

DNA quality assessment – what can we learn from our neighbours

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Agenda

1. Introduction

2. Molecular genetic EQA schemes of the RfB

3. FV

4. Conclusion

5. Future Development

Introduction

- External quality assessment schemes – definition and aim
- Unique characteristics of molecular genetic diagnostics to be considered
- Database concerning EQA provider: www.eurogentest.org/

www.ifcc.com

Cytochrom p450 2B6*6	RfB
Cytochrom p450 2C19	RfB INSTAND
Cytochrom p450 2C8 (CYP2C8) Gene: K399R	RfB ECAT
Cytochrom p450 2C9	RfB INSTAND
Cytochrom p450 2D6	RfB INSTAND
Cytochrom p450 3A4*22	RfB
Cytochrom p450 3A5*3	RfB
Dihydropyrimidin-Dehydrogenase (DPD) Gene: Exon-skipping mutation IVS14 G>A +1	RfB ECAT
DNA Isolation	RfB ECAT
DNA Sequencing	RfB CAP ECAT EMQN EQUALIS

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Molecular genetic proficiency testing of the RfB

Scope:

- DI – DNA Isolation
- FV – Genotyping
- SQ – Sequencing

DI-EQA

- Distribution twice a year by the „Referenzinstitut für Bioanalytik“ (www.dgkl-rfb.de)
- Offered since 2009
- Two different samples each á 500µl of human whole blood are provided
- Following parameters are requested:
 - Method used for DNA-isolation
 - DNA-concentration
 - Purification of the isolated DNA
- Following analytes can be determined:
 - FV-Leiden, FV-Hong-Kong, FV-Cambridge
 - Since 2012 MTHFR 677, FII, HFE

DI-EQA

n Resultatbox/Anzahl der Ergebnisse
 richtiges Ergebnis
 Ihr richtiges Ergebnis
 Ihr falsches Ergebnis

1 FV-Leiden (ARG506GLN)

Allele:	Probe/Sample	R/R	R/Q	Q/Q
R:R506	01	1	1	39
Q:Q506	02	41		

2 FV-H1299R (HIS1299ARG)

Allele:	Probe/Sample	H/H	H/R	R/R
H:H1299	01	5		
R:R1299	02	6		

3 FV-Cambridge (ARG306THR)

Allele:	Probe/Sample	R/R	R/T	T/T
R:R303	01	3		
T:T306	02	3		

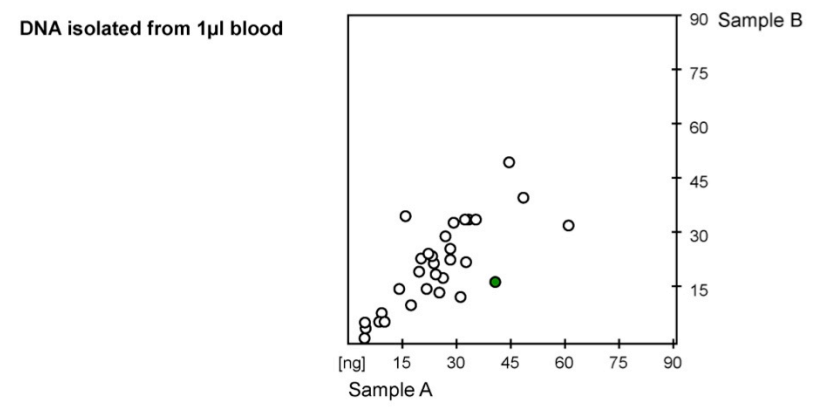
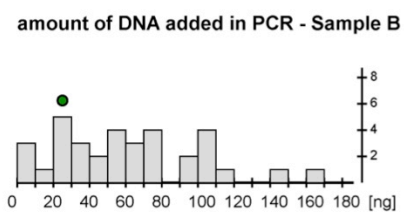
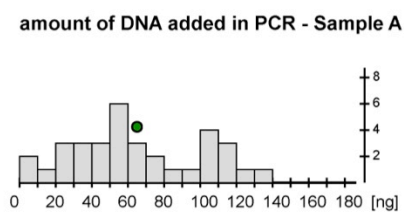
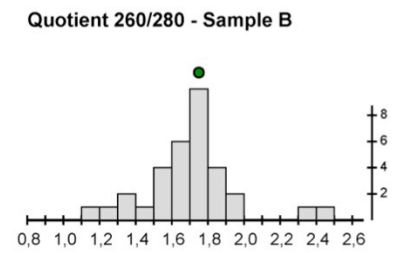
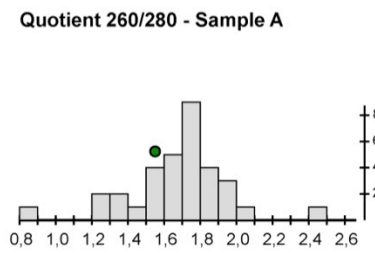
4 FV-Hong-Kong (ARG306GLY)

Allele:	Probe/Sample	R/R	R/G	G/G
R:R306	01	3		
G:G306	02	3		

- Your results are marked with a green dot -

D11/09
 Part.-No.
 0000023

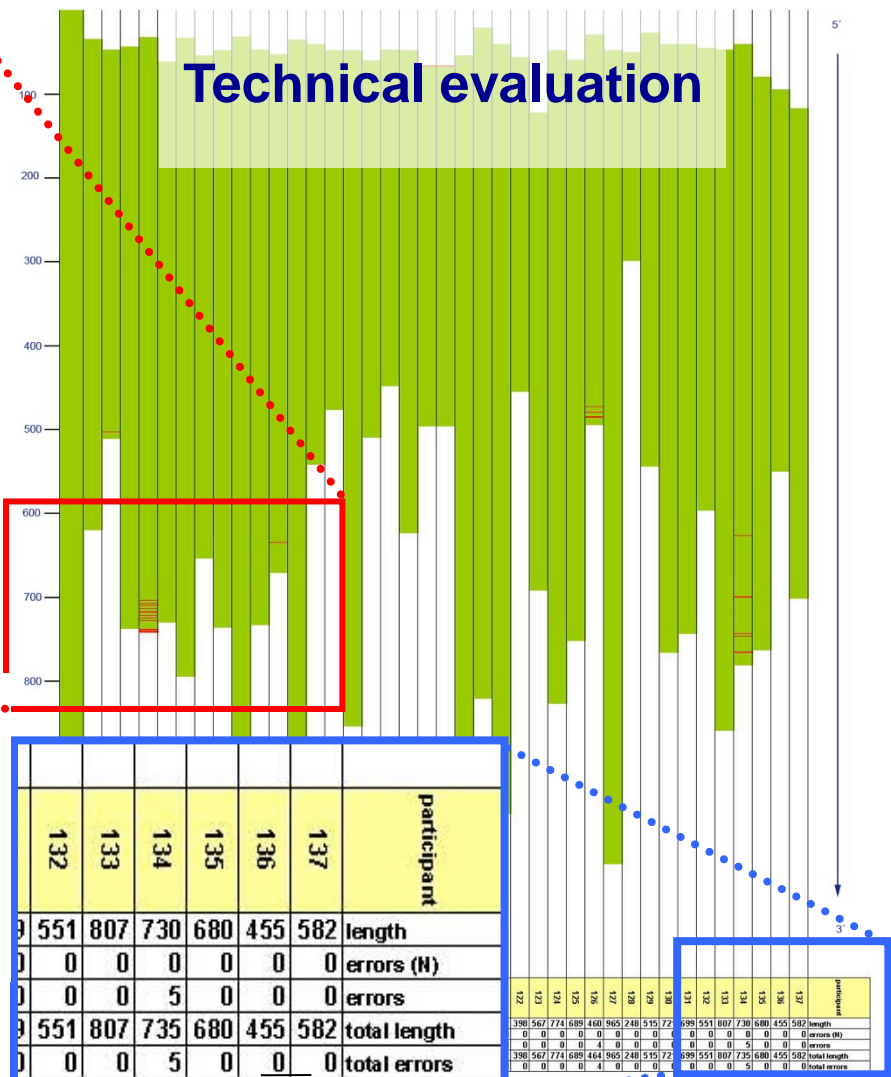
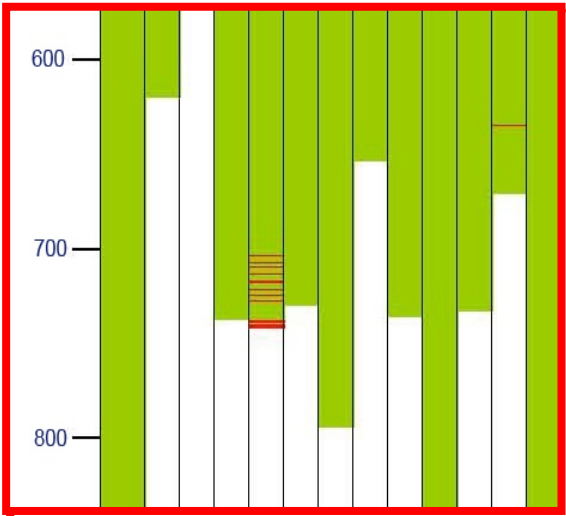
Used methods:
 automated: 26
 manually: 16



SQ-EQA

- Distribution twice a year by the „Referenzinstitut für Bioanalytik“ (www.dgkl-rfb.de)
- Offered since 2006
- Two different PCR-products as well as the sequencing primers are provided
- Separated into a technical and medical part
- Technical part:
 - Both samples have to be analyzed
 - The raw data has to be edited
- Medical part:
 - The patients history as well as other laboratory findings are provided
 - Only one sample has to be evaluated

SQ-EQA



DGKL EQA for DNA-sequencing

- Based on EQUAL-Seq und published reporting formats

132	133	134	135	136	137	participant
9 551	807	730	680	455	582	length
0 0	0 0	0 0	0 0	0 0	0 0	errors (N)
0 0	0 0	5 0	0 0	0 0	0 0	errors
9 551	807	735	680	455	582	total length
0 0	0 0	5 0	0 0	0 0	0 0	total errors

122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	participant
1398	567	774	689	460	965	248	535	72	599	551	607	730	680	455	582	length
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	errors (N)
0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	errors
1398	567	774	689	464	965	248	535	72	599	551	607	735	680	455	582	total length
0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	total errors

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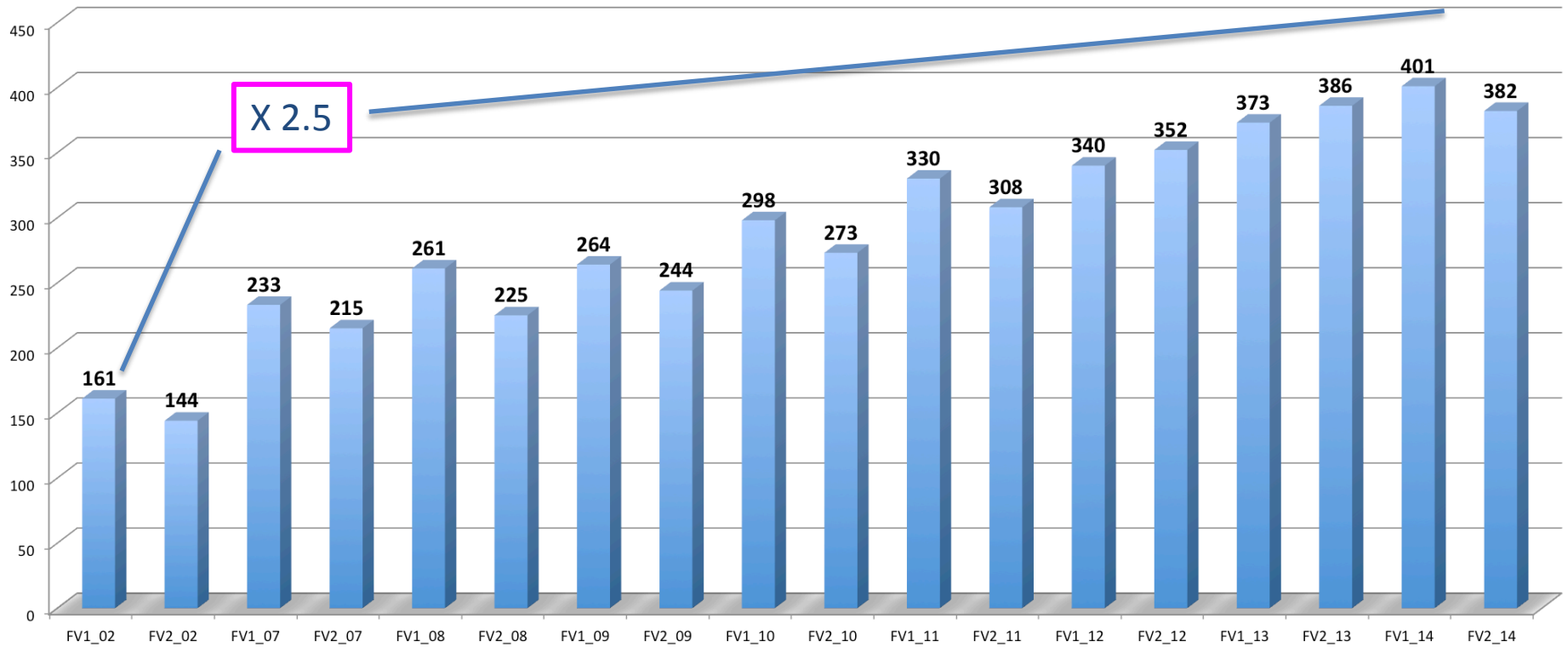
5. Future Development

FV-EQA

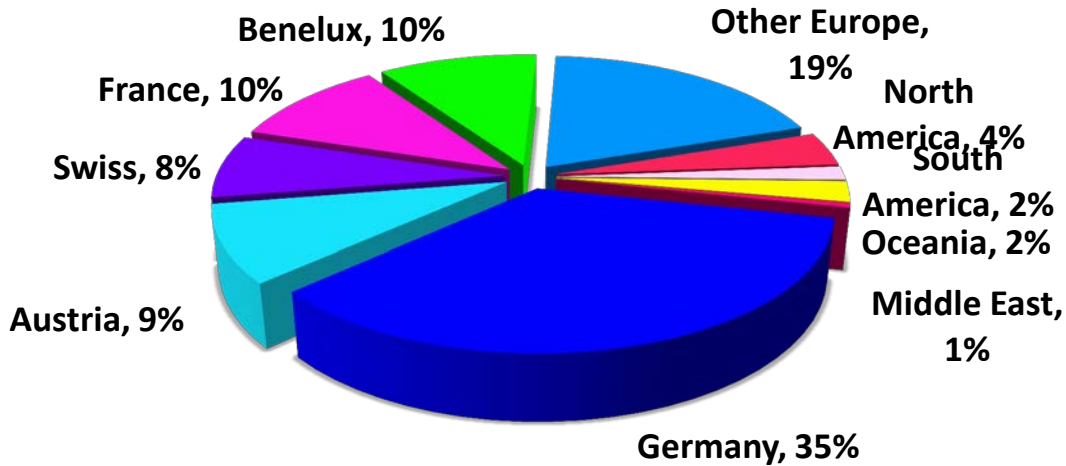
- Distribution twice a year by the „Referenzinstitut für Bioanalytik“ (www.dgkl-rfb.de)
- Offered since 2002
- Samples containing 500 to 1000 ng of lyophilized gDNA
- Since 2014 nine different sets (A-I) are provided
 - Each set is composed of two different samples
 - An average of four different analytes are provided per set
 - For sample validation two different methods are used

FV-EQA: scope

Number of participating laboratories

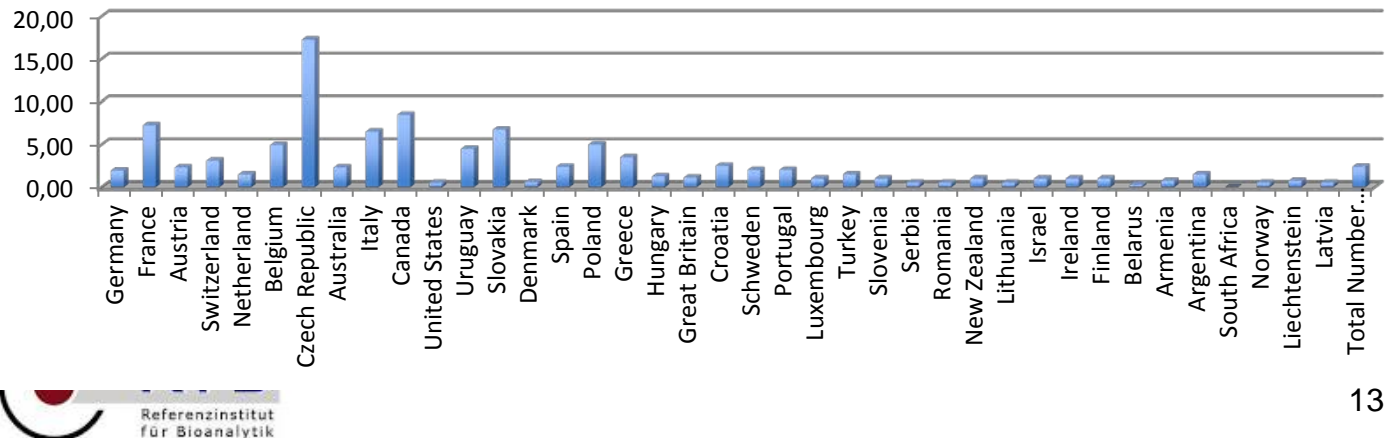


FV-EQA: scope



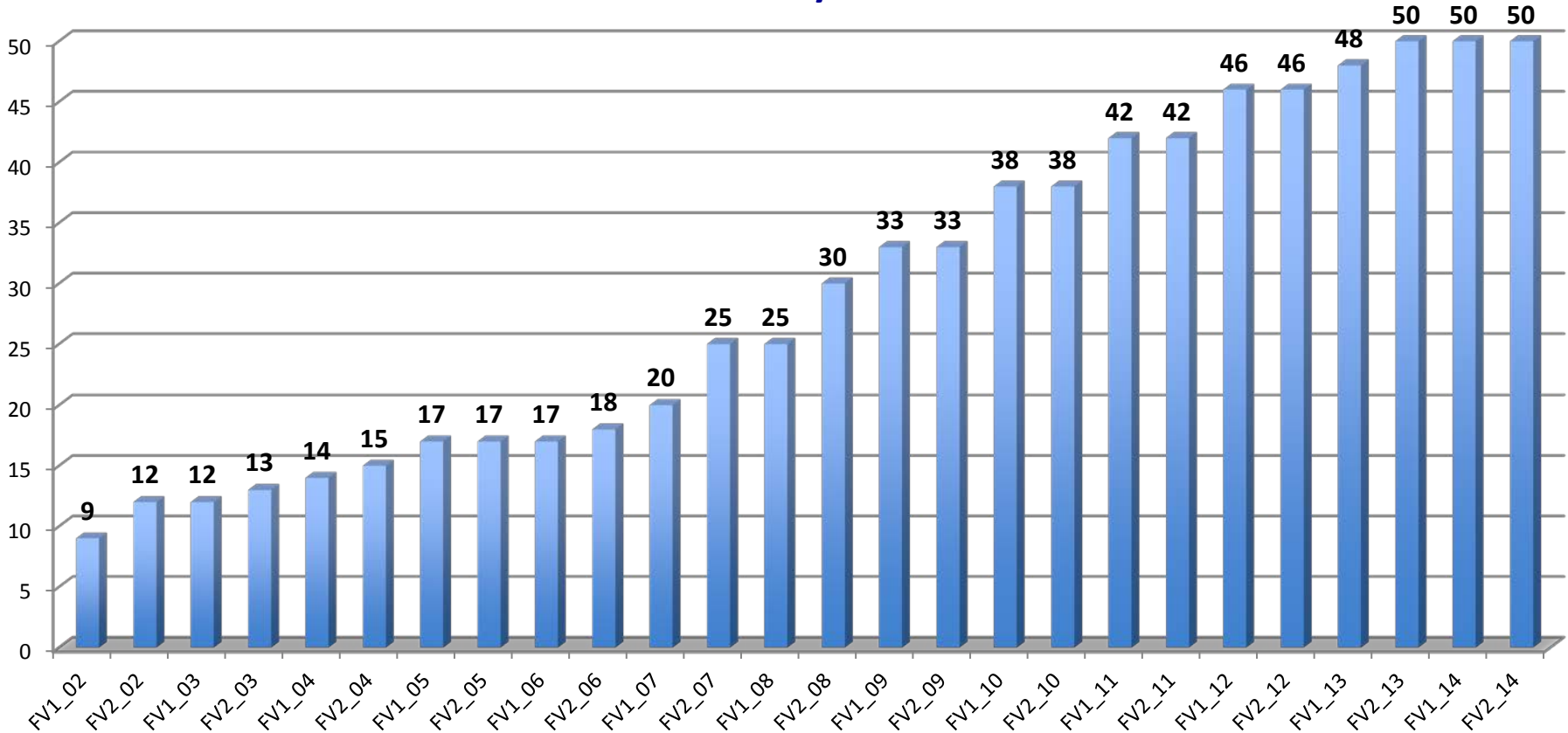
Countries	Number of Participants	Number of Participants (%)
Germany	131	35.1
Austria	34	9.1
Swiss	28	7.5
France	39	10.5
Benelux	39	10.5
Other Europe	71	19.0
North America	15	4.0
South America	6	1.6
Oceania	9	2.4
Middle East	1	0.3

Increment of participating laboratories in the last two EQAs compared to 2002



FV-EQA: scope

Number of analytes offered



FV-EQA: scope

Set A: FV-Leiden, Prothrombin, MTHFR (C677T, A1298C), PAI-I 4g5g

Set B: FXIII V34L, GPIIIa, β Fib g-455a, VKORC1 (g-1639a/c1173t), FXII c-46t, FV H1299R

Set C: a1 PI, Apo E, Apo B100, ACE, CETP

Set D: TPMT, Cyp2C19 *1/*2/*17, Cyp2C8 (K399R), Cyp2C9 *2/*3, UGT1a1 (*28), DPD Exon 14 skipping, BCHE A/K

Set E: ALDO B (149/174/334), HFE (H63D, C282Y, S65C), LCT c-13910t, NOD2 (R702W, G908R, L1007fins C)

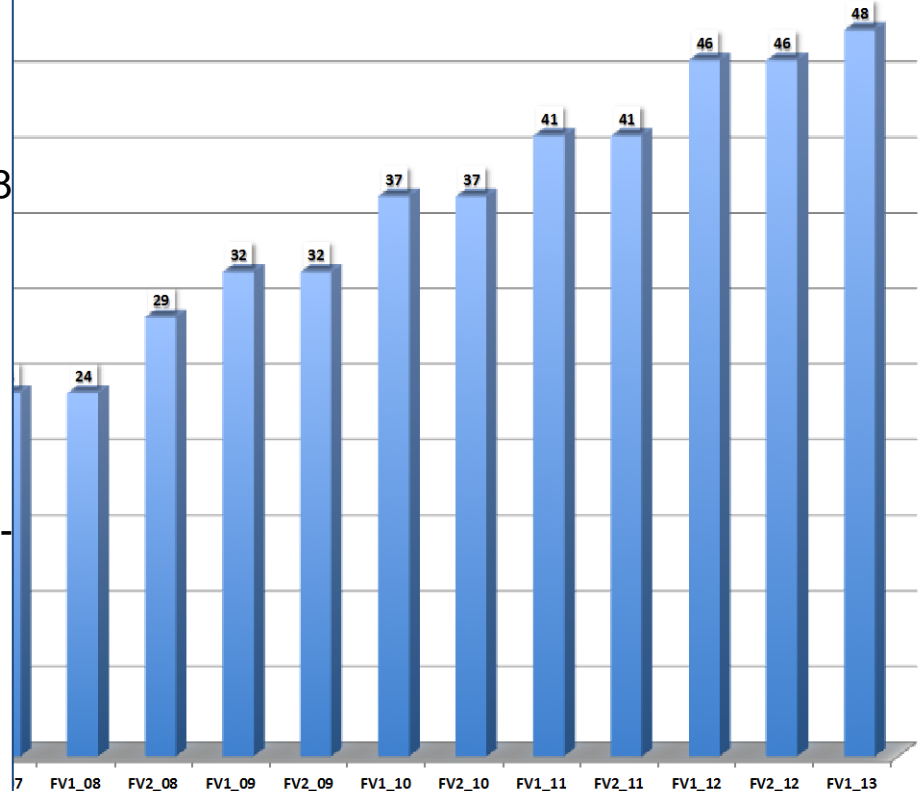
Set F: M. Wilson ATP7B-C3207 A, FSAP (Marburg-I), ITGA2 Gplalla C807T, Col1A1 SP1, VDR (BsmI, ApaI, TaqI)

Set G: K-Ras: Codon 12/13/61, BRAF V600E

Set H: HLA-B27

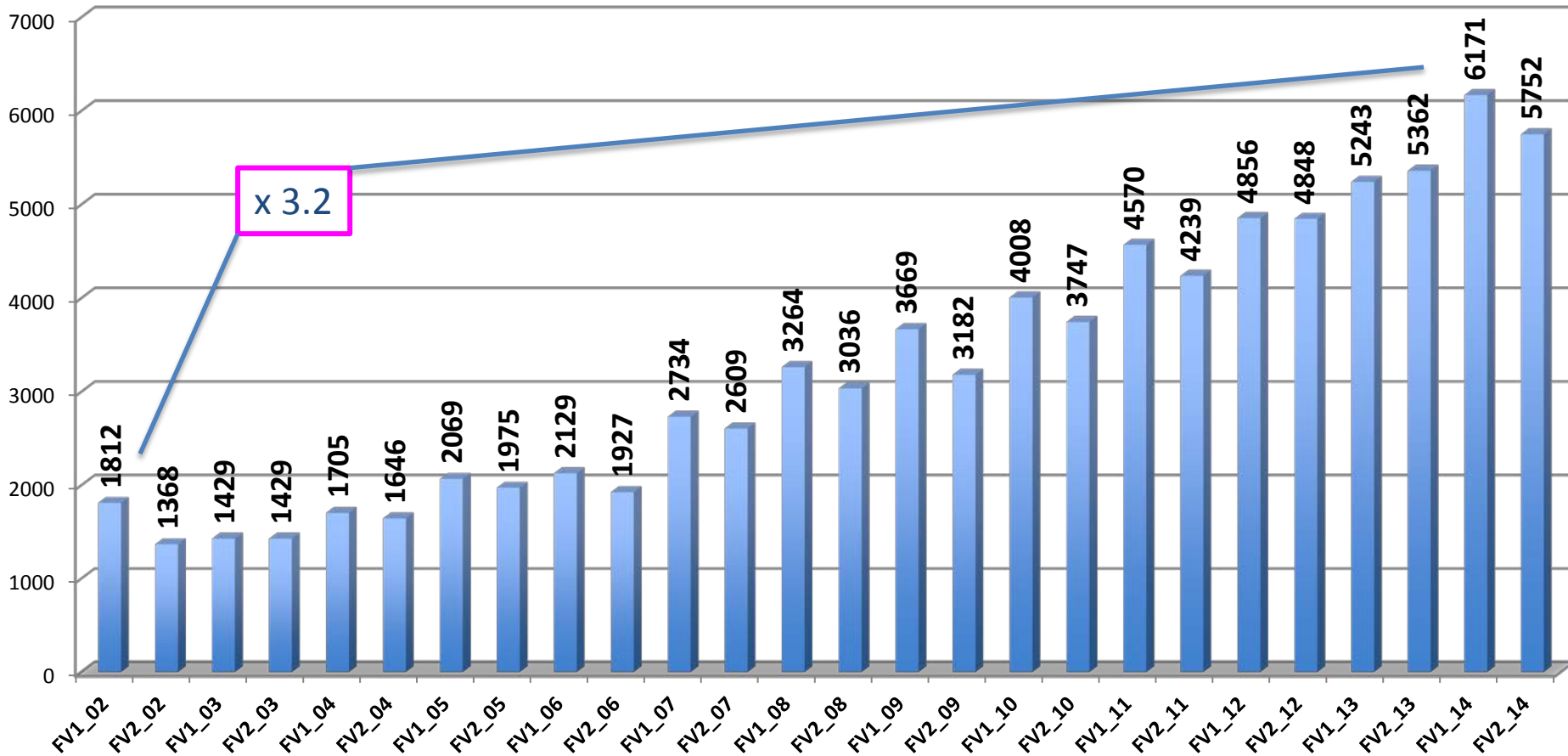
Set I: Cyp2D6

bytes offered



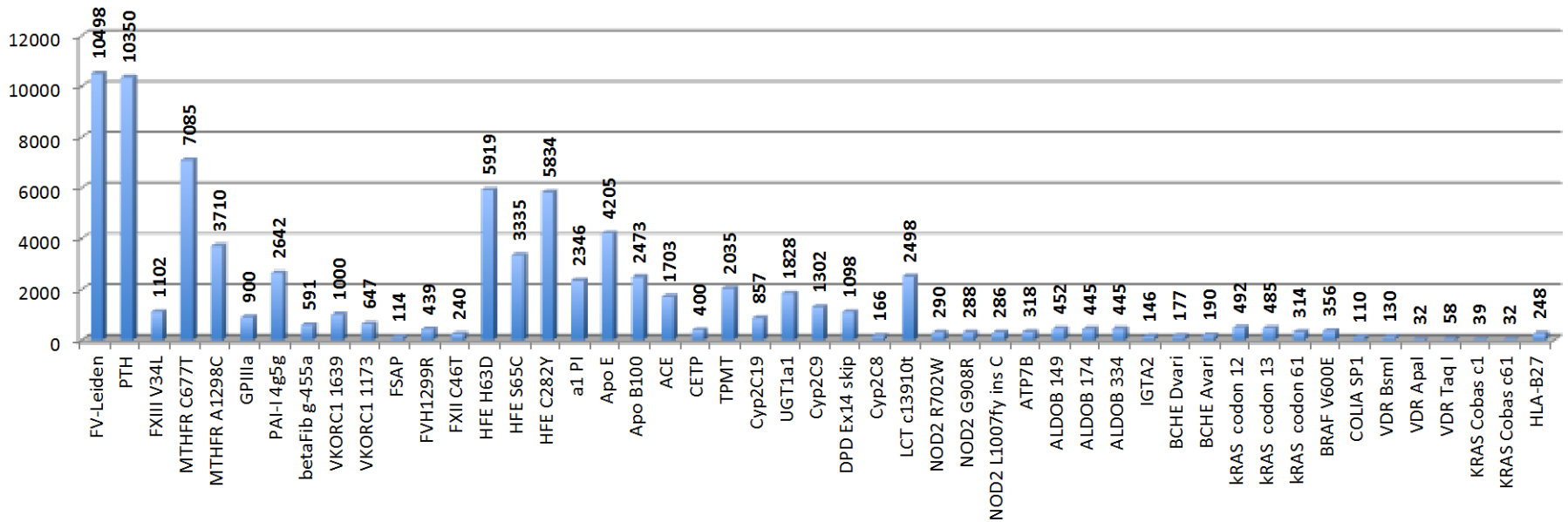
FV-EQA: scope

Number of genotypes determined

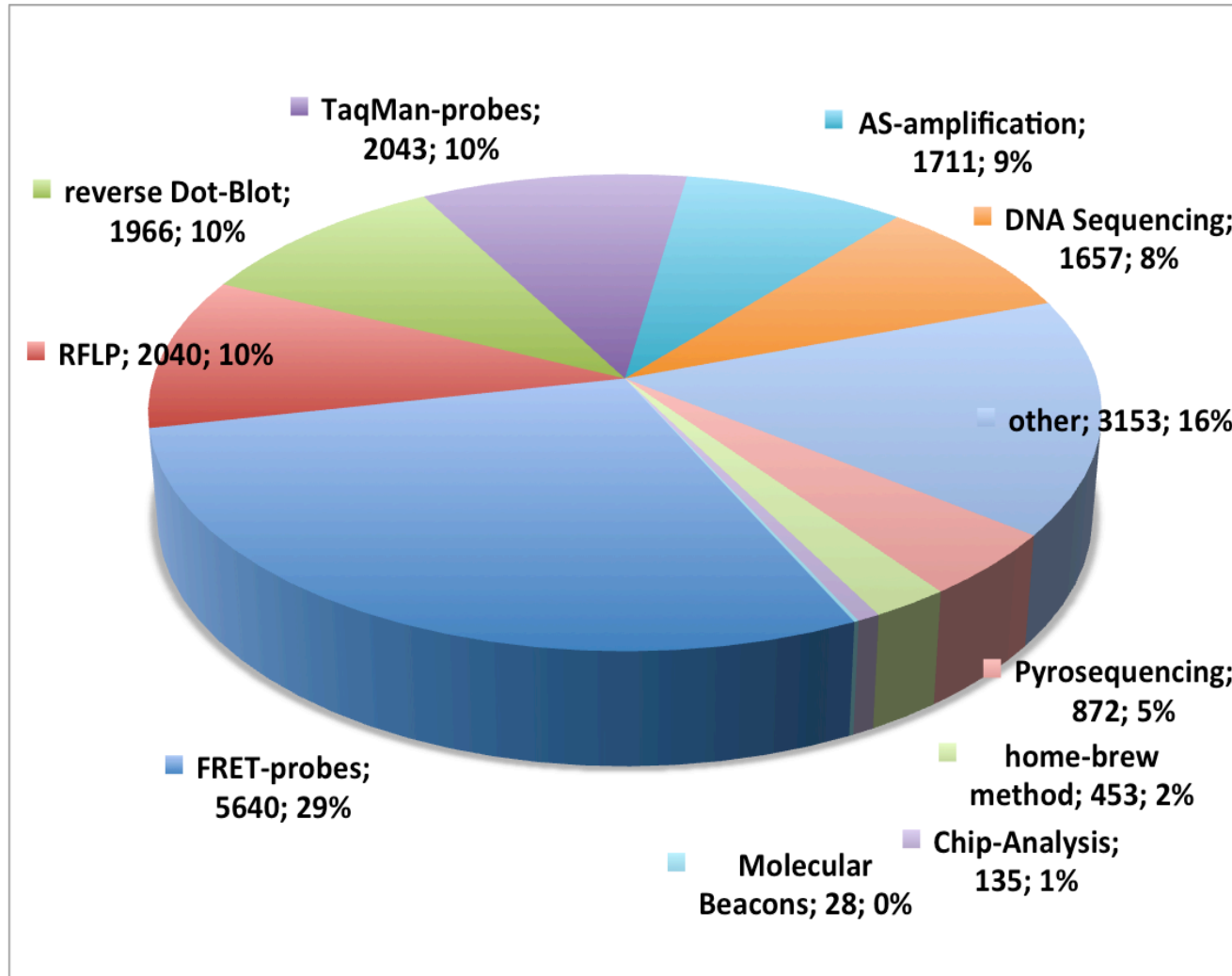


FV-EQA: scope

Number of genotypes determined per analyte

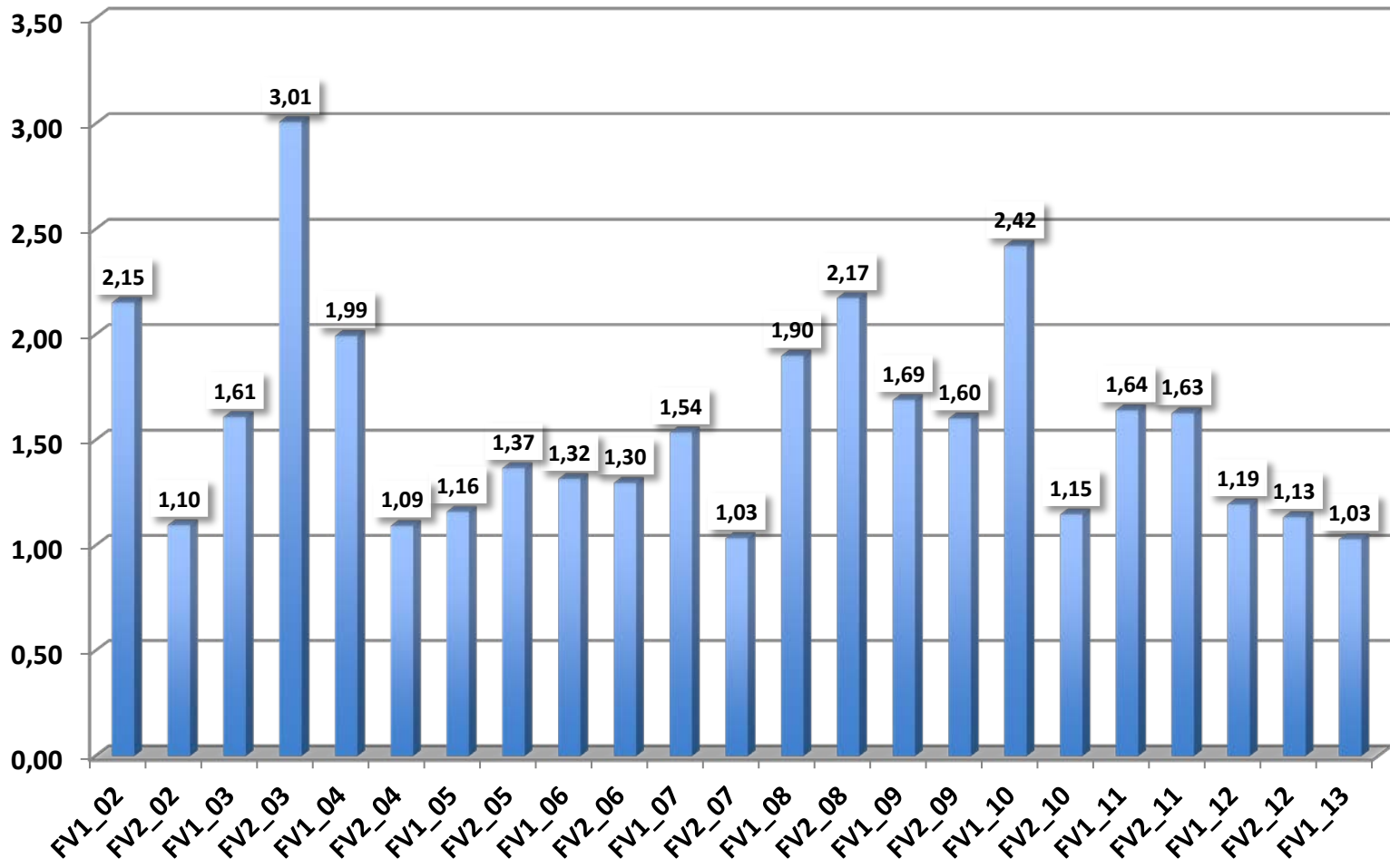


FV-EQA: methods



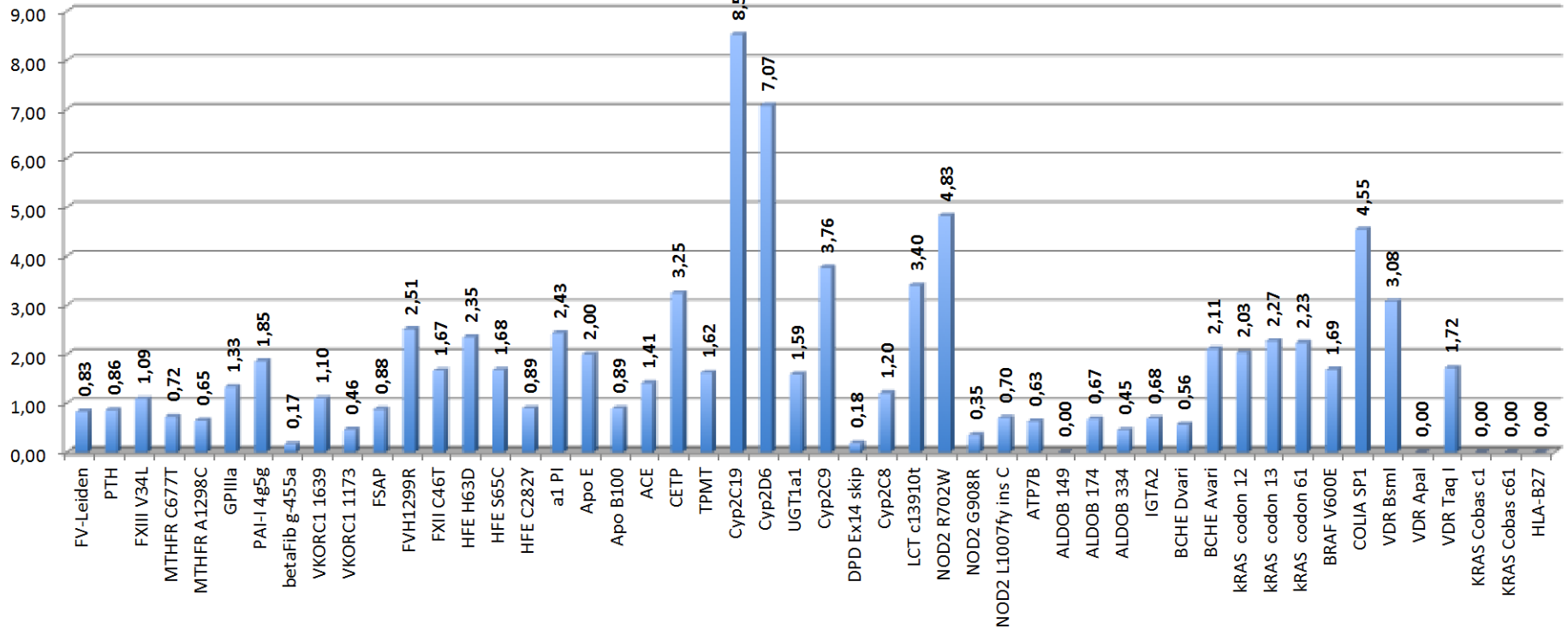
FV-EQA: error rate

Mean error rate (%)



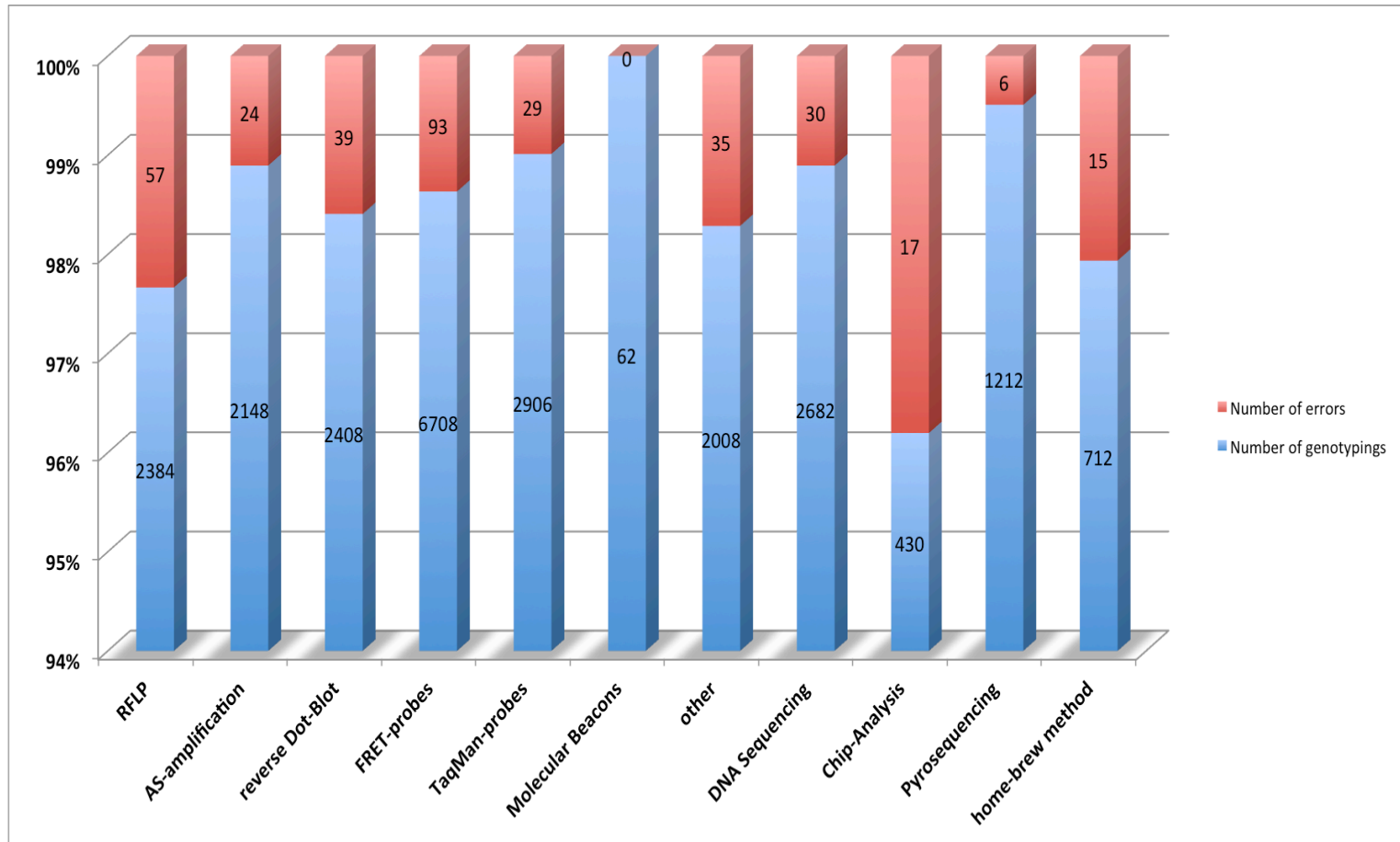
FV-EQA: error rate

Mean error rate for each analyte



FV-EQA: error rate

Error rate depending on method used



FV-EQA: error rate

rs-Number	Analyte	RFLP	ASA	rev Dotblot	FRET	Taqman	Molecular Beacons		DNA seq	Chip	Pyro	homebrew
		%error/ %method used	%error/ %method used	%error/ %method used	%error/ %method used	%error/ %method used	%error/ %method used	other	%error/ %method used	%error/ %method used	%error/ %method used	%error/ %method used
rs6647, rs17580, rs28929474	a1-Proteinase-Inhibitor	20/8,82	20/12,28	10/11,94	30/35,29	10/7,27	0/0	0/3,63	10/11,94	0/0	0/5,36	0/3,46
rs1799752	ACE	50/14,46	0/18,46	33,33/9,23	0/13,85	0/0,31	0/0	16,67/28	0/0,31	0/0	0/2,46	0/12,92
rs1800546, rs76917243, rs78340951	ALDO B	0/5,5	0/3,21	0/27,06	0/6,88	0/0,92	0/0	66,67/11,47	0/38,53	33,33/0,92	0/4,59	0/0,92
rs5742904	ApoB100	100/16,75	0/6,04	0/7,6	0/29,53	0/1,73	0/0	0/4,49	0/26,42	0/0	0/5,53	0/1,9
rs429358, rs7412	ApoE	16,67/21,72	0/6,66	5,56/11,58	44,44/32,14	5,56/7,72	0/0	11,11/2,41	5,56/11,78	11,11/0,48	0/4,05	0/1,45
rs76151636	ATP7B-C3207A	0/2,26	0/3,01	0/0	0/52,63	0/0,75	0/0	0/6,77	0/30,83	0/0	0/2,26	0/1,5
rs1799807, rs1803274	BCHE	0/2,22	0/6,67	0/0	0/10	25/7,78	0/0	25/5,56	50/56,67	0/0	0/11,11	0/0
rs1800790	beta-Fibrinogen g-455a	0/18,23	0/8,33	0/18,23	0/16,15	0/11,46	0/0	0/10,42	0/10,94	0/0,52	0/5,73	0/0
rs113488022	BRAF-V600E	0/0	20/4,22	0/9,04	20/6,02	20/16,27	0/0	0/3,61	40/33,13	0/0	0/21,08	0/6,63
rs708272	CETP	50/43,7	0/0,84	0/0	0/21,85	0/3,36	0/0	16,67/15,97	0/4,2	33,33/5,04	0/2,52	0/2,52
rs1800012	COLIA1 Sp1	25/33,33	0/0	25/17,65	0/3,92	50/11,76	0/0	0/13,73	0/7,84	0/0	0/5,88	0/5,88
rs4244285, rs12248560	CYP2C19	22,22/8,76	0/3,02	5,56/5,44	33,33/21,75	16,67/26,28	0/0,6	0/3,63	0/12,08	11,11/7,55	5,56/6,65	5,56/4,23
rs10509681	CYP2C8	0/0	0/0	0/0	0/0	0/21,62	0/0	0/9,46	100/54,05	0/0	0/14,86	0/0
rs1799853, rs1057910	CYP2C9	30/10,83	0/1,08	20/6,5	30/30,87	10/18,95	0/0	0/3,25	10/15,34	0/1,08	0/9,93	0/2,17
rs3918290	DPD Exon 14 skipping	0/6,71	0/5,7	0/3,69	100/44,3	0/12,58	0/0	0/2,18	0/9,23	0/0,5	0/12,42	0/2,68
rs5985	F XIII (V34L)	33,33/12,65	33,33/15,74	33,33/10,8	0/39,81	0/10,49	0/0	0/4,32	0/4,63	0/0,31	0/0,93	0/0,31
rs1799963	Faktor II 20210	30/9,72	10/12,52	10/11,99	40/36,83	0/14,92	0/0,33	0/7,97	0/1,54	0/0,69	0/1,83	10/1,67
rs6025	Faktor V (Leiden)	22,22/8,68	11,11/12,66	22,22/11,86	44,44/36,54	0/15,19	0/0,32	0/8,32	0/1,77	0/0,68	0/1,89	0/2,09
rs7080536	FSAP	0/18,18	0/9,09	0/0	0/13,64	0/0	0/0	0/18,18	0/15,91	0/0	0/25	0/0
rs1800595	FV H1299R	0/21,81	25/10,11	25/19,68	25/23,4	0/1,06	0/0	25/5,32	0/12,77	0/0	0/5,85	0/0
rs1801020	FXII c46t	0/10	0/0,91	0/0	66,67/10,91	33,33/32,73	0/0	0/10	0/15,45	0/0	0/18,18	0/1,82
rs5918	GP IIIa (L33P)	75/21,2	12,5/8,8	0/14,4	12,5/30	0/6,8	0/0	0/6	0/4,4	0/2,8	0/1,2	0/4,4
rs17999445, rs1800562, rs1800730	HFE	8,33/11,58	3,33/8,65	10/14,5	48,33/33,47	13,33/12,84	0/0,4	6,67/8,58	5/4,06	3,33/0,53	0/3,26	1,67/2,13
	HLA-B27	0/0	33,33/26,52	33,33/16,67	0/8,33	0/1,52	0/3,03	0/21,97	0/1,52	0/10,61	0/0	33,33/9,85
rs1126643	ITGA2 Gplalla	0/16,18	0/0	0/0	0/55,88	0/0	0/0	0/17,65	0/0	0/0	0/4,41	0/5,88
rs121913530, rs121913529, rs121913535, rs112445441, rs121913238, rs121913240, rs17851045	K-RAS	4,76/3,85	4,76/7,26	28,57/15,38	0/2,99	0/3,85	0/0	14,29/7,26	23,81/28,63	9,52/2,14	14,29/21,79	0/6,84
rs4988235	LCT C-13910-T	12,5/7,81	16,67/9,65	12,5/20,72	25/32,81	4,17/2,95	0/0,1	16,67/9,55	0/6,93	0/0,21	0/6,65	12,5/2,62
rs1801133, rs1801131	MTHFR	26,32/14,25	5,26/11,76	10,53/12,5	42,11/31,11	5,26/14,76	0/0,11	5,26/7,18	0/3,17	5,26/0,45	0/3,17	0/1,53
rs2066844, rs2066845, rs2066847	NOD2	0/11,21	0/2,59	0/1,72	33,33/11,21	0/18,1	0/0	0/7,76	66,67/44,83	0/0	0/2,59	0/0
rs1799889	PAI-1	25/9,28	25/17,31	25/15,81	12,5/25,85	0/6,9	0/0	0/8,66	0/8,28	12,5/0,75	0/5,4	0/1,76
rs1800460, rs1800462, rs1142345	TPMT	14,29/9,42	7,14/5,38	21,43/2,99	21,43/33,48	28,57/20,33	0/0	7,14/4,19	0/14,65	0/0	0/5,38	0/4,19
rs34815109	UGT1A1	0/0,17	0/1,02	0/1,36	22,8/26,32	0/0,51	0/0	34,2/21,56	34,2/31,75	0/0,17	5,7/8,66	3,11/8,49
rs7975232	VDR-Apal-rs7975232	0/11,76	0/0	0/0	0/0	0/0	0/0	0/29,41	0/35,29	0/0	0/17,65	0/5,88
rs1544410	VDR-BsmI-rs1544410	20/21,54	0/0	20/13,85	0/12,31	0/6,15	0/0	0/18,46	40/10,77	0/0	20/13,85	0/3,08
rs731236	VDR-TaqI-rs731236	0/20,69	0/0	0/0	0/0	0/0	0/0	100/27,59	0/34,48	0/0	0/10,34	0/6,9
rs9923231, rs9934438	VKORC 1	42,86/10,35	0/4,97	0/3,93	14,29/32,51	28,57/14,29	0/0	14,29/5,8	0/18,01	0/0,41	0/9,11	0/0,62

FV-EQA: error types

Evaluation of the FV1_09

n Resultatbox/Anzahl der Ergebnisse
 richtiges Ergebnis
 Ihr richtiges Ergebnis
 Ihr falsches Ergebnis

1 Faktor V (Leiden)

Allele:	Probe/Sample	R/R	R/Q	Q/Q
R: R506	01	224		1
Q: Q506	02	226		

2 Faktor II 20210

Allele:	Probe/Sample	G/G	G/A	A/A
G: G20210	01	7	1	219
A: A20210	02	223	1	2

3 MTHFR

Allele:	Probe/Sample	C/C	C/T	T/T	A'A'	A'C'	C'C'
C: C677	01	165	1				95
T: T677	02	167			94		

4 PAI-1

Allele:	Probe/Sample	4/4	4/5	5/5
4: 4G	01	62	1	
5: 5G	02		64	

Errors

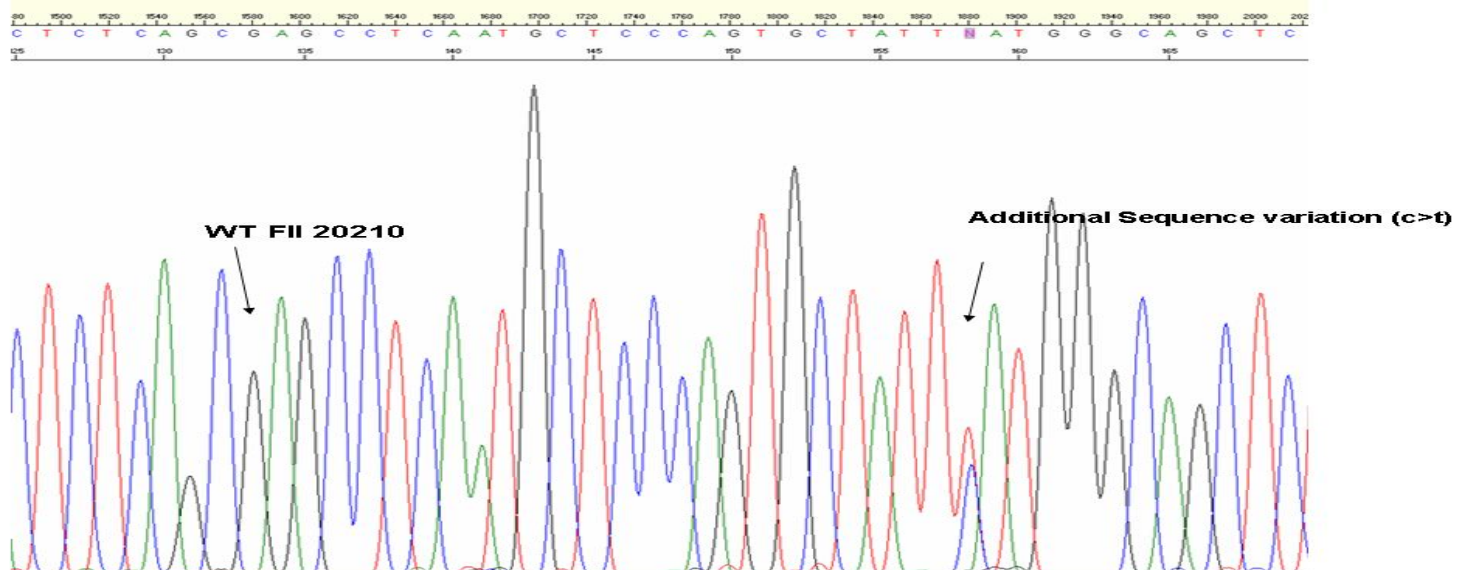
–Reporting (e.g. LCT)

–Analytic

❖ by rare sequence variations, which result in non-standard genotyping (frequently specific for a genotyping method)

❖ Inadequate genotyping (e.g. CYP2D6)

FV-EQA: error types



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Conclusions

1. Increasing number of participants, analytes offered and analytes determined per laboratory
2. Changes in respect to the methods used for genotyping over the years
3. Identification of best-in-class methods
4. Reduction of the overall error rate by EQA scheme participation
5. Determination of inappropriate methods per genotype

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Future development

Separation of FV into MG1 and MG2:

- Analytes being included: FVII R353Q, AT3 Cambridge, CYP3A5*3, TNFalpha 238 and 308, HLA-B*5701, CYP2B6, IL28B C/T polymorphism, IL6, CYP3A4*22

New EQAs

- EQA scheme for isolation of circulating nucleic acids
- EQA scheme for NGS

Questions?

