

Ringversuche für Mutationsanalytik

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Basic Truths about Quality

Murphy's Law

Anything that can go wrong, will
go wrong

W.I. Lenin

Trust is good, Control is better

Quality has its price



Genetics and the Control of Quality

What is Quality in Genetics ?

Quality Control in Genetics : Why ? Why Not ?

Available QC Procedures

Solutions for more and better QC

Challenges in Genetic QC

What is Quality in Genetics ?

Quality is the probability of correct results

Quality applies to

Qualitative results (Genotyping)

Quantitative results (Nucleic Acid Concentrations)

Concept : Quality in the Clinical Laboratory

Quality Control in Genetics : Why ?

- **Reliability** is essential for decisions : diagnostic, therapeutic or prognostic
- Genetic results reflect a permanent property of the individual (Genotype) of greater potential importance than most non-genetic parameters → **Ethics !**
- Genetic analyses may cost a lot more than non-genetic analyses → **Money !**

Quality Control in Genetics :

Why Not ?

- **Scarcity** of Control Materials : Rare Diseases / Ethical Constraints

- **Economy** in Testing for multiple Mutations

11 mutation AGS testing

1 patient + 1 wild type plus 11 controls = 1 result per 13 reactions

- „Our tests are inherently correct“

(Kit manufacturer statement, with CE !)

Quality Control in Genetics : Internal QC Materials

- **Contained** in some kits, usually platform-dependent (plasmids), some genomic DNA
- **Limited** commercial availability
- **Lacking** in many kits, particularly for testing of multiple mutations
- **Lacking** in products : „Oligo-Only-Companies“

Money : Costs up, Control down

- Basic Clinical Chemistry
K⁺ : < 10 CHF / < 1 €
Internal and External QC available and obligatory
- Basic Genetic Parameters
HFE Genotyping : 100 CHF / 70 €
Internal and External QC available but voluntary
- Complex Genetic Parameters
CYP21 AGS (11 mutations) : 1100 CHF / 770 €
Internal QC not, External QC available but voluntary

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Gute RV-Übersicht bei :

www.eurogentest.org



- EQUALIS¹⁵
- INSTAND¹¹
- LABQUALITY¹⁶ (in cooperation with EQUALIS)
- ÖQUASTA⁴ (in collaboration with DGKL)
- UKNEQAS^{13, 14}
- EMQN¹⁰
- UKNEQAS^{13, 14}
- DGKL²

Organisation

DGKL² (RfE)¹²

ECAT⁷ (in collaboration with DGKL)

ÖQUASTA⁴ (in collaboration with DGKL)

IEQA-ISS¹⁹

EMQN¹

● INSTAND¹¹

DGKL² (RfE)¹²

ECAT⁷ (in collaboration with DGKL)

● INSTAND¹¹

ÖQUASTA⁴ (in collaboration with DGKL)

DGKL² (RfE)¹²

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● INSTAND¹¹

ÖQUASTA⁴ (in collaboration with DGKL)

Instand - Ringversuche - Auswertungen - Microsoft Internet Explorer

Adresse <http://www.instandev.de/ringversuche/auswertungen/>

Instand e.V.
Gesellschaft zur Förderung der Qualitätssicherung in
medizinischen Laboratorien e.V.

2008	[Jahr]	700 Ethanol (Klin. Anordnungen)	CFTR del F508
2008 August , Gerinnung	Rv 845	710 Aminosaeuren	LCT T-13910C
2008 6 Oktober	Rv 806	730 Faktor V-Leiden(Vollblut)	UGT1A6 A541G
2008 5 August	Rv 805	731 Prothrombin 20210(Vollblut)	BCHE A Variant
2008 4 Juli	Rv 804	732 MTHFR C677T (Vollblut)	BCHE K Variant
2008 3 Mai	Rv 803	739 Kollagenrezeptor GP Ia/IIa	GHR Del exon 3
2008 2 März	Rv 802	750 Carb.Def.Transferrin	HLA B5701
2008 2 April, Infektionsserologie	Rv 832	760 Cardiac Marker	CYP2C9 *2 Allele
2008 1 Januar	Rv 801	761 Cardiac Marker BNP	CYP2C9 *3 Allele
2007	[Jahr]	770 Molekulargenetik III	
2007 6 Nov., Infektionsserologie	Rv 734	771 Molekulargenetik IV	
2007 6 Okt. / Nov.	Rv 706	772 Molekulargenetik V	
		773 Molekulargenetik VI	

LCT T-13910C

RV / Split	Prb	ZW	Bew. Bereich	MW	Vk	Anz	Quote (%)	ges. (%)
RV 2008 6 Oktober								
M.0-99	61		1, TT -13910			28	96.4	96.4
	62		1, TT -13910			28	96.4	
	63		2, T -13910 C			28	100.0	

Instand e.V. - Gesellschaft zur Förderung der Qualitätssicherung in medizinischen Laboratorien e.V. (vormals Hämometerprüfstelle)

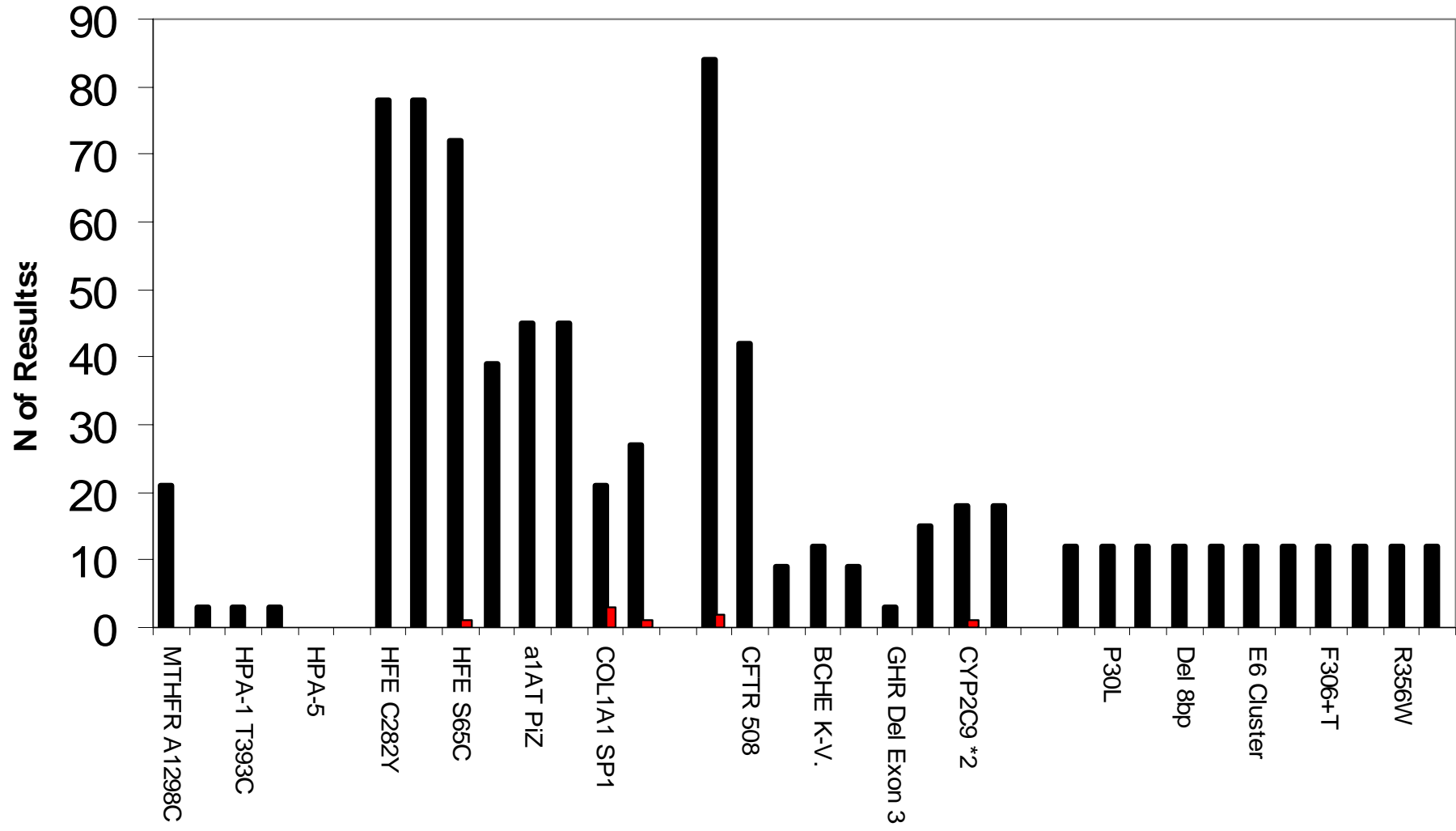
D-40223 Düsseldorf - Ubierstr. 20 - Postfach 250211 - Tel +49 211 159213 0 FAX +49 211 159213 30

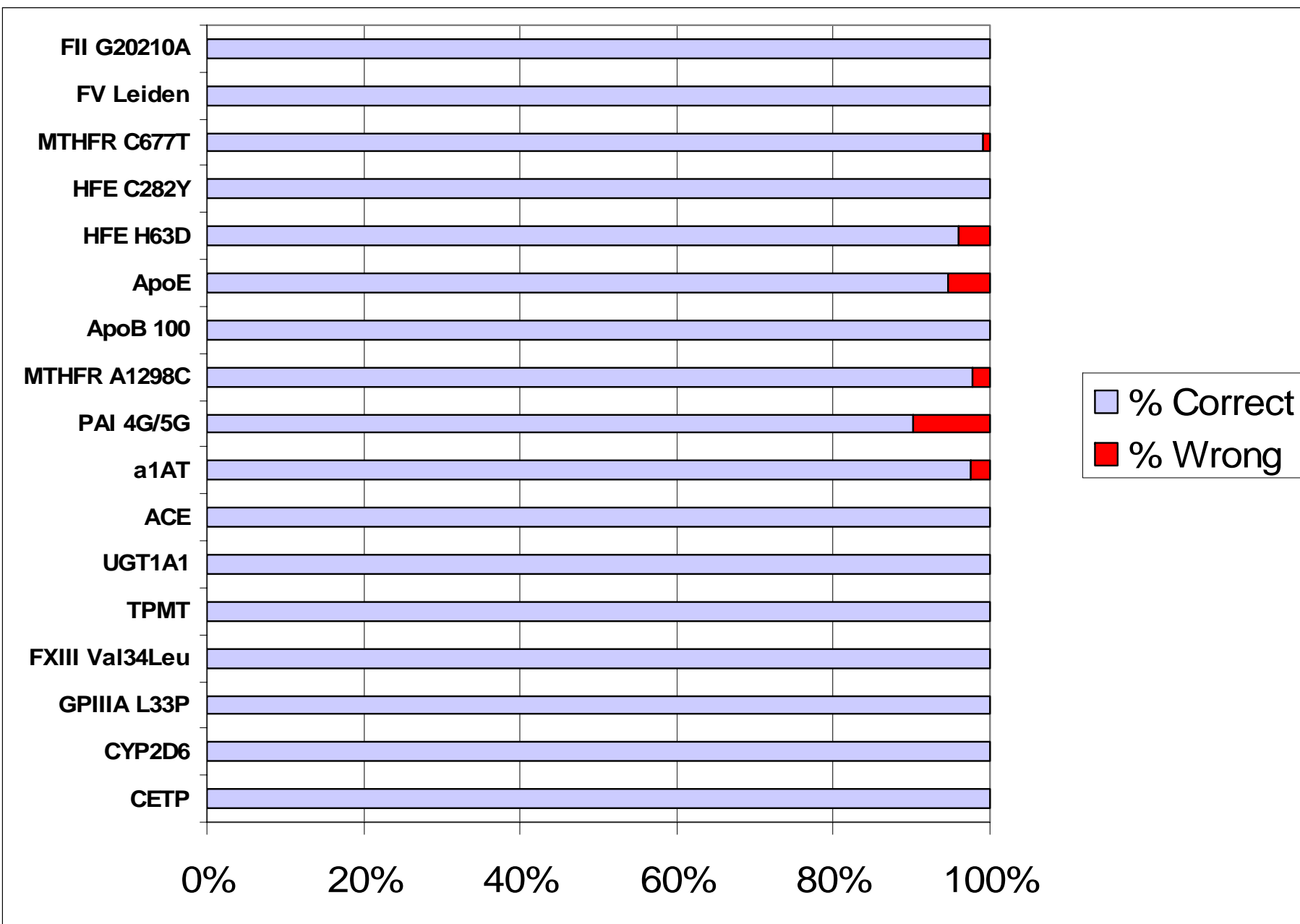
WHO Collaborating Centre for Quality Assurance and Standardization in Laboratory Medicine

Internet

Start | RV_770_7... | MD 2009 | Microsoft P... | RV 770_77... | Instand - R... | Instand - ... | 12:18

INSTAND RV 770 * 771 * 772 * 773 Maly Oct 08





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Postulates for a „Good Control“

- Well-defined, sustainable, reasonable cost
- Platform-independent (must work with different kits and home brews)
- Maximum information content (Multi-Heterozygotes)
- Extensively tested on multiple platforms

Sources of QC DNA available

- **Patients**

Limited availability, Ethical considerations, usually only single-locus data available

- **EBV-derived Cell Lines**

Several Collections exist but genotype information is limited

- **Whole Genome Amplification**

works well in QC but is cumbersome

- **BAC or YAC clones**

... Theoretical possibility

- **Synthetic Genes**

... Theoretical possibility



INSTAND : Single Target Concept (Flörke)

42 different samples to control 14 mutations

= 3 samples per target

Nr. 730 - 732 Molekulargenetik I

- je 2 Proben 0,5 ml humanes Vollblut - (Keine Mehrbedarfsbestellung möglich)

Nr. 730 Faktor V-Leiden-Mutation

Nr. 732 MTHFR C677T

Nr. 731 Prothrombin 20210

Nr. 739 - 749 Molekulargenetik II

- je 3 Proben, cDNA, in Wasser gelöst -

Set 1

Gerinnung

Nr. 740 Faktor V-Leiden-Mutation

Nr. 749 Fibrinogenrezeptor HPA 1a/1b

Nr. 739 Kollagenrezeptor C807T

Nr. 747 MTHFR C677T

Nr. 748 PAI-1 4G/5G

Nr. 746 Prothrombin 20210

Set 2

Enzyminhibitoren

Nr. 743 alpha1-Antitrypsin (PiM, PiS, PiZ)

Hämochromatose

Nr. 741 HFE (H63D, C282Y)

Lipoproteine

Nr. 744 Apolipoprotein E (E2, E3, E4)

M. Wilson

Nr. 745 Cu-Transporter-Protein: ATP7B (H1069Q)

Hyperbilirubinämie

Nr. 742 UDP-Glucuronyltransferase 1 (TATA-Box)




INSTAND : Multiple Target Concept (Maly)

18 different samples to control 48 mutations

= 0.375 samples per target

Nr. 770 - 775		Molekulargenetik III - VIII		(Maly / Fried)	
- 6 Sets zu je 3 Proben zu je 500 ng DNA -				Je Set	65,00 € / RV
					45,00 € Mehrbedarf
Nr. 770 Set 1			Nr. 771 Set 2		
F XIII Val34Leu	Gerinnung	HFE C282Y	Hämochromatose		
GPIA C807T	Gerinnung	HFE H63D	Hämochromatose		
HPA-1 T393C	Gerinnung	HFE S65C	Hämochromatose		
HPA-5 G1648A	Gerinnung	ApoE E2 E3 E4	Lipoproteine		
MTHFR A1298C	Gerinnung	a1-AT PiS	Enzyminhibitoren		
β-Fibrinogen G-455A	Gerinnung	a1-AT PiZ	Enzyminhibitoren		
F VII R353Q	Gerinnung	COL1A1 SP1 G/T	Kollagen (Osteoporose)		
Angiotensinogen M235T	Kreislauf	VDR Bsm1 B/b	Vitamin D Rezeptor (Osteoporose)		
ACE Ins / Del	Kreislauf				
Nr. 772 Set 3			Nr. 774 Set 5		
CFTR Delta F508	Cystische Fibrose	NOD2 R702W	Autoimmunität		
LCT T-13910C	Lactose-Intoleranz	NOD2 G908R	Autoimmunität		
UGT1A6 A541G	Pharmakogenetik	NOD2 L1007fs insC	Autoimmunität		
BCHE Atypische V.	Pharmakogenetik	MHC B27	Autoimmunität		
BCHE K-Variante	Pharmakogenetik	TNFα G-238A	Autoimmunität		
GH-R del exon 3	Pharmakogenetik	TNFα G-308A	Autoimmunität		
B5701	Pharmakogenetik, Abacavir-Hypersensitivität				
CYP2C9	Pharmakogenetik, Coumarin-Empfindlichkeit				
Nr. 773 Set 4			Nr. 775 Set 6		
CYP21B	21-Hydroxylase, AGS	AMH Ile 49 Ser	Pharmakogenetik		
		AMHR2 A-482G	Pharmakogenetik		
		FSH receptor	Pharmakogenetik		
		VKORC1 G-1639A	Pharmakogenetik		
		PRNP M129V	Nervensystem		
		Hämoglobin S	Sichelzell-Krankheit		

 Instand e.V. Postfach 25 02 11 D-40093 Düsseldorf BRD	Protokollbogen	RV 772
	Versand : 11. März 09 Einsendeschluss : 1. April 09	Molekulargenetik V 22.02.2009

Material : 40 ul gDNA in Gigen Elution Buffer (- 12.5 ng / ul) für 10 x PCR mit je 4ul.

Resultate : Bitte markieren Sie das entsprechende Feld durch ein Kreuz (X).

RV 772			▼	▼	▼			
	Probe →	772-1	772-2	772-3	Method	Reagenz	Bemerkungen	
1	CFTR F508	Wild Type	X					
		ΔF508 Het		X				
		ΔF508 HOM			X			
2	LCT T-13910C	TT -13910	X					
		T -13910 C		X				
		-13910 CC			X			
3	UGT1A6 A541G	AA 541						
		A 541 G						
		541 GG						
4	BCHE A Variant	Asp 70 (wt)	X					
		Asp 70 Gly		X	X			
		70 Gly Gly						
5	BCHE K Variant	Ala 539 (wt)						
		Ala 539 Thr						
		539 Thr Thr						
6	GHR Del exon 3	Wild Type						
		Del e3 Het						
		Del e3 HOM						
7	HLA B5701	NEGATIVE	X					
		POSITIVE		X				
8	CYP2C9 *2 Allele	CC 430						
		C430T						
		(Cys144Arg) 430TT						
9	CYP2C9 *3 Allele	AA 1075						
		A1075C						
		(Ile359Pro) 1075CC						

Methoden (Code) : RFLP (RFLP) * ASA - PCR (ASA) * Biot (Biot) * LightCycler (LC) * Rotorgene (RG) * Sequencing (SQ) * Andere (OTH)

Reagenz (Code) : Roche (RO) * Genes-4U (G4U) * Vienna Lab (VL) * Applied Biosys. (AB) * Qiagen (QG) * Hain (H) * Andere (OTH)

Ich bestätige, die Untersuchung selbst oder durch mir unterstellte Mitarbeiter in der eigenen Praxis/ in dem von mir gezielten Laboratorium durchgeführt und wie Patientenproben behandelt zu haben.



Stempel _____



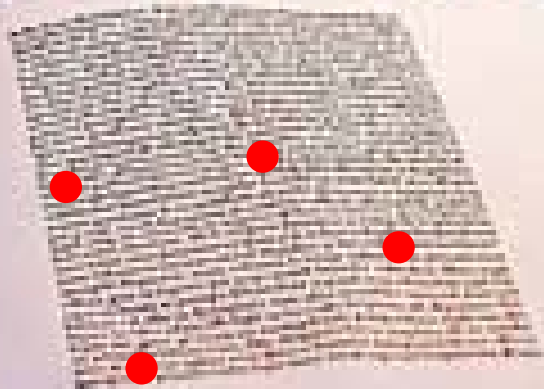
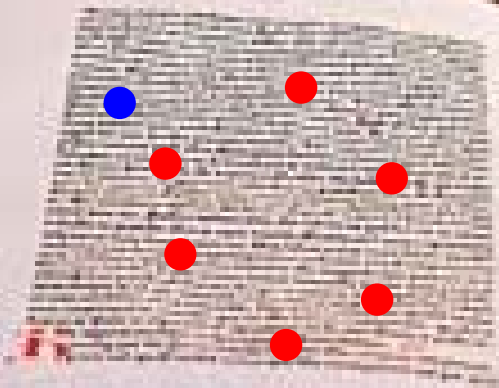
Unterschrift _____



Datum _____

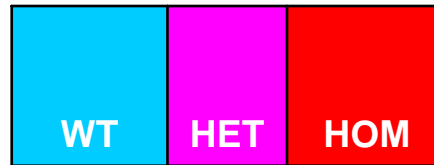
Single target

Multiple targets



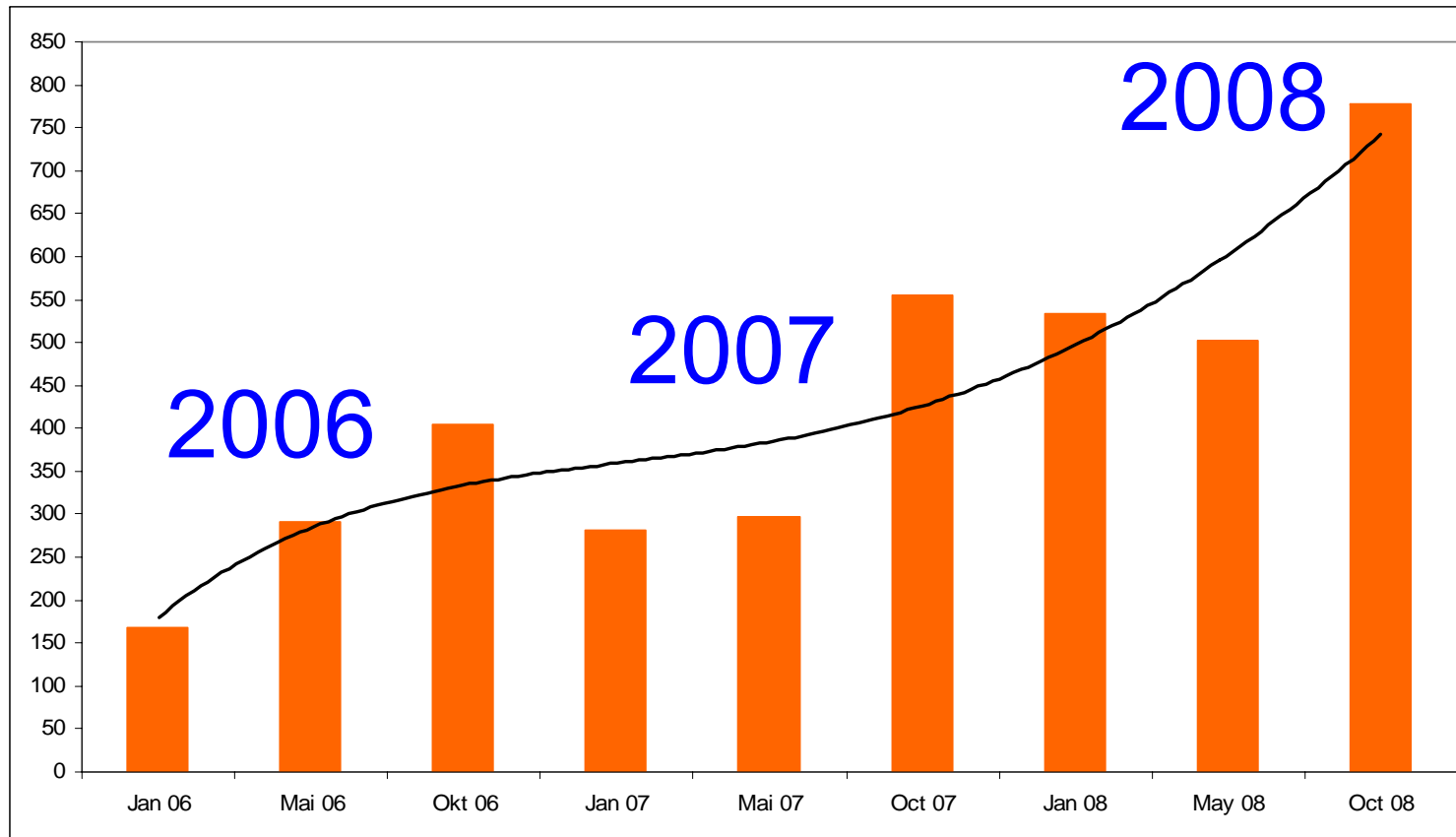
Multiple Target Concept

RV
772



Line Name	CFTR del F508	LCT T-13910C	UGT1A6 A541G	BCHE-Atyp.	BCH E KV	GHR fl/d3	HL A B57 01	CYP 2C9*2 C430T	CYP 2C9*3 A1075C
A	No Mut = wt	TT-13910	AA541	wt	HET	d3/d3	NEG	CC	AA
B	No Mut = wt	TT-13910	AA541	wt	HET	fl/d3	NEG	CC	AC
C	No Mut = wt	T-13910C	A541G	wt	wt	fl/fl	POS	CC	AA
D	No Mut = wt	T-13910C	A541G	HET	HOM	fl/fl	POS	CT	AA
E	No Mut = wt	TT-13910	A541G	wt	wt	fl/fl	POS	CT	AA
F	No Mut = wt	T-13910C	A541G	wt	wt	fl/fl	NEG	CT	AA
G	No Mut = wt	T-13910C	AA541	wt	wt	fl/fl	NEG	CC	AA
H	No Mut = wt	-13910 CC	A541G	HET	HET	fl/fl	NEG	CC	AA
I	dF508 HET	TT-13910	A541G	wt	wt	fl/fl	NEG	CT	AA

of Results in INSTAND Multiple Target Genetic EQA scheme



Genetics and the Control of Quality

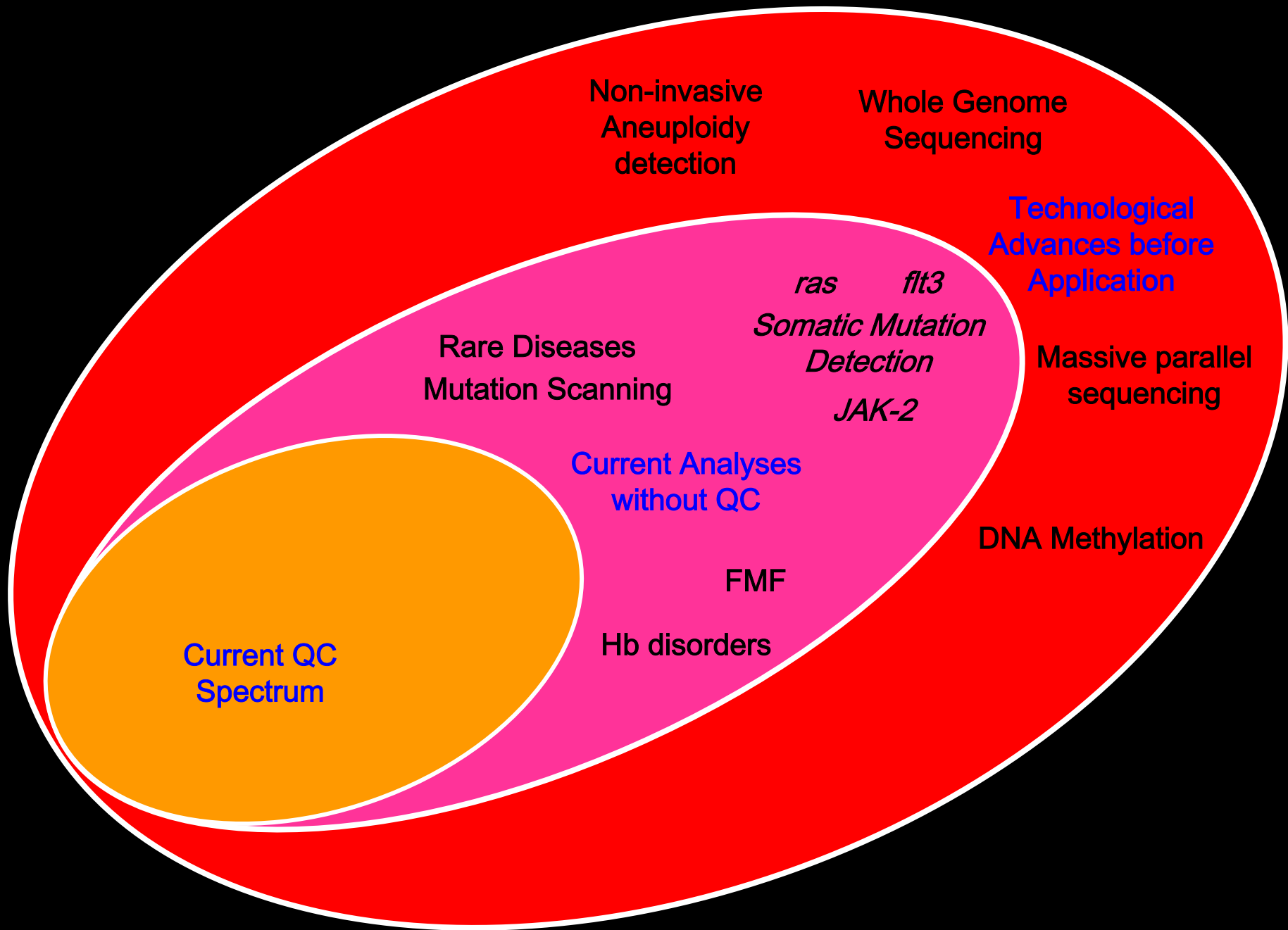
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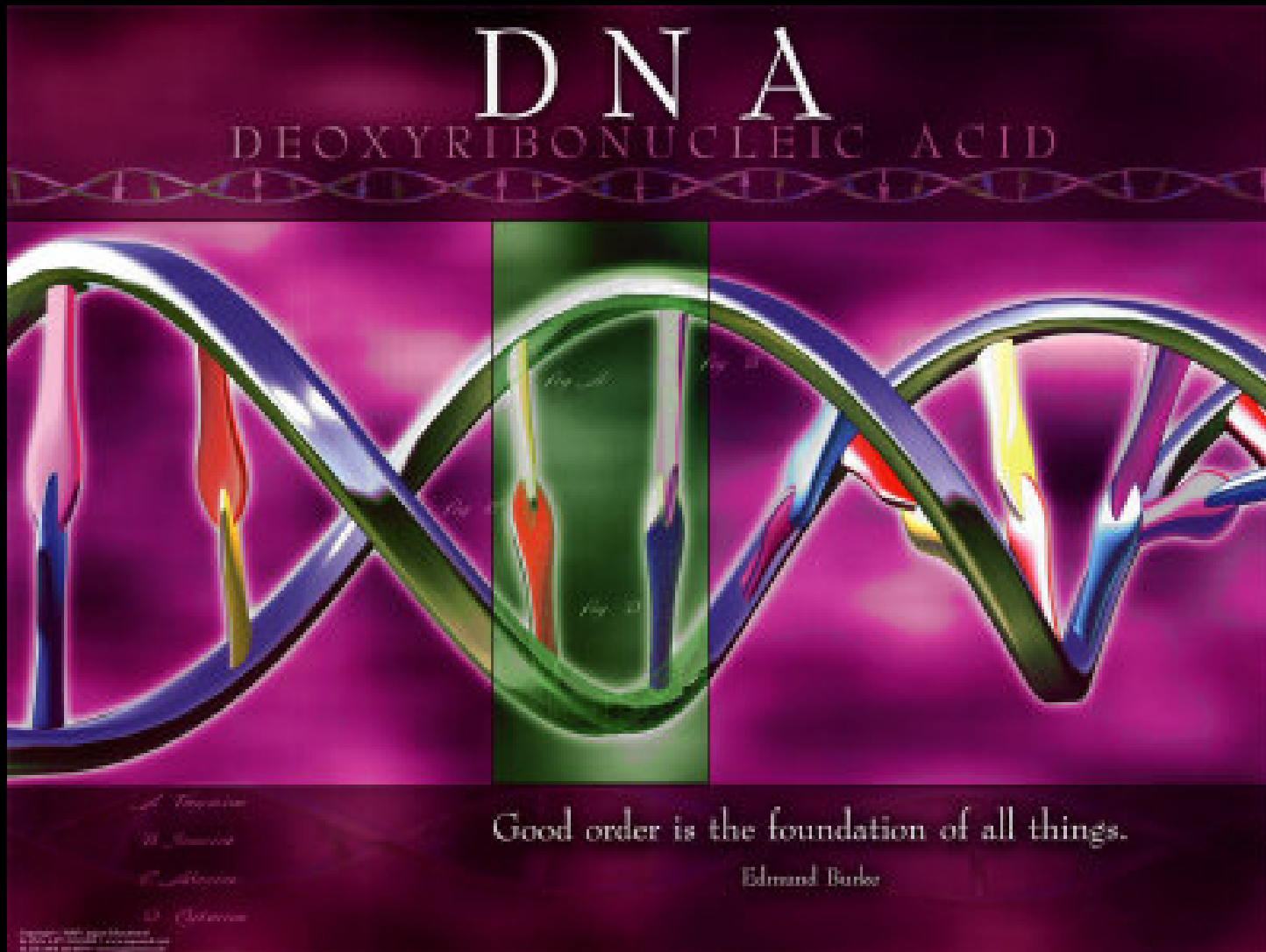
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Thanks for listening



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