraphPad Prism 7.05 (GraphPad Software, San Diego, CA, USA) was used for statistical analyses. $P<0.05$ was considered statistically significant.

RESULTS

Laboratory characteristics

Seventeen laboratories (11 university hospitals and six referral medical laboratories) responded. The median number of LC-MS/MS instruments was three (range, 1–10). The median number of laboratory medicine physicians was one (range, 1–3), and the median number of laboratory technicians was two (range, 1–5). Nine laboratory directors had >10 years and 35% had 5–10 years of experience with clinical LC-MS/MS tests (for a list of tests, see Table 1).

When the general characteristics of LC-MS/MS clinical laboratories were compared according to the type of institution, the median number of LC-MS/MS instruments was higher in referral medical laboratories than university hospitals ($P=0.0300$). Referral medical laboratories performed more NSTs ($P=0.0022$) and tended to perform more tacrolimus tests ($P=0.0833$) than university hospitals. Referral medical laboratories tended to have a faster TAT for NST than university hospitals ($P=0.0606$). The number of LC-MS/MS instruments moderately correlated with the number of laboratory medicine physicians ($r=0.5482$, $P=0.0227$), strongly correlated with the number of laboratory tech-

Table 2. Laboratory operation/management issues

		Laboratories, N (%)			
		NST (N=11)	Tacrolimus (N=9)	Vitamin D (N=9)	Plasma metanephrines (N=6)
Test volume (per month)	<50	5 (45)	2 (22)	2 (22)	1 (17)
	50–100		1 (11)	0	1 (17)
	100–200		3 (33)	3 (33)	1 (17)
	200–500		1 (11)	2 (22)	2 (33)
	500–2,500		2 (22)	2 (22)	1 (17)
	2,500–5,000	3 (27)	0	0	0
	5,000≤	3 (27)	0	0	0
Batch size	<10	NA	2 (22)	2 (22)	0
	10–20		3 (33)	1 (11)	2 (33)
	20–50		3 (33)	3 (33)	1 (17)
	50≤		1 (11)	3 (33)	3 (50)
TAT	1 day	0	4 (44)	0	0
	<3 days	8 (72)	5 (55)	8 (88)	5 (83)
	<7 days	3 (27)	0	0	1 (17)
Testing frequency	Daily	6 (55)	6 (66)	3 (33)	0
	2–4/week	4 (36)	3 (33)	4 (44)	6 (100)
	Once/week	1 (9)	0	2 (22)	0
	Other	0	Weekends 5 (55)	0	0
Test order and reporting	Manual	3 (27)	6 (67)	6 (67)	5 (83)
	Unidirectional interface	8 (73)	3 (33)	3 (33)	1 (17)
Type of reporting	Numerical report	0	9 (100)	8 (88)	6 (100)
	Text report	11 (100)	3 (33)	2 (22)	1 (17)

Abbreviations: NST, newborn screening test; TAT, turnaround time; NA, not available.

nicians (r=0.6793, *P*=0.0027), and strongly correlated with the number of clinical MS tests (r=0.7146, *P*=0.0013).

LC-MS/MS tests

Tables 2 and 3 summarize responses on test-specific laboratory operational/management issues and methodological issues, respectively.

NSTs represented the highest volume of LC-MS/MS tests, with over half of the laboratories performing >2,500 tests per month. For tacrolimus, vitamin D, and plasma metanephrine tests, over half of the laboratories performed <200 tests per month. Over half reported a target TAT of <72 hours for all four tests (Table 2).

All laboratories used a stable isotope-labeled internal standard for the vitamin D and plasma metanephrine tests. However, for tacrolimus testing, 78% of the laboratories used a non-isotopic internal standard, ascomycin. The majority of laboratories used

Table 3. Test-specific methodological issues

		Laboratories, N (%)			
		NST (N=11)	Tacrolimus (N=9)	Vitamin D (N=9)	Plasma metanephrines (N=6)
Sample pretreatment		Derivatization 6 (55) Non-derivatized 5 (45)	Protein precipitation with acetonitrile 8 (89) Protein precipitation with methanol 1 (11)	Solid phase extraction 1 (11) Liquid-liquid extraction 6 (67) Protein precipitation 3 (33)	Solid phase extraction 5 (83) Protein precipitation 1 (17)
Type of reagent	Prepared in-house	7 (64)	0	3 (33)	5 (83)
	Commercially purchased	5 (45)	9 (100)	3 (33)	
	Both			3 (33)	1 (17)
Type of internal standard	Isotope-labeled	NA	2 (22)	9 (100)	6 (100)
	Non-isotope-labeled	NA	7 (78)	0	0
Type of calibration material	Commercially purchased	NA	9 (100)	8 (89)	0
	Prepared in-house	NA	0	1 (11)	6 (100)
Type of matrix	Matrix-matched	NA	9 (100)	9 (100)	4 (66)
	Matrix-unmatched	NA	0	0	2 (33)
Calibrators, N	3,4	NA	0	2 (22)	2 (33)
	5,6	NA	7 (78)	2 (22)	2 (33)
	>6	NA	2 (22)	5 (56)	2 (33)
Type of QC material	Commercially purchased	6 (55)	9 (100)	9 (100)	4 (66)
	Prepared in-house	1 (9)	0	0	2 (33)
	Others	CDC 7 (64)			
Concentrations of QC material [median (range)]		AA 2 (2–4) AC 2 (2–4)	3 (3–4)	2 (2–3)	2 (2–3)
QC frequency	per plate	55 (6)	1 (11)	2 (22)	1 (17)
	per run	0	2 (22)	3 (33)	1 (17)
	daily	5 (45)	6 (67)	4 (45)	4 (66)
Participating EQA programs	KEQAS	11 (100)	9 (100)	5 (63)	6 (100)
	CDC	11 (100)	NA	1 (13)	NA
	CAP	0	5 (56)	8 (100)	1 (17)
	Others	PPFK 10 (92)		DEQAS 2 (25) VitDQAP 1 (13)	RCPA 2 (33)

Abbreviations: NST, newborn screening test; NA, not available; CDC, Centers for Disease Control and Prevention; AA, amino acids; AC, acylcarnitines; KEQAS, Korean Association of External Quality Assessment Service; PPFK, Planned Population Federation of Korea; CAP, College of American Pathologists; DEQAS, Vitamin D External Quality Assessment Scheme; VitDQAP, Vitamin D Metabolites Quality Assurance Program; RCPA, The Royal College of Pathologists of Australasia; EQA, external quality assessment.

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Table 4. Components of method validation

	Laboratories, N (%)			
	NST (N=9)	Tacrolimus (N=8)	Vitamin D (N=9)	Plasma metanephrines (N=6)
Accuracy	6 (67)	7 (88)	9 (100)	6 (100)
Precision	9 (100)	8 (100)	9 (100)	6 (100)
Linearity	5 (56)	8 (100)	9 (100)	6 (100)
Comparison	6 (67)	8 (100)	9 (100)	4 (67)
Carry-over	6 (67)	7 (88)	9 (100)	6 (100)
Recovery	4 (44)	7 (88)	8 (89)	6 (100)
Limit of detection	3 (33)	3 (38)	4 (44)	3 (50)
Limit of quantification	2 (22)	8 (100)	8 (89)	6 (100)
Ion suppression and matrix effect	2 (22)	7 (88)	6 (67)	5 (83)
Batch size	1 (11)	1 (13)	1 (11)	1 (17)
SST	0	1 (13)	2 (22)	1 (17)
Interferences	0	3 (38)	3 (33)	2 (33)
Stability	0	6 (75)	2 (22)	3 (50)

Abbreviations: SST, system suitability test; NST, newborn screening test.

commercial calibrators for the tacrolimus (100%) and vitamin D (89%) tests. However, all laboratories used in-house prepared calibrators for the plasma metanephrine test. The majority of laboratories used matrix-appropriate calibrators for the tacrolimus (100%), vitamin D (100%), and plasma metanephrine (66%) tests (Table 3).

The majority of laboratories used commercially available QC materials for all four tests. A median of two or three concentrations of QC materials was used, and the most common frequency of QC analysis was "per plate" for the NST (55%) and "per day" for the tacrolimus (67%), vitamin D (45%), and plasma metanephrine (66%) tests. The majority of laboratories used mean±3 SDs as the QC acceptability criterion (66%) for the NST, while mean±2 SDs was the most commonly used QC acceptability criterion for the tacrolimus (83%), vitamin D (66%), and plasma metanephrine (66%) tests. The majority of laboratories participated in the Korean Association of External Quality Assessment Service (KEQAS) external quality assessment (EQA) scheme, a national laboratory PT scheme, as well as other test-specific global EQA schemes. In addition, the majority of laboratories validated their tests for accuracy, precision, linearity, method comparison, carry-over, recovery, lower limit of quantification, ion suppression, and matrix effect (Table 4).

DISCUSSION

This survey summarizes the current practices and characteristics of clinical LC-MS/MS laboratories in Korea. It shows that LC-MS/MS has been successfully introduced in many clinical laboratories, with major applications in newborn screening, therapeutic drug monitoring, endocrinology, and nutritional assessment. The expansion and integration of LC-MS/MS into clinical laboratories are reflected in its proportion in EQA programs and in the fact that 7% and 2% of all participating laboratories in the 2018 KEQAS EQA scheme use LC-MS/MS for tacrolimus and vitamin D testing, respectively.

However, the number of clinical LC-MS/MS laboratories in Korea (19) is still small, only 6% of the total number of clinical laboratories accredited by the KOLAS (305). Further, clinical LC-MS/MS laboratories are limited to university hospitals (65%) and large referral medical laboratories (35%). The proportion of LC-MS/MS in Korean EQA programs is lower than that in global EQA programs (17% and 10% of all participating laboratories for tacrolimus and vitamin D tests, respectively) [5]. Therefore, considering the global growth of clinical LC-MS/MS tests, there is still room for further expansion of LC-MS/MS in Korea.

The original sample preparation methods for the LC-MS/MS NST used butyl esterification (derivatization). However, with the improved sensitivity of MS instruments, it is possible to detect amino acids and acylcarnitines as their native free acids (non-derivatized). We found that the proportion of derivatization and non-derivatized sample preparation methods used was similar, in accordance with recent CDC NST QC data summaries [6]. Protein precipitation is the most commonly used method for extracting immunosuppressants because it is simple, cheap, and less time-consuming [7]. For the vitamin D tests, 67% and 33% of all laboratories used liquid-liquid extraction and protein precipitation methods, respectively, similar to the proportions reported in the vitamin D EQA Scheme (DEQAS) data [8]. To analyze plasma metanephrines, solid phase extraction has been the sample preparation method of choice [9]. In the present survey, all but one laboratory used solid phase extraction for sample preparation.

The addition of an internal standard to samples before analysis represents the single most valuable method enhancement that MS can offer [10]. All responding laboratories used methods that included the use of an internal standard. The majority of laboratories used a stable isotope-labeled form of the measured analyte. However, for tacrolimus, a non-isotopic internal standard, ascomycin, was used by the majority of laboratories.

Although a recent study did not observe any differences between the use of ascomycin and an isotope-labeled internal standard, tacrolimus-$^{13}C,D_2$ [11], other studies reported better results using tacrolimus-$^{13}C,D_2$ than ascomycin [12]. With the recent availability of commercial isotope-labeled internal standards for immunosuppressants (e.g., Chromsystems, Munich, Germany, or Recipe, Munich, Germany), the use of isotope-labeled internal standards in immunosuppressant LC-MS/MS tests is likely to increase in the near future.

Regarding quality assurance and QC for LC-MS/MS, the CLSI document C62-A suggests that a minimum of two concentrations of QC materials should be measured at least every 24 hours during patient testing [10]. We found that at least two concentrations of QC materials were measured for each LC-MS/MS test, and all laboratories were using QC acceptability criteria based on their own established QC means and SDs derived from repetitive analysis of control materials. All laboratories reported participation in one or more EQA programs. As EQA is one of the most preferred methods for assessing test accuracy, these laboratories reported using LC-MS/MS within a quality framework that addresses both internal and external quality assurance.

One of the potential obstacles preventing the adoption of LC-MS/MS in routine clinical laboratories is the seemingly overwhelming method validation requirements required to meet the guidelines for clinical applications. Although there are multiple guidelines for the validation of bioanalytical methods [13-15], including the CLSI C62-A [10], regulatory requirements differ somewhat across countries and laboratory accreditation agencies. In this regard, the development of regional/national guidelines for LC-MS/MS validation for clinical laboratories that address regional regulations could facilitate the use of LC-MS/MS in medium-sized community hospitals and regional clinical laboratories in Korea.

This study has some limitations. Because this survey was an initiative of the CMSRC of the KSCC, it focused primarily on LC-MS/MS testing in clinical chemistry applications. Thus, other areas of clinical MS, such as gas chromatography-MS, inductively coupled plasma MS, and matrix-assisted laser desorption ionization time-of-flight MS (MALDI-TOF MS), in clinical microbiology were not assessed. Another limitation was the fact that the number of responding laboratories was relatively low, making it difficult to draw definitive conclusions.

However, this survey is the first to describe various aspects of LC-MS/MS in clinical laboratories in Korea. Furthermore, it is important to understand the current status of clinical LC-MS/MS laboratory practices to form a basis upon which future harmonization and advancements can be made. Further studies attempting to harmonize and enhance the analytical robustness of LC-MS methods on a nationwide basis will be necessary.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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ORCID

Hyojin Chae https://orcid.org/0000-0001-5870-1887
Sung-Eun Cho https://orcid.org/0000-0001-8082-3100
Hyung-Doo Park https://orcid.org/0000-0003-1798-773X
Sail Chun https://orcid.org/0000-0002-5792-973X
Yong-Wha Lee https://orcid.org/0000-0002-4459-6286
Yeo-Min Yun https://orcid.org/0000-0002-5485-8331
Sang-Hoon Song https://orcid.org/0000-0002-5084-1137
Sang-Guk Lee https://orcid.org/0000-0003-3862-3660
Kyunghoon Lee https://orcid.org/0000-0002-3154-0347
Junghan Song https://orcid.org/0000-0003-0576-9938
Soo-Youn Lee https://orcid.org/0000-0001-7595-4042

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