Report on the External Quality Assessment Scheme for Molecular Diagnostics in Korea (2017)

Man Jin Kim, Mi-Hye Yoon, Ji Yun Song, Sung Im Cho, Sung-Sup Park, and Moon-Woo Seong

Department of Laboratory Medicine, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea

Corresponding author:

Moon-Woo Seong
Department of Laboratory Medicine, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea
Tel: +82-2-2072-4180
Fax: +82-2-747-0359
E-mail: MWSeong@snu.ac.kr

pISSN: 2384-2458
eISSN: 2288-7261

Quality control for genetic analysis has become more important with a drastic increase in testing volume and clinical demands. The molecular diagnostics division of the Korean Association of Quality Assurance for Clinical Laboratory conducted two trials in 2017 on the basis of molecular diagnostics surveys, involving 53 laboratories. The molecular diagnostics surveys included 37 tests: gene rearrangement tests for leukemia (*BCR-ABL1*, *PML-RARA*, *AML1-ETO*, and *TEL-AML1*), genetic tests for Janus kinase 2, FMS-like tyrosine kinase 3-internal tandem duplication, FMS-like tyrosine kinase 3-tyrosine kinase domain, nucleophosmin, cancer-associated genes (*KRAS*, *EGFR*, *KIT*, and *BRAF*), hereditary breast and ovarian cancer genes (*BRCA1* and *BRCA2*), Li-Fraumeni syndrome (*TP53*), Wilson disease (*ATP7B*), achondroplasia (*FGFR3*), hearing loss and deafness (*GJB2*), Avellino (*TGFBI*), multiple endocrine neoplasia 2 (*RET*), Huntington disease, spinocerebellar ataxia, spinal and bulbar muscular atrophy, mitochondrial encephalopathy with lactic acidosis and stroke-like episodes, myoclonic epilepsy ragged red fibre, Leber hereditary optic neuropathy, Prader-raderd Angelman syndrome, Duchenne muscular dystrophy, spinal muscular atrophy, fragile X syndrome, apolipoprotein E genotyping, methylenetetrahydrofolate reductase genotyping, and *ABO* genotyping. Molecular genetic surveys revealed excellent results for most participants. The external quality assessment program for genetic analysis in 2017 proved useful for continuous education and the evaluation of quality improvement.

(J Lab Med Qual Assur 2018;40:199-210)

Key Words: Laboratory proficiency testing, Molecular pathology, Molecular genetics

서론

2017년도 분자진단검사 신빙도조사는 전체 회원기관에게 참가 신청조사를 하여 총 2회 시행하였고 회차별 37종목에 각 2종의 검체를 참여기관에 배포하여 다양한 돌연변이 및 유전 질환을 포함하였다.

재료 및 방법

1. 분자진단검사

1차 신빙도조사는 2017년 4월 17일에 검체를 발송하고 2018년 1월 10일에 결과를 통보하였고, 2차 신빙도조사는 2017년 9월 19일에 검체를 발송하고 2018년 2월 10일에 결과를 통보하였다. 신빙도조사 물질의 회차별 검체와 검체번호, 검체구성은 Table 1과 같다.

각 기관의 보고에 따라 검사방법, 검사결과 및 검사방법별 검사결과를 비교 분석하였으며 기관별 보고결과에 대하여 기본적인 평가(acceptable, unacceptable)를 실시하였다. 합격/불합격의 기준은 10기관 미만의 참여항목의 경우 주관기관의 검증결과와 일치하는 경우 acceptable이라고 판정하였으며 10기관 이상의 참여항목의 경우 주관기관의 검증결과와 참여기관 중 80% 이상의 일치가 있을 경우 acceptable이라고 판정하였다. 삼염기 반복 유전질환인 Huntington disease (HD), spinocerebellar ataxia (SCA1, SCA2, SCA3, SCA6, SCA7), spinal and bulbar muscular atrophy (SBMA), fragile X syndrome (*FMR1*) 검사의 정량값은 평가에 포함시키지 않았다.

Table 1. Survey tests and specimens for external quality assessment of the molecular diagnostics test survey in 2017

Tests	Specimens	Test specimens of 1st trial	Specimens	Test specimens of 2nd trial
Leukemia (<i>BCR-ABL1</i>)	GH 17-01	Normal cell line	GH 17-11	Leukemia cell line (<i>BCR/ABL1</i> positive)
	GH 17-02	Normal cell line	GH 17-12	Normal cell line
Leukemia (<i>PML-RARA</i>)	GH 17-01	Normal cell line	GH 17-11	Normal cell line
	GH 17-02	Leukemia cell line (<i>PML/RARA</i> positive)	GH 17-12	Normal cell line
Leukemia (<i>AML1-ETO</i>)	GH 17-01	Normal cell line	GH 17-11	Normal cell line
	GH 17-02	Normal cell line	GH 17-12	Normal cell line
Leukemia (<i>TEL-AML1</i>)	GH 17-01	Leukemia cell line (<i>TEL/AML1</i> positive)	GH 17-11	Normal cell line
	GH 17-02	Normal cell line	GH 17-12	Leukemia cell line (<i>TEL/AML1</i> positive)
<i>JAK2</i>	GH 17-03	Extracted DNA with <i>JAK2</i> mutation	GH 17-13	Normal DNA
	GH 17-04	Normal DNA	GH 17-14	Extracted DNA with <i>JAK2</i> mutation
<i>FLT3</i> -ITD	GH 17-05	Normal DNA	GH 17-15	Normal DNA
	GH 17-06	Extracted DNA with <i>FLT3</i> -ITD mutation	GH 17-16	Extracted DNA with <i>FLT3</i> -ITD mutation
<i>FLT3</i> -TKD	GH 17-07	Extracted DNA with <i>FLT3</i> -TKD mutation	GH 17-17	Extracted DNA with <i>FLT3</i> -TKD mutation
	GH 17-08	Normal DNA	GH 17-18	Normal DNA
<i>NPM1</i>	GH 17-09	Extracted DNA with <i>NPM1</i> mutation	GH 17-19	Normal DNA
	GH 17-10	Normal DNA	GH 17-20	Extracted DNA with <i>NPM1</i> mutation
<i>KRAS</i>	GS 17-01	Extracted DNA with <i>KRAS</i> mutation	GS 17-09	Normal DNA
	GS 17-02	Normal DNA	GS 17-10	Extracted DNA with <i>KRAS</i> mutation
<i>EGFR</i>	GS 17-03	Extracted DNA with <i>EGFR</i> mutation	GS 17-11	Extracted DNA with <i>EGFR</i> mutation
	GS 17-04	Normal DNA	GS 17-12	Normal DNA
<i>BRAF</i>	GS 17-05	Normal DNA	GS 17-13	Extracted DNA with <i>BRAF</i> mutation
	GS 17-06	Extracted DNA with <i>BRAF</i> mutation	GS 17-14	Normal DNA
<i>KIT</i>	GS 17-07	Normal DNA	GS 17-15	Normal DNA
	GS 17-08	Extracted DNA with <i>KIT</i> mutation	GS 17-16	Extracted DNA with <i>KIT</i> mutation
<i>BRCA1</i>	GG 17-01	Extracted DNA with <i>BRCA1</i> mutation	GG 17-41	Normal DNA
	GG 17-02	Normal DNA	GG 17-42	Extracted DNA with <i>BRCA1</i> mutation
<i>BRCA2</i>	GG 17-03	Normal DNA	GG 17-43	Extracted DNA with <i>BRCA2</i> mutation
	GG 17-04	Extracted DNA with <i>BRCA2</i> mutation	GG 17-44	Normal DNA
<i>TP53</i>	GG 17-05	Extracted DNA with <i>TP53</i> mutation	GG 17-45	Normal DNA
	GG 17-06	Normal DNA	GG 17-46	Extracted DNA with <i>TP53</i> mutation
<i>ATP7B</i>	GG 17-07	Extracted DNA with <i>ATP7B</i> mutation	GG 17-47	Extracted DNA with <i>ATP7B</i> mutation
	GG 17-08	Normal DNA	GG 17-48	Normal DNA
MELAS	GG 17-09	Extracted DNA with MELAS mutation	GG 17-49	Normal DNA
	GG 17-10	Normal DNA	GG 17-50	Extracted DNA with MELAS mutation
MERRF	GG 17-11	Normal DNA	GG 17-51	Extracted DNA with MERRF mutation
	GG 17-12	Extracted DNA with MERRF mutation	GG 17-52	Normal DNA
<i>GJB2</i>	GG 17-13	Extracted DNA with <i>GJB2</i> variant	GG 17-53	Extracted DNA with <i>GJB2</i> variant
	GG 17-14	Normal DNA	GG 17-54	Normal DNA
LHON	GG 17-15	Extracted DNA with LHON mutation	GG 17-55	Extracted DNA with LHON mutation
	GG 17-16	Normal DNA	GG 17-56	Normal DNA

(Continued to the next page)

Table 1. Continued

Tests	Specimens	Test specimens of 1st trial	Specimens	Test specimens of 2nd trial
<i>RET</i>	GG 17-17	Extracted DNA with <i>RET</i> mutation	GG 17-57	Extracted DNA with <i>RET</i> mutation
	GG 17-18	Normal DNA	GG 17-58	Normal DNA
SCA1	GG 17-19	Normal DNA	GG 17-59	Normal DNA
	GG 17-20	Extracted DNA with SCA1 mutation	GG 17-60	Normal DNA
SCA2	GG 17-19	Normal DNA	GG 17-59	Normal DNA
	GG 17-20	Normal DNA	GG 17-60	Normal DNA
SCA3	GG 17-19	Normal DNA	GG 17-59	Normal DNA
	GG 17-20	Normal DNA	GG 17-60	Normal DNA
SCA6	GG 17-19	Normal DNA	GG 17-59	Extracted DNA with SCA6 mutation
	GG 17-20	Normal DNA	GG 17-60	Normal DNA
SCA7	GG 17-19	Normal DNA	GG 17-59	Normal DNA
	GG 17-20	Normal DNA	GG 17-60	Normal DNA
APOE genotype	GG 17-21	Extracted DNA with APOE $\epsilon 3/\epsilon 3$ genotype	GG 17-61	Extracted DNA with APOE $\epsilon 4/\epsilon 4$ genotype
	GG 17-22	Extracted DNA with APOE $\epsilon 4/\epsilon 4$ genotype	GG 17-62	Extracted DNA with APOE $\epsilon 3/\epsilon 4$ genotype
Achondroplasia	GG 17-23	Normal DNA	GG 17-63	Extracted DNA with <i>FGFR3</i> mutation
	GG 17-24	Extracted DNA with <i>FGFR3</i> mutation	GG 17-64	Normal DNA
MTHFR genotype	GG 17-25	Extracted DNA with <i>MTHFR</i> 677T/T homozygote	GG 17-65	Extracted DNA with <i>MTHFR</i> 677C/T heterozygous
	GG 17-26	Normal DNA	GG 17-66	Extracted DNA with <i>MTHFR</i> 677T/T homozygote
PWS/AS	GG 17-27	Normal DNA	GG 17-67	Extracted DNA with PWS mutation
	GG 17-28	Extracted DNA with AS mutation	GG 17-68	Normal DNA
DMD	GG 17-29	Normal DNA	GG 17-69	Extracted DNA with <i>DMD</i> mutation
	GG 17-30	Extracted DNA with <i>DMD</i> mutation	GG 17-70	Normal DNA
HD	GG 17-31	Extracted DNA with HD mutation	GG 17-71	Extracted DNA with HD mutation
	GG 17-32	Normal DNA	GG 17-72	Normal DNA
<i>FMRI</i>	GG 17-33	Normal DNA	GG 17-73	Normal DNA
	GG 17-34	Extracted DNA with <i>FMRI</i> mutation	GG 17-74	Extracted DNA with <i>FMRI</i> mutation
<i>TGFBI</i>	GG 17-35	Extracted DNA with <i>TGFBI</i> mutation	GG 17-75	Normal DNA
	GG 17-36	Normal DNA	GG 17-76	Extracted DNA with <i>TGFBI</i> mutation
SBMA	GG 17-37	Normal DNA	GG 17-77	Normal DNA
	GG 17-38	Extracted DNA with <i>AR</i> mutation	GG 17-78	Extracted DNA with <i>AR</i> mutation
SMA	GG 17-39	Extracted DNA with <i>SMN1</i> mutation	GG 17-79	Extracted DNA with <i>SMN1</i> mutation
	GG 17-40	Normal DNA	GG 17-80	Normal DNA
ABO genotype	GO 17-01	Extracted DNA with <i>cis-AB/B</i> genotype	GO 17-03	Extracted DNA with <i>B/O</i> genotype
	GO 17-02	Extracted DNA with <i>A/O</i> genotype	GO 17-04	Extracted DNA with <i>cis-AB/A</i> genotype

Abbreviations: *JAK2*, Janus kinase 2; *FLT3*, FMS-like tyrosine kinase 3; ITD, internal tandem duplication; TKD, tyrosine kinase domain; *NPM1*, nucleophosmin; *EGFR*, epidermal growth factor receptor; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibres; LHON, Leber hereditary optic neuropathy; *RET*, multiple endocrine neoplasia 2; SCA, spinocerebellar ataxia; *APOE*, apolipoprotein E; *MTHFR*, methylenetetrahydrofolate reductase; PWS/AS, Prader-Willi/Angelman syndrome; DMD, Duchenne muscular dystrophy; HD, Huntington disease; *FMRI*, fragile X mental retardation-1; *TGFBI*, transforming growth factor beta induced; SBMA, spinal and bulbar muscular atrophy; SMA, spinal muscular atrophy.

결과

1. 분자진단검사

1) 참여기관 및 검사항목

2017년 1차 및 2차 신빙도조사에 참여한 기관은 53개 기관이었다. 분자진단검사는 혈액종양 8종목, 고형종양 4종목, 선천성 유전질환 8종목, 미토콘드리아 유전질환 3종, 삼염기 반복 유전질환 7종, 신경근육 유전질환 1종, 기타 유전질환 3종, 유전형검사 3종목 등 총 37종목에 대해 시행하였다.

혈액종양 분자진단검사 8종목인 leukemia and lymphomas (*BCR-ABL1*, *PML-RARA*, *AML1-ETO*, *TEL-AML1*), *JAK2*, FMS-like tyrosine kinase 3 (*FLT3*-internal tandem duplication [ITD], *FLT3*-tyrosine kinase domain [TKD]), nucleophosmin (*NPM1*) 분자진단검사는 1차에서 각각 36기관, 34기관, 33기관, 31기관, 30기관, 19기관, 17기관, 16기관이, 2차에서 37기관, 36기관, 35기관, 33기관, 31기관, 20기관, 18기관, 18기관이 참가하였다. 고형종양 분자진단검사 4종목인 *KRAS*, *BRAF*, *EGFR*, *KIT* 분자진단검사는 1차에서 10기관, 15기관, 9기관, 10기관이 참가하였고, 2차에서는 11기관, 14기관, 10기관, 10기관이 참가하였다. 유전질환 8종목인 *BRCA1*, *BRCA2*, *TP53*, *ATP7B*, *FGFR3*, *GJB2*, *TGFBI*, *RET* 분자진단검사는 1차에서 각각 16기관, 16기관, 6기관, 8기관, 9기관, 10기관, 13기관, 7기관이 참가하였으며, 2차에서 15기관, 15기관, 6기관, 8기관, 9기관, 10기관, 13기관, 7기관이 참가하였다. 삼염기 반복 유전질환과 신경근육 유전질환 종목인 HD, SCA1, SCA2, SCA3, SCA6, SCA7, SBMA, *FMR1* 분자진단검사에는 1차에서 각각 5기관, 6기관, 7기관, 6기관, 6기관, 5기관, 10기관이 참가하였고, 2차에서 5기관, 6기관, 7기관, 6기관, 6기관, 6기관, 5기관, 10기관이 참가하였다. 미토콘드리아 유전질환에 속하는 종목인 mitochondrial encephalopathy with lactic acidosis and stroke like episodes (MELAS), myoclonic epilepsy ragged red fibre (MERRF), Leber hereditary optic neuropathy (LHON—major mutation) 검사에는 1차에서 각각 6기관, 5기관, 6기관이 참가하였고, 2차에서 6기관, 5기관, 6기관이 각각 참가하였다. Prader-Willi/Angelman syndrome (PWS/AS), Duchenne muscular dystrophy (DMD), spinal muscular atrophy (SMA) 분자진단검사는 1차에서 각각 5기관, 6기관, 6기관이 각각 참가하였고, 2차에서 5기관, 6기관, 6기관이 각각 참가하였다. 유전형검사에 속하는 종목인 apolipoprotein E (*APOE*) genotyping,

methylenetetrahydro-folate reductase (*MTHFR*) genotyping, *ABO* genotyping 분자진단검사는 1차에서 각각 32기관, 13기관, 13기관이 참가하였으며, 2차에서 34기관, 14기관, 13기관이 참가하였다.

2) 검사방법

각 기관별 사용하는 검사법은 Table 2와 같다.

3) 신빙도조사 결과

Leukemia and lymphomas 분자진단검사는 1차에서 *TEL-AML1* 재배열과 *PML-RARA* 재배열 검체를, 2차에서는 *TEL-AML1* 재배열과 *BCR-ABL1* 재배열 검체로 구성하였다. 1차 평가에서 *PML-RARA*, *AML1-ETO* 재배열 문항에 대하여 참여한 모든 기관이 예상결과와 일치하는 결과를 보고하였고, *BCR-ABL1* 재배열 문항은 1개 기관이 다른 subtype을 보고하였다. *TEL-AML1* 재배열 문항은 31개 기관 중 1개 기관이 예상결과와 상이한 결과를 보고하였다. 2차에서는 *BCR-ABL1*, *PML-RARA*, *AML1-ETO* 재배열 문항에 대하여 참여한 모든 기관이 예상결과와 일치하는 결과를 보고하였고 *TEL-AML1* 재배열 문항은 33개 기관 중 2개 기관이 예상결과와 상이한 결과를 보고하였다.

JAK2, *FLT3*-ITD, *FLT3*-TKD, *NPM1* 분자진단검사에서는 *JAK2*, *NPM1*에 참여한 모든 기관이 1차와 2차에서 예상결과와 일치하는 결과를 보고하였다. *FLT3*-ITD 분자진단검사에서는 1차에 참여한 모든 기관이 예상결과와 일치하는 결과를 보고하였으나 2차에서는 1개 기관이 정상 검체에서 변이를 발견해 보고하였으며 *FLT3*-TKD 분자진단검사에서는 1차에서 1개 기관이 변이를 발견하지 못하였으며 2차에서는 전체 응답결과의 80% 이상의 consensus를 보이지 않아 전체 기관의 결과를 “ungraded”로 보고하였다.

KRAS, *BRAF*, *EGFR*, *KIT* 분자진단검사에서는 *KRAS*, *BRAF*, *EGFR* 분자진단검사에 참여한 모든 기관이 1차와 2차에서 예상결과와 일치하는 결과를 보고하였다. *KIT* 분자진단검사에서는 1차에서 1개 기관이 예상결과와 상이한 결과를 보고하였고 2차에서는 모든 기관이 예상결과와 일치하는 결과를 보고하였다.

BRCA1, *BRCA2*, *TP53*, *ATP7B*, *GJB2*, *FGFR3*, *TGFBI*, *RET* 분자진단검사에서는 *BRCA2*, *TP53*, *FGFR3*, *TGFBI*에 참여한 모든 기관이 1차와 2차에서 예상결과와 일치하는 결과를 보고하였다. *BRCA1* 분자진단검사에서는 2차에 참여기관 중 1개 기관이 예상결과와 다른 염기변이를 보고하였다. *ATP7B* 분자진단검사에서는 1차에 참여한 1개 기관

Table 2. Mutation detection methods of the molecular diagnostics test survey in 2017

Tests	Mutation detection methods (lab-developed test)	Response	Mutation detection methods (commercial kit)	Response
Leukemia (<i>BCR-ABL1</i>)	Allele-specific PCR	1	Hemavision	29
	PCR (include multiplex), gel electrophoresis	1		
	PCR and gel electrophoresis	2		
	Real-time PCR	4		
Leukemia (<i>PML-RARA</i>)	Allele-specific PCR	1	Hemavision	29
	PCR (include multiplex), gel electrophoresis	1		
	PCR and gel electrophoresis	2		
	Real-time PCR	3		
Leukemia (<i>AML1-ETO</i>)	Allele-specific PCR	1	Hemavision	29
	PCR (include multiplex), gel electrophoresis	1		
	PCR and gel electrophoresis	2		
	Real-time PCR	2		
Leukemia (<i>TEL-AML1</i>)	Allele-specific PCR	1	Hemavision	29
	PCR (include multiplex), gel electrophoresis	1		
	PCR and gel electrophoresis	2		
	Real-time PCR	2		
<i>JAK2</i>	Allele-specific PCR	7		
	Pyrosequencing	1		
	Real-time PCR	10		
	Real-time PCR, hydrolysis probe	2		
	Sanger sequencing	10		
	Single base extension (SNaPshot)	1		
<i>FLT3</i> -ITD	Allele-specific PCR	1		
	PCR and fluorescent based capillary electrophoresis (fragment length analysis)	3		
	PCR (include multiplex), gel electrophoresis	1		
	PCR and gel electrophoresis	1		
	RFLP	1		
<i>FLT3</i> -TKD	Sanger sequencing	12		
	Allele-specific PCR	1		
	PCR and fluorescent based capillary electrophoresis (fragment length analysis)	1		
	PCR (include multiplex), gel electrophoresis	1		
	PCR and gel electrophoresis	10		
	Sanger sequencing	2		
<i>NPM1</i>	Other*	2		
	Real-time PCR	1		
	RFLP	1		
	Sanger sequencing	16		

(Continued to the next page)

Table 2. Continued

Tests	Mutation detection methods (lab-developed test)	Response	Mutation detection methods (commercial kit)	Response
<i>KRAS</i>	Codon12-pyrosequencing	2		
	Codon13-pyrosequencing	2		
	Codon61-pyrosequencing	2		
	Codon12-Sanger sequencing	8		
	Codon13-Sanger sequencing	8		
	Codon61-Sanger sequencing	8		
<i>BRAF</i>	codon 600 (p.V600E)-pyrosequencing	2		
	codon 600 (p.V600E)-real-time PCR	6		
	codon 600 (p.V600E)-real-time PCR, hydrolysis probe	3		
	codon 600 (p.V600E)-Sanger sequencing	4		
	codon 600 (p.V600K)-pyrosequencing	2		
	codon 600 (p.V600K)-Sanger sequencing	4		
	codon 600 (p.V600K)-pyrosequencing	2		
	codon 600 (p.V600K)-Sanger sequencing	4		
<i>EGFR</i>	Codon719-pyrosequencing	1		
	Codon790-pyrosequencing	1		
	Codon858-pyrosequencing	1		
	Codon861-pyrosequencing	1		
	Exon 19 deletion-pyrosequencing	1		
	Codon719-Sanger sequencing	8		
	Codon790-Sanger sequencing	8		
	Codon858-Sanger sequencing	8		
	Codon861-Sanger sequencing	8		
	Exon 19 deletion-Sanger sequencing	8		
<i>KIT</i>	codon 816-real-time PCR, hydrolysis probe	1		
	codon 816-Sanger sequencing	8		
	codon 816-other	1		
	Exon 9-Sanger sequencing	6		
	Exon 9-other	1		
	Exon 11-Sanger sequencing	6		
	Exon 11-other	1		
	Exon 13-Sanger sequencing	6		
	Exon 13-other	1		
	Exon 17-Sanger sequencing	7		
	Exon 17-other	1		
<i>BRCA1</i>	Sanger sequencing	16		
<i>BRCA2</i>	Sanger sequencing	16		
<i>TP53</i>	Sanger sequencing	6		
<i>ATP7B</i>	Sanger sequencing	8		
Achondroplasia	Sanger sequencing	9		

(Continued to the next page)

Table 2. Continued

Tests	Mutation detection methods (lab-developed test)	Response	Mutation detection methods (commercial kit)	Response
<i>GJB2</i>	Sanger sequencing	10		
<i>TGFBI</i>	Real-time PCR	3		
	Sanger sequencing	10		
<i>RET</i>	Sanger sequencing	7		
HD	PCR and fluorescent based capillary electrophoresis (fragment length analysis)	5		
SCA1	PCR and fluorescent based capillary electrophoresis (fragment length analysis)	6		
SCA2	PCR and fluorescent based capillary electrophoresis (fragment length analysis)	7		
SCA3	PCR and fluorescent based capillary electrophoresis (fragment length analysis)	6		
SCA6	PCR and fluorescent based capillary electrophoresis (fragment length analysis)	6		
SCA7	PCR and fluorescent based capillary electrophoresis (fragment length analysis)	6		
SBMA	PCR and fluorescent based capillary electrophoresis (fragment length analysis)	5		
LHON	Sanger sequencing	5		
	Single base extension (SNaPshot)	1		
MELAS	Sanger sequencing	6		
MERRF	Sanger sequencing	5		
PWS/AS	PCR and gel electrophoresis, methylation specific	5		
DMD	MLPA	6		
SMA	MLPA	4		
	RFLP	2		
<i>APOE</i> genotype	Allele-specific PCR	5		
	PCR (include multiplex), gel electrophoresis	3		
	PCR and gel electrophoresis	1		
	Real-time PCR	17		
	Real-time PCR, hydrolysis probe	1		
	Real-time PCR, melting curve analysis	1		
	RFLP	1		
	Sanger sequencing	2		
	Line probe assay	1		
<i>MTHFR</i> genotype	Allele-specific PCR	2		
	PCR and gel electrophoresis	1		
	Real-time PCR	1		
	Real-time PCR, hydrolysis probe	1		
	Real-time PCR, melting curve analysis	1		
	RFLP	4		

(Continued to the next page)

Table 2. Continued

Tests	Mutation detection methods (lab-developed test)	Response	Mutation detection methods (commercial kit)	Response
ABO genotype	Sanger sequencing	2		
	Single base extension (SNaPshot)	1		
	Allele-specific PCR	1		
	RFLP	1		
FMR1	Sanger sequencing	9		
	Single base extension (SNaPshot)	2		
	PCR and fluorescent based capillary electrophoresis (fragment length analysis)	6		
	Southern blot	1		
	Triplet repeat primed PCR and fluorescent based capillary electrophoresis	2		

Abbreviations: PCR, polymerase chain reaction; *JAK2*, Janus kinase 2; *FLT3*, FMS-like tyrosine kinase 3; ITD, internal tandem duplication; RFLP, restriction fragment length polymorphism; TKD, tyrosine kinase domain; *NPM1*, nucleophosmin; *EGFR*, epidermal growth factor receptor; *RET*, multiple endocrine neoplasia 2; HD, Huntington disease; SCA, spinocerebellar ataxia; SBMA, spinal and bulbar muscular atrophy; LHON, Leber hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibres; PWS/AS, Prader-Willi/Angelman syndrome; *DMD*, Duchenne muscular dystrophy; MLPA, multiple ligation probe amplification; SMA, spinal muscular atrophy; *APOE*, apolipoprotein E; *MTHFR*, methylenetetrahydrofolate reductase; *FMR1*, fragile X mental retardation-1.

*No response.

이 예상 변이부위와 상이한 부위의 변이를 보고하였으나 2차에서는 예상결과와 일치하는 결과를 보고하였다. *GJB2* 분자진단검사에서는 1차와 2차에 참여한 각 1개 기관이 예상 변이부위와 상이한 부위의 변이를 보고하여 평가에서 제외되었다. *RET* 분자진단검사에서는 1차에서 예상결과와 일치하는 결과를 보고하였으나 2차에서는 1개 기관이 예상결과와 다른 단백질을 보고하였다.

HD, SCA1, SCA2, SCA3, SCA6, SCA7, SBMA 분자진단검사에서는 SCA1, SCA2, SCA3, SCA6, SCA7 분자진단검사에 참여한 모든 기관이 예상결과와 일치하는 결과를 보고하였다. HD, SBMA 분자진단검사에서는 2차에 참여한 기관 중 각각 2개 기관이 예상결과와 상이한 결과를 보고하였다.

MELAS, MERRF, LHON 분자진단검사에서는 MERRF, LHON 분자진단검사에 참여한 모든 기관에서 1차와 2차에서 예상결과와 일치하는 결과를 보고하였다. MELAS 분자진단검사에서는 1차에서 1개 기관이 예상결과와 상이한 결과를 보고하였으나 2차에서는 참여한 모든 기관에서 예상결과와 일치하는 결과를 보고하였다. PWS/AS, DMD, SMA 분자진단검사에서는 참여한 모든 기관이 1차와 2차에서 예상결과와 일치하는 결과를 보고하였다.

APOE, *MTHFR*, ABO genotyping 분자진단검사에서는 *MTHFR* genotyping 분자진단검사에 참여한 모든 기관이 1차

와 2차에서 예상결과와 일치하는 결과를 보고하였다. *APOE* genotyping 분자진단검사에서는 1차에 제공한 1번 검체에 대하여 1개 기관이 예상결과와 상이한 결과를 보고하였다. ABO genotyping 분자진단검사에서는 1차에 제공한 1번 검체에 대하여 2개 기관이 예상결과와 상이한 결과를 보고하였으며 2번 검체에 대하여 1개 기관이 예상결과와 상이한 결과를 보고하였다.

FMR1 분자진단검사에서는 1차에 참여한 1개 기관이 평가용 검체로 제공한 2종류의 검체 모두에서 예상결과와 다른 결과를 보고하였으나 2차에서는 참여한 모든 기관이 예상결과와 일치하는 결과를 보고하였다. 각 기관별 보고결과를 정리한 정오답 통계는 Table 3과 같다.

고찰

2017년도 분자진단검사 신병도조사는 전체적으로 매우 우수한 결과를 보였다(1-4). *BCR-ABL1*, *PML-RARA*, *AML1-ETO* 재배열 문항, *JAK2*, *NPM1*, *KRAS*, *BRAF*, *EGFR*, *BRCA1*, *BRCA2*, *TP53*, *FGFR3*, *TGFBI*, SCA1, SCA2, SCA3, SCA6, SCA7, MERRF, LHON PWS/AS, DMD, SMA, *MTHFR* genotyping의 항목은 참여한 모든 기관이 1차와 2차에서 예상결과와 일치한 결과를 보고하였다.

Table 3. Results of the molecular diagnostics test survey carried out in 2017

Tests	Specimens of 1st trial	Intended responses	Total no. of participants/ acceptable response	Specimens of 2nd trial	Intended responses	Total no. of participants/ acceptable response
Leukemia (BCR-ABL1)	GH 17-01	Mutation not detected	36/36	GH 17-11	BCR/ABL1 positive	37/37
	GH 17-02	Mutation not detected	36/36	GH 17-12	Mutation not detected	37/37
Leukemia (PML-RARA)	GH 17-01	Mutation not detected	34/34	GH 17-11	Mutation not detected	36/36
	GH 17-02	PML/RARA positive	34/34	GH 17-12	Mutation not detected	36/36
Leukemia (AML1-ETO)	GH 17-01	Mutation not detected	33/33	GH 17-11	Mutation not detected	35/35
	GH 17-02	Mutation not detected	33/33	GH 17-12	Mutation not detected	35/35
Leukemia (TEL-AML1)	GH 17-01	TEL/AML1 positive	31/30	GH 17-11	Mutation not detected	33/33
	GH 17-02	Mutation not detected	31/31	GH 17-12	TEL/AML1 positive	33/31
JAK2	GH 17-03	p.Val617Phe mutation	30/30	GH 17-13	Mutation not detected	31/31
	GH 17-04	Mutation not detected	30/30	GH 17-14	p.Val617Phe mutation	31/31
FLT3-ITD	GH 17-05	Mutation not detected	19/19	GH 17-15	Mutation not detected	20/19
	GH 17-06	FLT3-ITD mutation	19/19	GH 17-16	FLT3-ITD mutation	20/20
FLT3-TKD	GH 17-07	FLT3- TKD mutation	17/16	GH 17-17	FLT3-TKD mutation	18/all ungraded
	GH 17-08	Mutation not detected	17/17	GH 17-18	Mutation not detected	18/18
NPM1	GH 17-09	NPM1 mutation	16/16	GH 17-19	Mutation not detected	18/18
	GH 17-10	Mutation not detected	16/16	GH 17-20	NPM1 mutation	18/18
KRAS	GS 17-01	Codon 12 mutation	10/10	GS 17-09	Mutation not detected	11/11
	GS 17-02	Mutation not detected	10/10	GS 17-10	Codon 12 mutation	11/11
EGFR	GS 17-03	Exon 19 deletion	9/9	GS 17-11	Exon 19 deletion	10/10
	GS 17-04	Mutation not detected	9/9	GS 17-12	EGFR mutation	10/10
BRAF	GS 17-05	Mutation not detected	15/15	GS 17-13	p.Val600Glu	14/14
	GS 17-06	p.Val600Glu	15/15	GS 17-14	Mutation not detected	14/14
KIT	GS 17-07	Mutation not detected	10/10	GS 17-15	Mutation not detected	10/10
	GS 17-08	Codon 816 mutation	10/9	GS 17-16	Codon 816 Mutation	12/12
BRCA1	GG 17-01	c.5074+1G>T, heterozygote	16/16	GG 17-41	Mutation not detected	15/15
	GG 17-02	Mutation not detected	16/16	GG 17-42	c.3442delG, p.Glu1148Argfs*7, heterozygote	15/14
BRCA2	GG 17-03	Mutation not detected	16/16	GG 17-43	Mutation not detected	15/15
	GG 17-04	c.8140C>T, p.Gln2714*, heterozygote	16/16	GG 17-44	c.7480C>T, p.Arg2494*, heterozygote	15/15
TP53	GG 17-05	c.742C>T, p.Arg248Trp, heterozygote	6/6	GG 17-45	Mutation not detected	6/6
	GG 17-06	Mutation not detected	6/6	GG 17-46	c.742C>T, p.Arg248Trp, heterozygote	6/6

(Continued to the next page)

Table 3. Continued

Tests	Specimens of 1st trial	Intended responses	Total no. of participants/ acceptable response	Specimens of 2nd trial	Intended responses	Total no. of participants/ acceptable response
ATP7B	GG 17-07	c.3809A>G, p.Asn1270Ser, heterozygote c.3247C>T, p.Leu1083Phe, heterozygote	8/7	GG 17-47	c.3104G>T, p.Gly1035Val, heterozygote c.3247C>T, p.Leu1083Phe, heterozygote	8/8
	GG 17-08	Mutation not detected	8/8	GG 17-48	Mutation not detected	8/8
MELAS	GG 17-09	m.3243A>G	6/5	GG 17-49	Mutation not detected	6/6
	GG 17-10	Mutation not detected	6/6	GG 17-50	m.3243A>G	6/6
MERRF	GG 17-11	Mutation not detected	5/5	GG 17-51	m.8344A>G	5/5
	GG 17-12	m.8344A>G	5/5	GG 17-52	Mutation not detected	5/5
GJB2	GG 17-13	c.35delG, p.Gly12Valfs*, heterozygote	10/9	GG 17-53	c.35delG, p.Gly12Valfs*, heterozygote	10/10
	GG 17-14	Mutation not detected	10/10	GG 17-54	Mutation not detected	10/10
LHON	GG 17-15	m.11778G>A	6/6	GG 17-55	Mutation not detected	6/6
	GG 17-16	Mutation not detected	6/6	GG 17-56	m.11778G>A	6/6
RET	GG 17-17	c.2753T>C, p.Met918Thr, heterozygote	7/7	GG 17-57	c.1859G>C, p.Cys620Ser, heterozygote	7/6
	GG 17-18	Mutation not detected	7/7	GG 17-58	Mutation not detected	7/7
SCA1	GG 17-19	Mutation not detected	6/6	GG 17-59	Mutation not detected	6/6
	GG 17-20	SCA1 mutation	6/6	GG 17-60	Mutation not detected	6/6
SCA2	GG 17-19	Mutation not detected	7/7	GG 17-59	Mutation not detected	7/7
	GG 17-20	Mutation not detected	7/7	GG 17-60	Mutation not detected	7/7
SCA3	GG 17-19	Mutation not detected	6/6	GG 17-59	Mutation not detected	6/6
	GG 17-20	Mutation not detected	6/6	GG 17-60	Mutation not detected	6/6
SCA6	GG 17-19	Mutation not detected	6/6	GG 17-59	SCA6 mutation	6/6
	GG 17-20	Mutation not detected	6/6	GG 17-60	Mutation not detected	6/6
SCA7	GG 17-19	Mutation not detected	6/6	GG 17-59	Mutation not detected	6/6
	GG 17-20	Mutation not detected	6/6	GG 17-60	Mutation not detected	6/6
APOE genotype	GG 17-21	APOE $\epsilon 3/\epsilon 3$ genotype	32/31	GG 17-61	APOE $\epsilon 4/\epsilon 4$ genotype	34/34
	GG 17-22	APOE $\epsilon 4/\epsilon 4$ genotype	32/31	GG 17-62	APOE $\epsilon 3/\epsilon 4$ genotype	34/34
Achondroplasia	GG 17-23	Mutation not detected	9/9	GG 17-63	p.Gly380Arg	9/9
	GG 17-24	p.Gly380Arg	9/9	GG 17-64	Mutation not detected	9/9
MTHFR genotype	GG 17-25	MTHFR 677T/T homozygous genotype	13/13	GG 17-65	MTHFR 677C/T heterozygous genotype	14/14
	GG 17-26	Mutation not detected	13/13	GG 17-66	MTHFR 677T/T homozygous genotype	14/14
PWS/AS	GG 17-27	Mutation not detected	5/5	GG 17-67	Prader-Willi syndrome	5/5
	GG 17-28	Angelman syndrome	5/5	GG 17-68	Mutation not detected	5/5
DMD	GG 17-29	Mutation not detected	6/6	GG 17-69	Exon 56-57 deletion	6/6
	GG 17-30	Exon 18-44 deletion	6/6	GG 17-70	Mutation not detected	6/6

(Continued to the next page)

Table 3. Continued

Tests	Specimens of 1st trial	Intended responses	Total no. of participants/ acceptable response	Specimens of 2nd trial	Intended responses	Total no. of participants/ acceptable response
HD	GG 17-31	Full penetrance	5/5	GG 17-71	Full penetrance	5/5
	GG 17-32	Mutation not detected	5/5	GG 17-72	Mutation not detected	5/3
<i>FMRI</i>	GG 17-33	Full mutation	10/9	GG 17-73	Mutation not detected	10/10
	GG 17-34	Mutation not detected	10/9	GG 17-74	Full mutation	10/10
<i>TGFBI</i>	GG 17-35	p.Arg124His	13/13	GG 17-75	Mutation not detected	13/13
	GG 17-36	Mutation not detected	13/13	GG 17-76	p.Arg124His	13/13
SBMA	GG 17-37	Mutation not detected	5/5	GG 17-77	Full penetrance	5/3
	GG 17-38	Full penetrance	5/5	GG 17-78	Mutation not detected	5/5
SMA	GG 17-39	<i>SMN1</i> , Exon7, homozygous deletion	6/6	GG 17-79	<i>SMN1</i> , Exon7, homozygous deletion	6/6
	GG 17-40	Mutation not detected	6/6	GG 17-80	Mutation not detected	6/6
ABO genotype	GO 17-01	<i>cis-AB/B</i> genotype	13/11	GO 17-03	<i>B/O</i> genotype	13/13
	GO 17-02	<i>A/O</i> genotype	13/12	GO 17-04	<i>cis-AB/A</i> genotype	13/13

Abbreviations: *FLT3*, FMS-like tyrosine kinase 3; ITD, internal tandem duplication; TKD, tyrosine kinase domain; *NPM1*, nucleophosmin; *EFGR*, epidermal growth factor receptor; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRE, myoclonic epilepsy with ragged red fibres; LHON, Leber hereditary optic neuropathy; *RET*, multiple endocrine neoplasia 2; SCA, spinocerebellar ataxia; *APOE*, apolipoprotein E; *MTHFR*, methylenetetrahydrofolate reductase; PWS/AS, Prader-Willi/Angelman syndrome; DMD, Duchenne muscular dystrophy; HD, Huntington's disease; *FMRI*, fragile X mental retardation-1; *TGFBI*, transforming growth factor beta induced; SBMA, spinal and bulbar muscular atrophy; SMA, spinal muscular atrophy.

검사범위가 한정된 KIT를 사용하는 기관에서는 검사부위 이외의 부위에서도 변이를 검출할 수 있는 대안을 마련하길 권장한다. 검체가 바뀌어 분석되었다고 예상되는 기관이 있어왔으므로 해당 기관에서는 매 회차마다 분석과정을 반드시 확인해야 한다.

HGVS에서는 오류를 방지하는 차원에서 triple-letter amino acid code 사용을 권장하고 있으므로 모든 참여기관들에서는 protein level에서의 변이에 대해 triple-letter amino acid code를 사용한 결과입력을 권장하며 특히 유전자변이 명명에서 잘못된 변이부위를 보고하거나 단백질변이 오기가 많이 발생하므로 최종보고 전 확인할 수 있는 체계를 구성하는 것을 권장한다.

동일 유전자를 검사하는 여러 기관에서 각기 다른 reference transcript를 사용하는 경우가 있으므로 검사 담당자는 주기적으로 reference transcript를 확인해야 한다. 변이에 따른 해석 보고와 분석한 정량값으로 유전질환을 진단하는 경우 변이 해석과 정량값의 기준이 변하는 경우가 종종 있으므로 관련 문헌 및 참고자료를 주기적으로 확인해야 하며 HGVS 및 ACMG 관련 지침을 준수하는 것을 권장한다[5-7].

REFERENCES

- Kim SH, Ki CS, Kim S, Kwon MJ, Kim JW, Park SS, et al. Annual report on external quality assessment in diagnostic genetics in Korea (2009). *J Lab Med Qual Assur* 2010;32:147-70.
- Kim SH, Ki CS, Jeon BR, Lee ST, Yoo EH, Kim JW, et al. Annual report on external quality assessment in diagnostic genetics in Korea (2008). *J Lab Med Qual Assur* 2009; 31:161-81.
- Kim SH, Ki CS, Kim JH, Oh SH, Kim JW, Park SS, et al. Annual report on external quality assessment in diagnostic genetics in Korea (2007). *J Lab Med Qual Assur* 2008; 30:167-87.
- Kim SH, Kim JW, Ki CS, Kim IS, Nam MH, Cho HJ, et al. Annual report on external quality assessment in diagnostic genetics in Korea (2005). *J Lab Med Qual Assur* 2006; 28:153-67.
- Shaffer LG, McGowan-Jordan J, Schmid M; International Standing Committee on Human Cytogenetic Nomencla-

- ture. ISCN 2013: an international system for human cytogenetic nomenclature (2013). Basel: S. Karger, 2013.
6. Richards CS, Bale S, Bellissimo DB, Das S, Grody WW, Hegde MR, et al. ACMG recommendations for standards for interpretation and reporting of sequence variations: revisions 2007. *Genet Med* 2008;10:294-300.
7. Zhang VW, Wang J. Determination of the clinical significance of an unclassified variant. *Methods Mol Biol* 2012; 837:337-48.