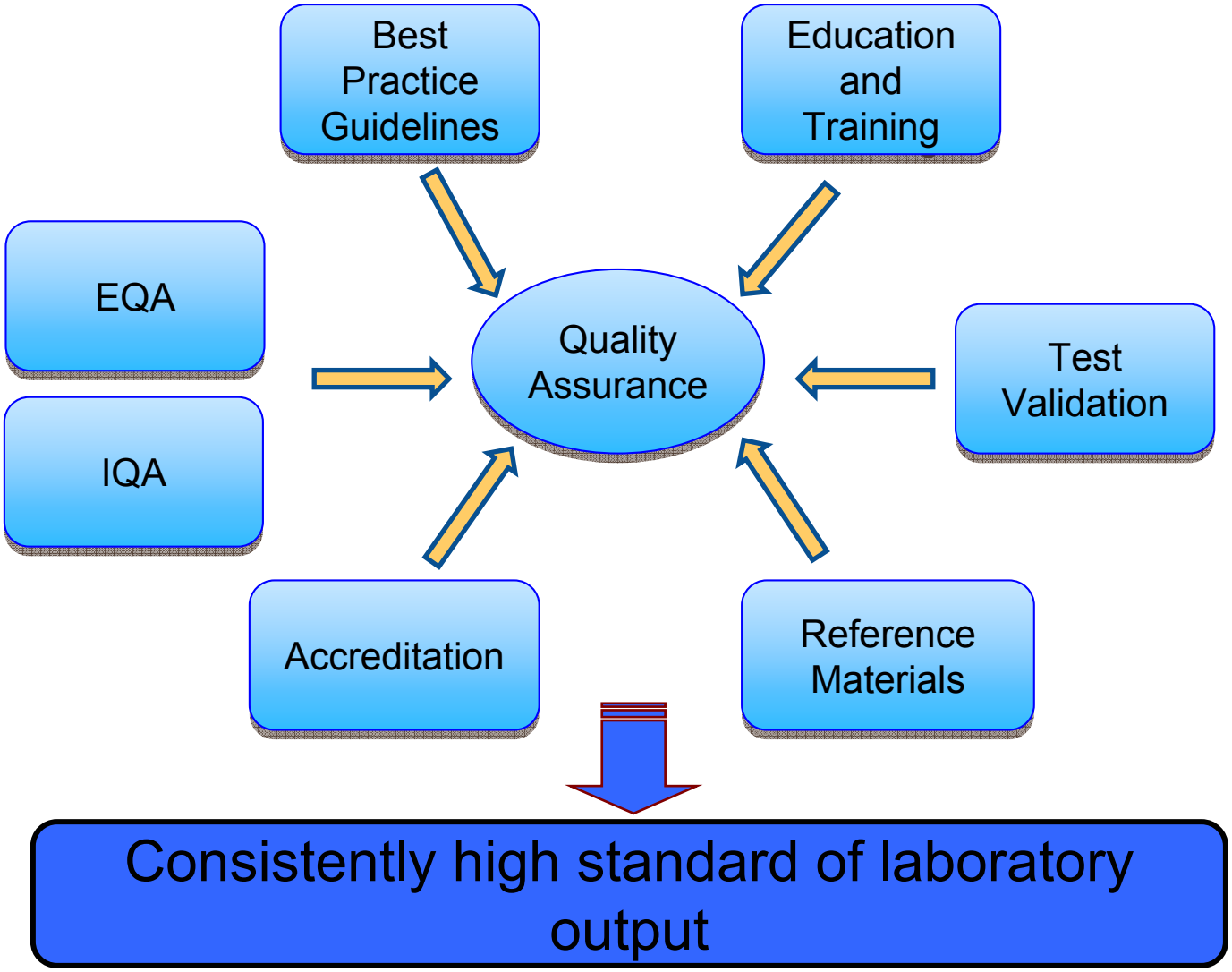
EMQN
The European Molecular Genetics Quality Network

Quality in the World of Molecular Genetics

David Barton, EMQN
National Centre for Medical Genetics
Dublin, Ireland

Quality Assured Genetic Testing

EMQN
The European Molecular Genetics Quality Network



Molecular Genetics Testing

EMQN
The European Molecular Genetics Quality Network

- Mostly yes / no answers
- TAT 3 to 40 days
- Scientists give the clinical interpretation of results
- Permanent medical record

Current Molecular Genetics Testing

EMQN
The European Molecular Genetics Quality Network

Mendelian disorders

- Cancer
- Neurological disorders
- Developmental delay
- Dysmorphology
- Etc.

Chromosomal imbalance

Molecular pathology

KRAS, GIST

Non-invasive prenatal diagnosis

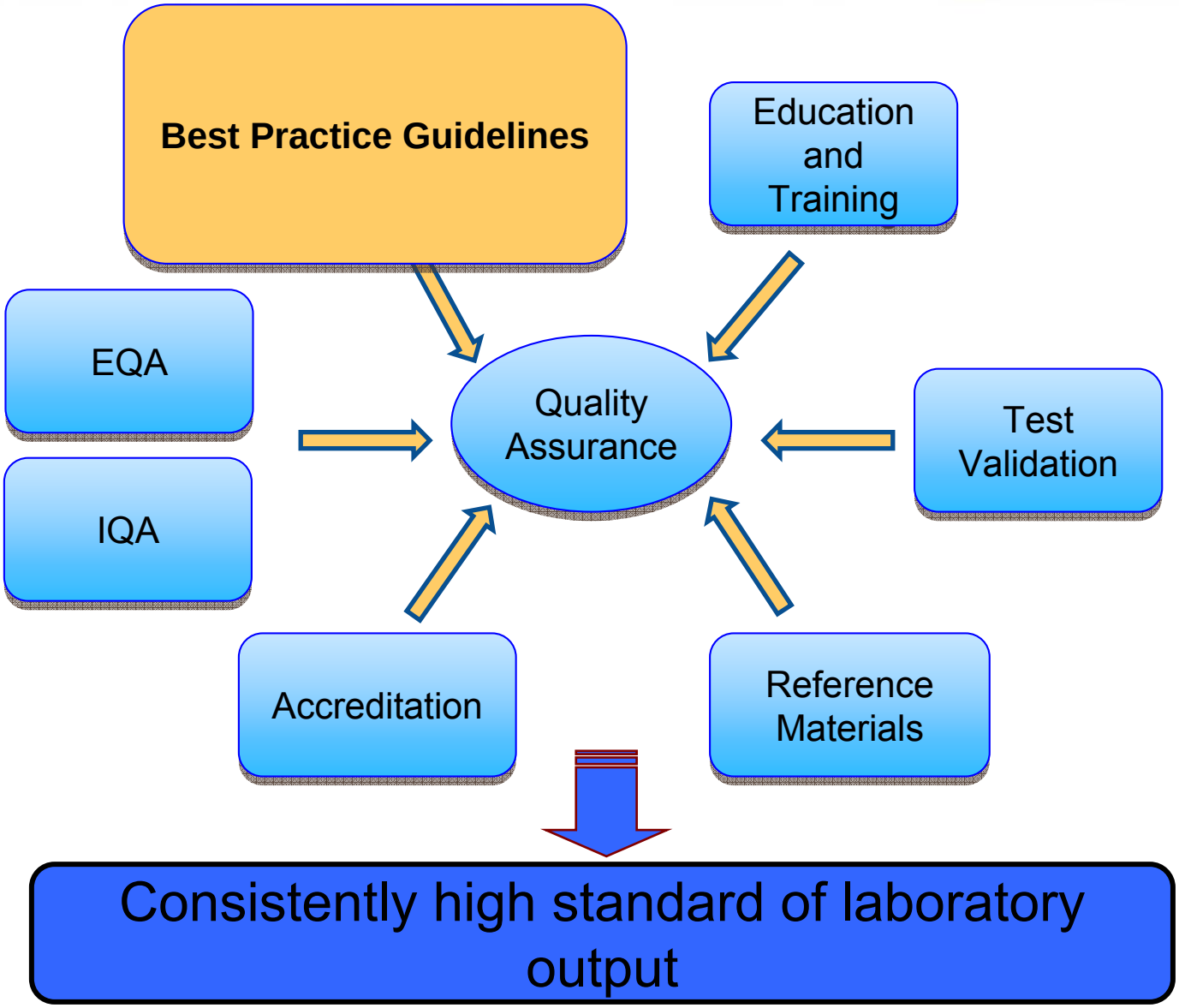
Future directions

EMQN
The European Molecular Genetics Quality Network

Free fetal DNA analysis

Next generation sequencing multi-gene disorders

Pharmacogenetics


Best Practice Guidelines
Education and Training
EQA
IQA
Quality Assurance
Test Validation
Accreditation
Reference Materials
Consistently high standard of laboratory output

Best practice

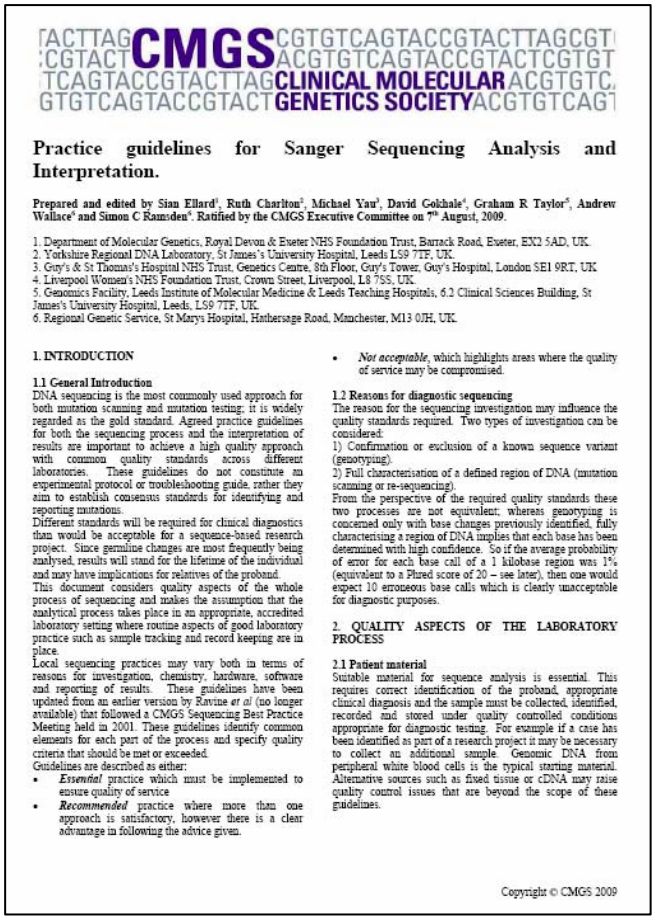
EMQN
The European Molecular Genetics Quality Network

- Sequencing
- Single nucleotide polymorphisms
- Unclassified variants – pathogenic or not?

Best practice

EMQN
The European Molecular Genetics Quality Network

- Sequencing
  - Uni – or bi-directional?
  - Genotyping
    - Confirmation or exclusion of known sequence variant
  - Mutation scanning
    - Full characterisation of a region of DNA



CMGS
CLINICAL MOLECULAR GENETICS SOCIETY

Practice guidelines for Sanger Sequencing Analysis and Interpretation.

Prepared and edited by Sian Ellard[1], Ruth Charlton[2], Michael Yau[3], David Gokhale[4], Graham R Taylor[5], Andrew Wallace[6] and Simon C Ramsden[6]. Ratified by the CMGS Executive Committee on 7th August, 2009.

1. Department of Molecular Genetics, Royal Devon & Exeter NHS Foundation Trust, Barrack Road, Exeter, EX2 5AD, UK.
2. Yorkshire Regional DNA Laboratory, St James's University Hospital, Leeds LS9 7TF, UK.
3. Guy's & St Thomas's Hospital NHS Trust, Genetics Centre, 8th Floor, Guy's Tower, Guy's Hospital, London SE1 9RT, UK.
4. Liverpool Women's NHS Foundation Trust, Crown Street, Liverpool, L8 7SS, UK.
5. Genomics Facility, Leeds Institute of Molecular Medicine & Leeds Teaching Hospitals, 6.2 Clinical Sciences Building, St James's University Hospital, Leeds, LS9 7TF, UK.
6. Regional Genetic Service, St Marys Hospital, Hathersage Road, Manchester, M13 0JH, UK.

1. INTRODUCTION

1.1 General Introduction

DNA sequencing is the most commonly used approach for both mutation scanning and mutation testing; it is widely regarded as the gold standard. Agreed practice guidelines for both the sequencing process and the interpretation of results are important to achieve a high quality approach with common quality standards across different laboratories. These guidelines do not constitute an experimental protocol or troubleshooting guide, rather they aim to establish consensus standards for identifying and reporting mutations.

Different standards will be required for clinical diagnostics than would be acceptable for a sequence-based research project. Since germline changes are most frequently being analysed, results will stand for the lifetime of the individual and may have implications for relatives of the proband.

This document considers quality aspects of the whole process of sequencing and makes the assumption that the analytical process takes place in an appropriate, accredited laboratory setting where routine aspects of good laboratory practice such as sample tracking and record keeping are in place.

Local sequencing practices may vary both in terms of reasons for investigation, chemistry, hardware, software and reporting of results. These guidelines have been updated from an earlier version by Ravine *et al* (no longer available) that followed a CMGS Sequencing Best Practice Meeting held in 2001. These guidelines identify common elements for each part of the process and specify quality criteria that should be met or exceeded.

Guidelines are described as either:

- *Essential* practice which must be implemented to ensure quality of service
- *Recommended* practice where more than one approach is satisfactory, however there is a clear advantage in following the advice given.
- *Not acceptable*, which highlights areas where the quality of service may be compromised.

1.2 Reasons for diagnostic sequencing

The reason for the sequencing investigation may influence the quality standards required. Two types of investigation can be considered:

1) Confirmation or exclusion of a known sequence variant (genotyping).

2) Full characterisation of a defined region of DNA (mutation scanning or re-sequencing).

From the perspective of the required quality standards these two processes are not equivalent; whereas genotyping is concerned only with base changes previously identified, fully characterising a region of DNA implies that each base has been determined with high confidence. So if the average probability of error for each base call of a 1 kilobase region was 1% (equivalent to a Phred score of 20 – see later), then one would expect 10 erroneous base calls which is clearly unacceptable for diagnostic purposes.

2. QUALITY ASPECTS OF THE LABORATORY PROCESS

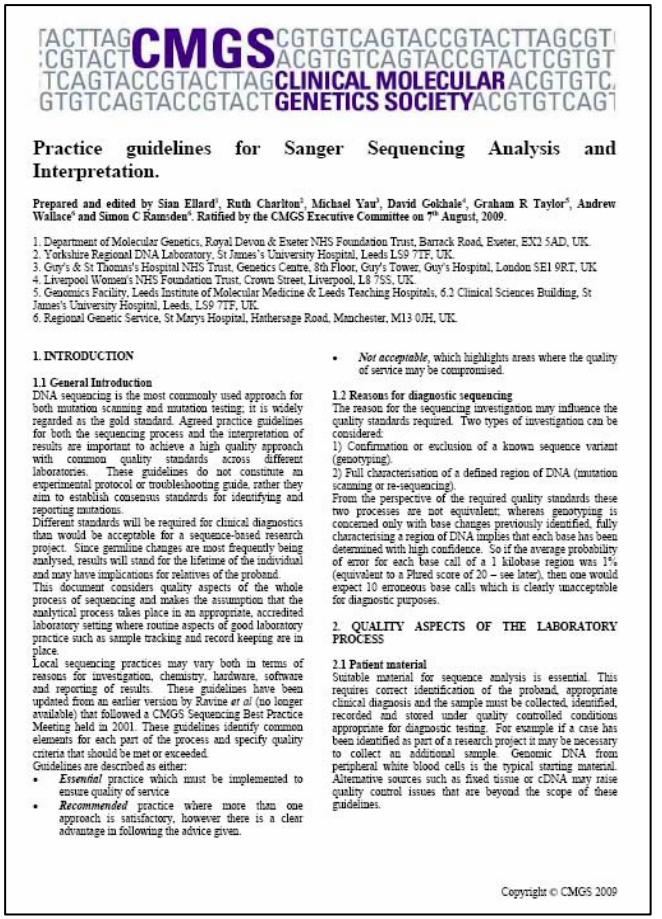
2.1 Patient material

Suitable material for sequence analysis is essential. This requires correct identification of the proband, appropriate clinical diagnosis and the sample must be collected, identified, recorded and stored under quality controlled conditions appropriate for diagnostic testing. For example if a case has been identified as part of a research project it may be necessary to collect an additional sample. Genomic DNA from peripheral white blood cells is the typical starting material. Alternative sources such as fixed tissue or cDNA may raise quality control issues that are beyond the scope of these guidelines.

http://www.cmgs.org/BPGs/Best_Practice_Guidelines.htm

Best practice

- Sequencing
  - Quality parameters not standardized
  - Negative results



CMGS CLINICAL MOLECULAR GENETICS SOCIETY

Practice guidelines for Sanger Sequencing Analysis and Interpretation.

Prepared and edited by Sian Ellard[1], Ruth Charlton[2], Michael Yau[3], David Gokhale[4], Graham R Taylor[5], Andrew Wallace[6] and Simon C Ramsden[6]. Ratified by the CMGS Executive Committee on 7th August, 2009.

1. Department of Molecular Genetics, Royal Devon & Exeter NHS Foundation Trust, Barrack Road, Exeter, EX2 5AD, UK.
2. Yorkshire Regional DNA Laboratory, St James's University Hospital, Leeds LS9 7TF, UK.
3. Guy's & St Thomas's Hospital NHS Trust, Genetics Centre, 8th Floor, Guy's Tower, Guy's Hospital, London SE1 9RT, UK.
4. Liverpool Women's NHS Foundation Trust, Crown Street, Liverpool, L8 7SS, UK.
5. Genomics Facility, Leeds Institute of Molecular Medicine & Leeds Teaching Hospitals, 6.2 Clinical Sciences Building, St James's University Hospital, Leeds, LS9 7TF, UK.
6. Regional Genetic Service, St Marys Hospital, Hathersage Road, Manchester, M13 0JH, UK.

1. INTRODUCTION

1.1 General Introduction

DNA sequencing is the most commonly used approach for both mutation scanning and mutation testing; it is widely regarded as the gold standard. Agreed practice guidelines for both the sequencing process and the interpretation of results are important to achieve a high quality approach with common quality standards across different laboratories. These guidelines do not constitute an experimental protocol or troubleshooting guide, rather they aim to establish consensus standards for identifying and reporting mutations.

Different standards will be required for clinical diagnostics than would be acceptable for a sequence-based research project. Since germline changes are most frequently being analysed, results will stand for the lifetime of the individual and may have implications for relatives of the proband.

This document considers quality aspects of the whole process of sequencing and makes the assumption that the analytical process takes place in an appropriate, accredited laboratory setting where routine aspects of good laboratory practice such as sample tracking and record keeping are in place.

Local sequencing practices may vary both in terms of reasons for investigation, chemistry, hardware, software and reporting of results. These guidelines have been updated from an earlier version by Ravine *et al* (no longer available) that followed a CMGS Sequencing Best Practice Meeting held in 2001. These guidelines identify common elements for each part of the process and specify quality criteria that should be met or exceeded.

Guidelines are described as either:

- ***Essential*** practice which must be implemented to ensure quality of service
- ***Recommended*** practice where more than one approach is satisfactory, however there is a clear advantage in following the advice given.
- ***Not acceptable***, which highlights areas where the quality of service may be compromised.

1.2 Reasons for diagnostic sequencing

The reason for the sequencing investigation may influence the quality standards required. Two types of investigation can be considered:

1) Confirmation or exclusion of a known sequence variant (genotyping).

2) Full characterisation of a defined region of DNA (mutation scanning or re-sequencing).

From the perspective of the required quality standards these two processes are not equivalent; whereas genotyping is concerned only with base changes previously identified, fully characterising a region of DNA implies that each base has been determined with high confidence. So if the average probability of error for each base call of a 1 kilobase region was 1% (equivalent to a Phred score of 20 – see later), then one would expect 10 erroneous base calls which is clearly unacceptable for diagnostic purposes.

2. QUALITY ASPECTS OF THE LABORATORY PROCESS

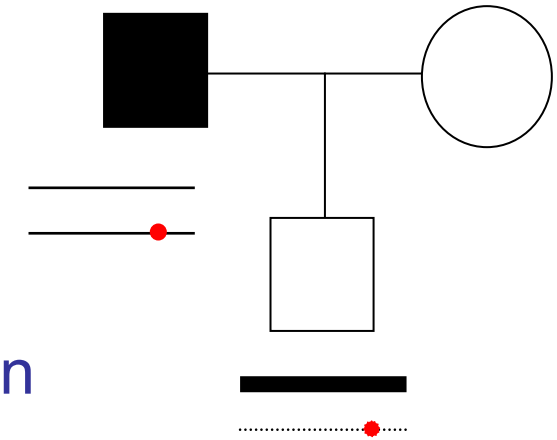
2.1 Patient material

Suitable material for sequence analysis is essential. This requires correct identification of the proband, appropriate clinical diagnosis and the sample must be collected, identified, recorded and stored under quality controlled conditions appropriate for diagnostic testing. For example if a case has been identified as part of a research project it may be necessary to collect an additional sample. Genomic DNA from peripheral white blood cells is the typical starting material. Alternative sources such as fixed tissue or cDNA may raise quality control issues that are beyond the scope of these guidelines.

http://www.cmgs.org/BPGs/Best_Practice_Guidelines.htm

Best practice

SNP check



Case:

- HNPCC family
- Father affected – known mutation
- Test result was negative for the son

- Need to check if there are any known SNPs under primer binding sites - SNPcheck

EQA genotyping results

	Genotyping errors per case			Genotyping errors per allele		
	No. of cases completed	No. of errors	Error rate (%)	No. of alleles analysed	No. of errors	Error Rate (%)
Case 1	45	0	0.0	90	0	0.0
Case 2	**47**	**10**	**21.2**	**94**	**10**	**10.6**

Re-design primers

- Move primer binding site
- Use wobble primers

Diagnostic error rate

Best practice

EMQN
The European Molecular Genetics Quality Network

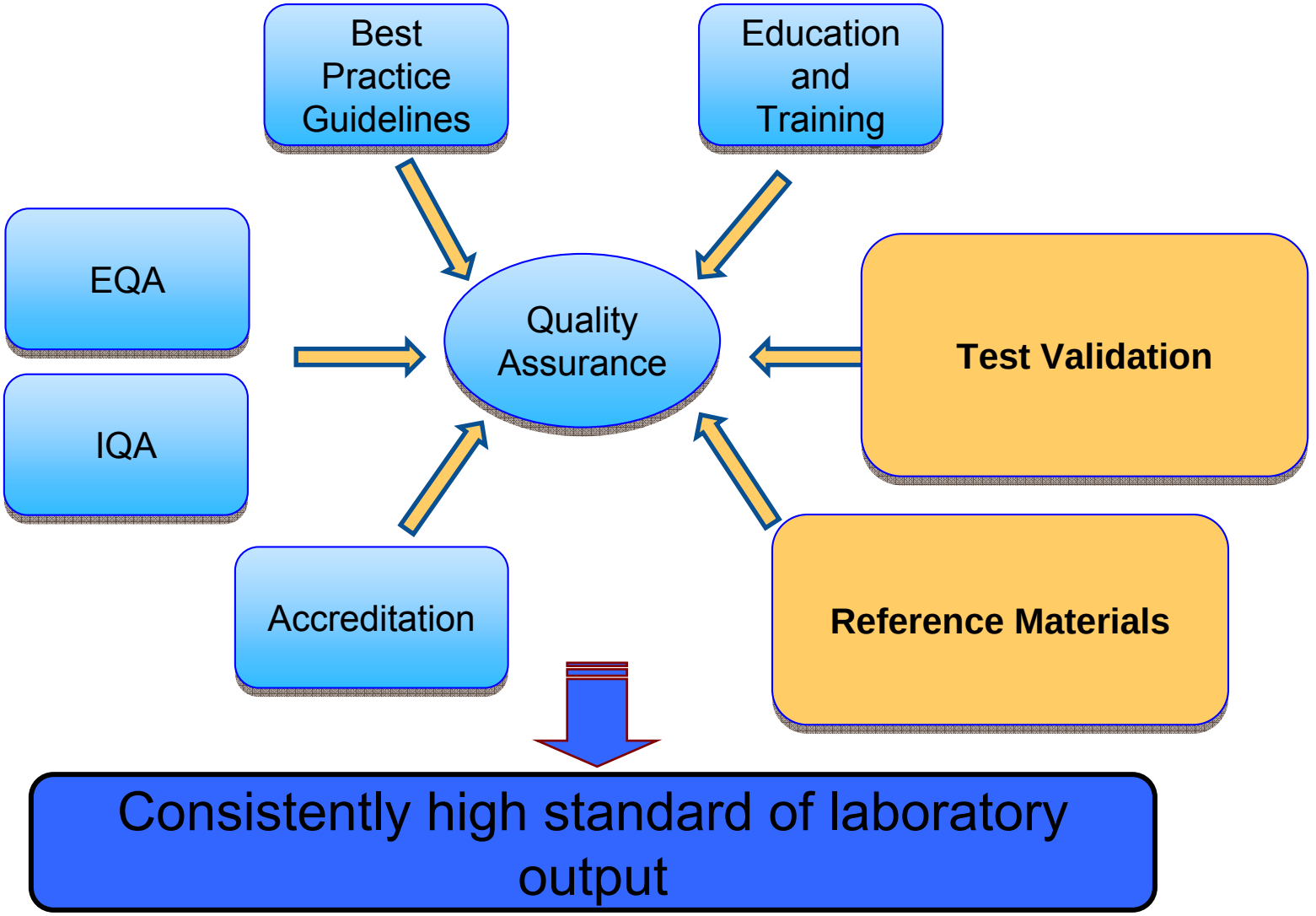
- Unclassified variants
  - Mutation or normal variant?
- Guidelines published
- Bioinformatics tools
  - Training/experience/caution required
  - Very time-consuming
- Novel approaches
  - Alamut
  - Bayesian Classification (Tavtigian, BRCA)

Best practice

- Unclassified variants
  - DMuDB – Diagnostic Mutation Database

Quality Assured Genetic Testing

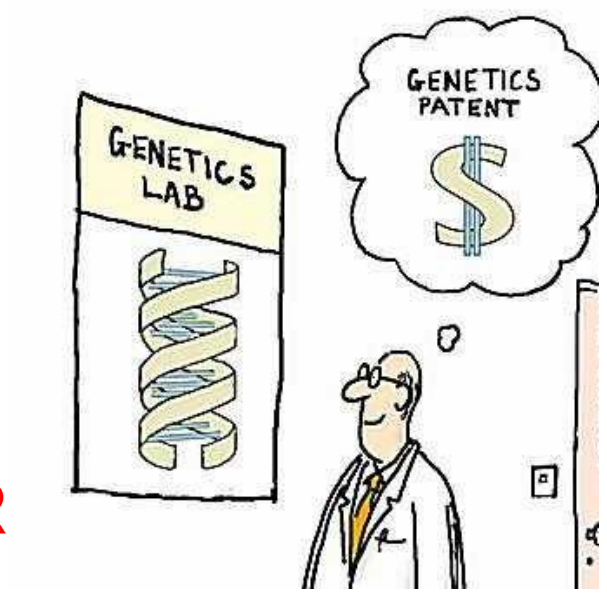
EMQN
The European Molecular Genetics Quality Network



Test validation

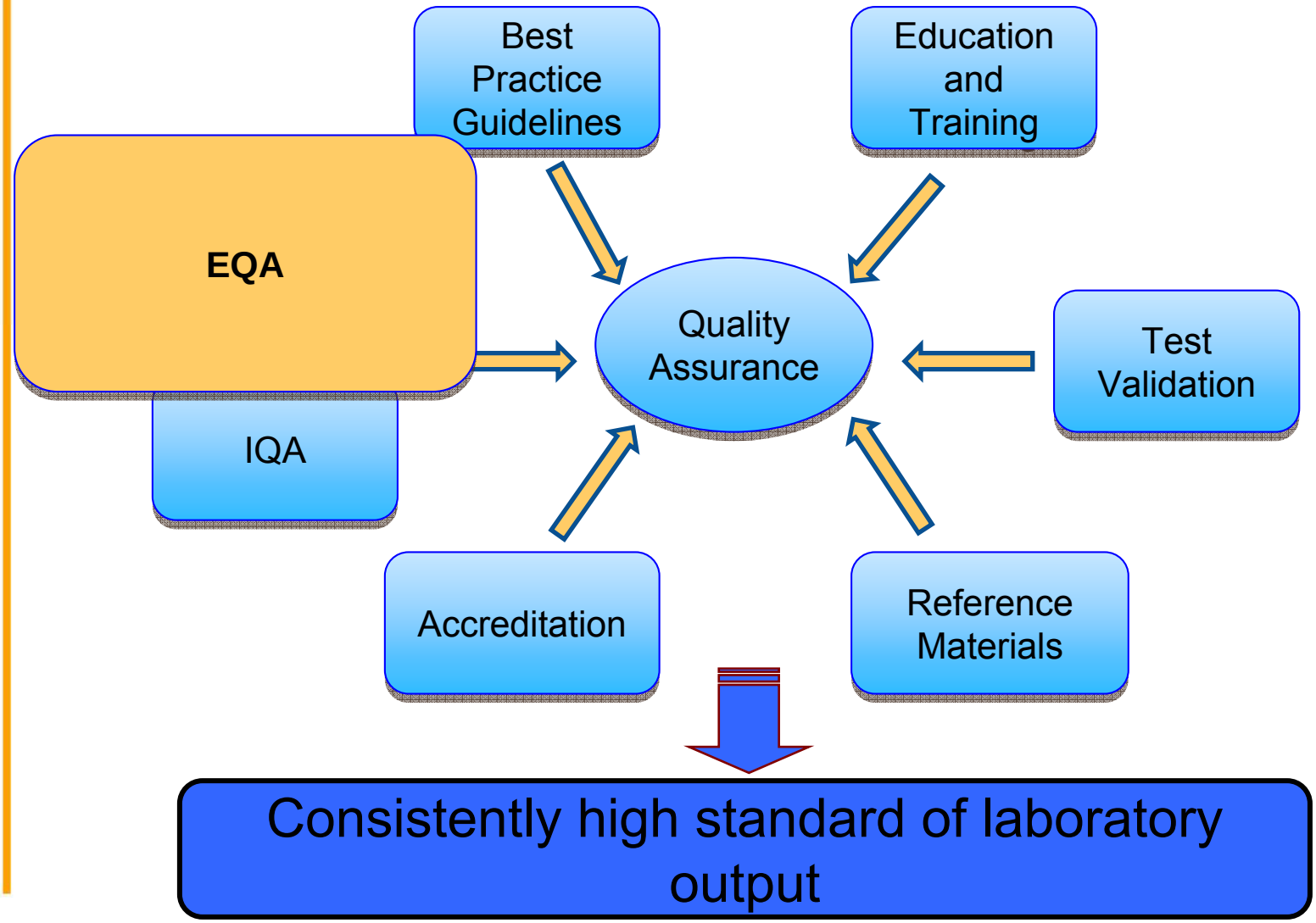
EMQN
The European Molecular Genetics Quality Network

- What is a new test?
- Most tests used are ‘homebrews’
- Lack of reference material
  - Panel for FRAX 2008
  - **Panel for PWS/AS**
  - **Panel for BCR-ABL Q-PCR**



Quality Assured Genetic Testing

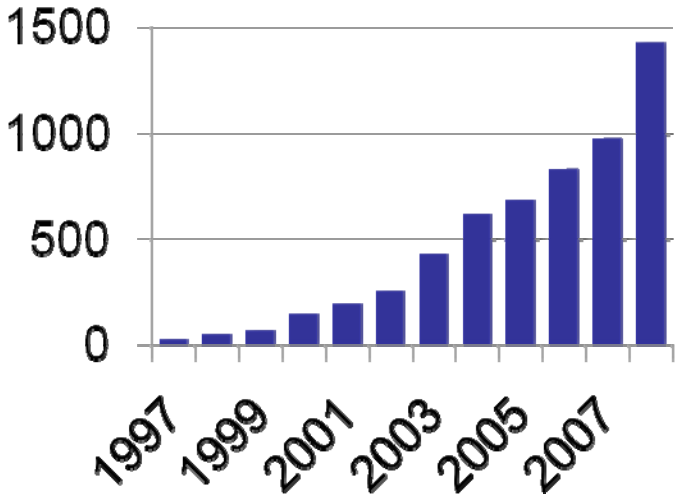
EMQN
The European Molecular Genetics Quality Network

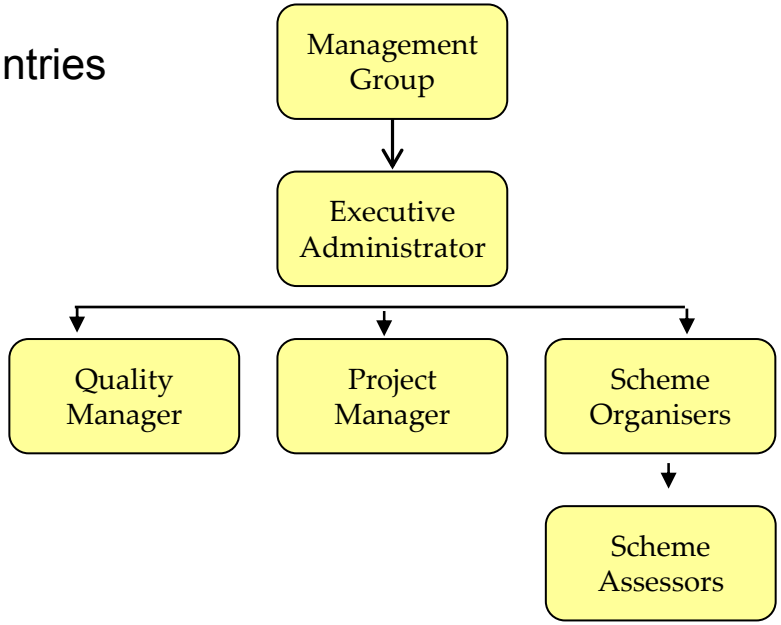


EMQN – network at a glance

- Manchester based international EQA organiser
- Network started in 1997 (EU funding)
- A management group of **9** scientists from **7** countries

- **52** scheme organisers and assessors from 11 countries
- **586** registered members
- **1400** participations





EQA schemes: Offered

EMQN
The European Molecular Genetics Quality Network

97/98	99/00	01/02/03	04/05	06	07	08	Provider
HD	HD	HD	HD	HD	HD	HD	NL
DMD	DMD	DMD	DMD	DMD	DMD	DMD	UK / NL
FRDA	FRDA	FRDA	FRDA	FRDA	FRDA	FRDA	IRL
	BRCA	BRCA	BRCA	BRCA	BRCA	BRCA	GER
	CMT	CMT	CMT	CMT	CMT	CMT	GER
	CF *	CF *	CF *	CF *	CF *	CF *	BEL
	AZF+	AZF+	AZF+	AZF+	AZF+	AZF+	GER
		RB	RB	RB	RB	RB	GER
		PW/AS	PW/AS	PW/AS	PW/AS	PW/AS	FRA
		FRAX	FRAX	FRAX	FRAX	FRAX	FRA
		HFE	HFE	HFE	HFE	HFE	UK
		HNPCC	HNPCC	HNPCC	HNPCC	HNPCC	FRA
		DNA-SEQ	DNA-SEQ	DNA-SEQ	DNA-SEQ	DNA-SEQ	UK
			SCA	SCA	SCA	SCA	POR
			PKU	PKU	PKU	PKU	NOR
				WIL#	WIL#	WIL#	UK
				SMA	SMA	SMA	TUR
				MODY	MODY	MonoDiab	UK
					MSCAN	MSCAN	UK
					DM	DM	DK
						CNX26	NL
						VHL	GER
						CAH	GER
						Men2	GER

*European Thematic Network for Cystic Fibrosis

+ European Academy of Andrology

^ European Porphyria Initiative

EuroWilson Group

Scheme participation

EMQN
The European Molecular Genetics Quality Network

Scheme	2004	2005	2006	2007	2008	Difference
AZF	91	88	97	96	125	+29
BRCA	51	58	70	77	101	+34
CMT	37	37	42	50	73	+12
CAH	0	0	0	0	38	+38
CNX26	0	0	0	0	30	+30
DM	0	0	0	31	73	+42
DMD	33	42	41	46	58	+12
FRAX	54	55	79	75	102	+27
FRDA	26	33	33	34	52	+18
HD	39	45	59	57	87	+30
HFE	58	55	60	61	67	+7
HNPCC	42	49	51	63	81	+18
Men2	0	0	0	0	44	+44
MonoDiab	0	0	12	13	20	+7
PKU	11	9	14	17	15	-2
POR	0	9	11	16	15	-1
PWAS	44	47	47	49	82	+33
RB	10	10	11	14	14	0
SCA	32	37	28	34	62	28
SMA	0	0	32	57	73	+16
VHL	0	0	0	0	20	+20
WIL	0	0	22	23	23	0
SEQ	95	114	122	127	136	9
MSCAN	0	0	0	41	36	-5
Total:	**623**	**688**	**831**	**981**	**1427**	**+446 (+45%)**

International schemes

EMQN
The European Molecular Genetics Quality Network

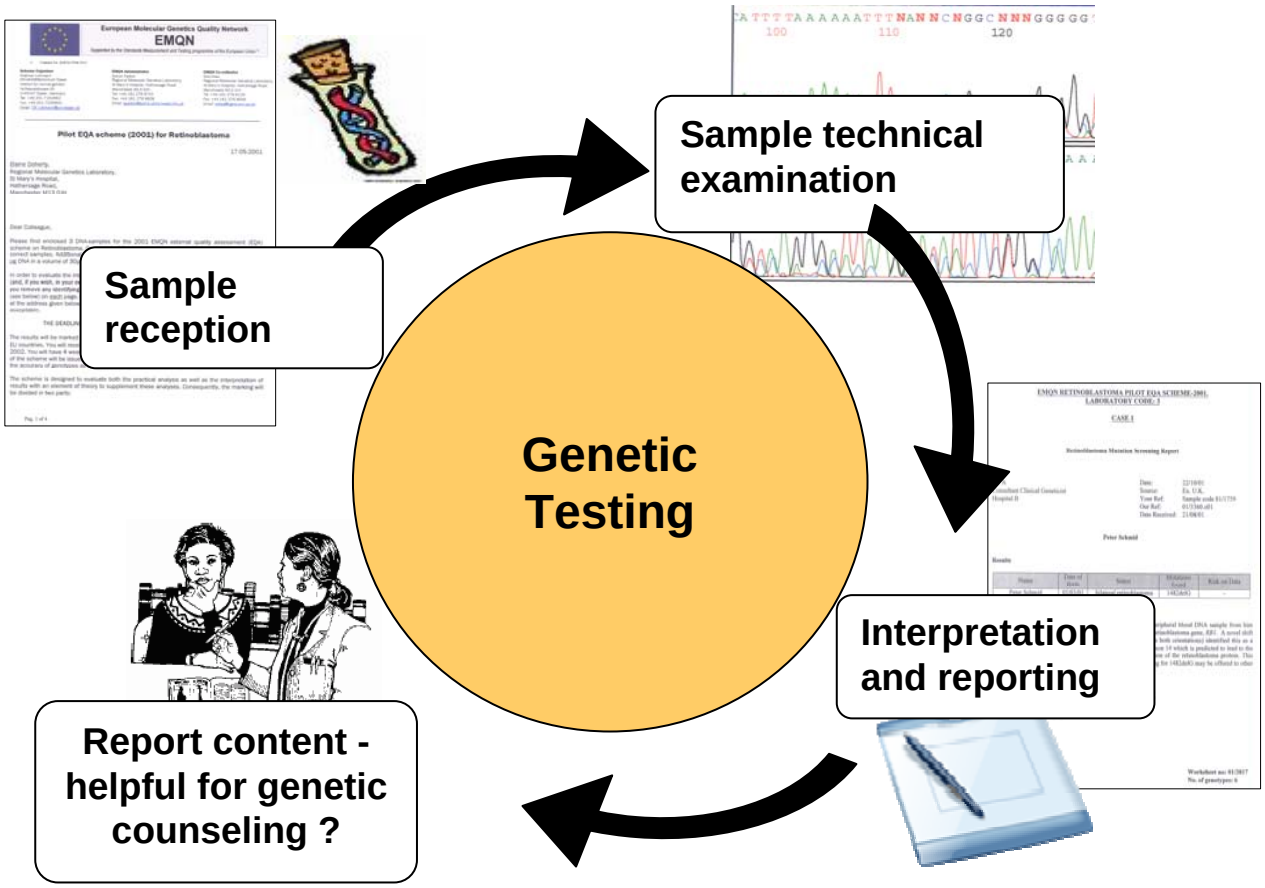
Types of schemes

EMQN
The European Molecular Genetics Quality Network

- Single gene disorder schemes
- Technique specific schemes
  - Qualitative
  - Quantitative

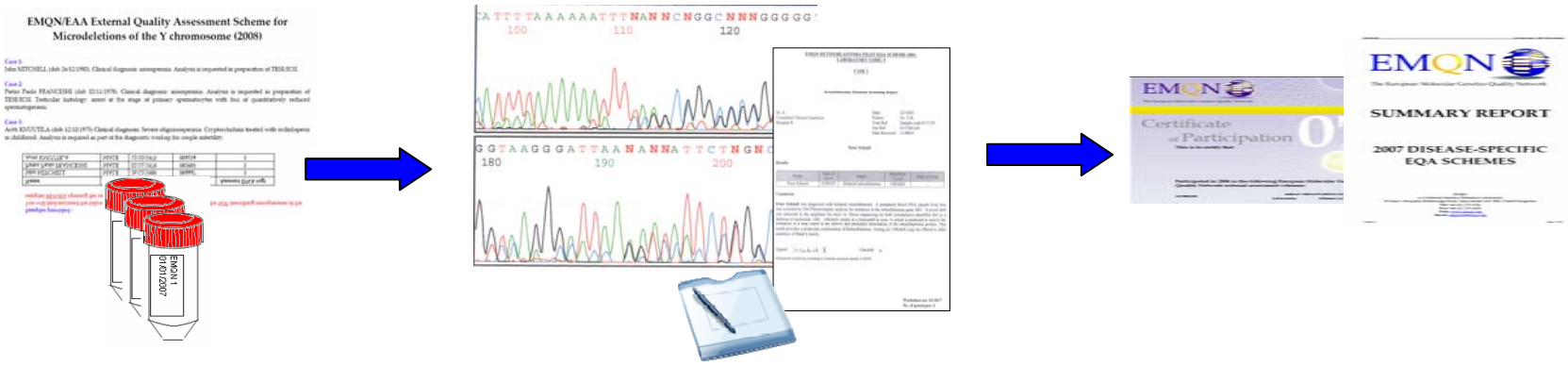
EQA

EMQN
The European Molecular Genetics Quality Network



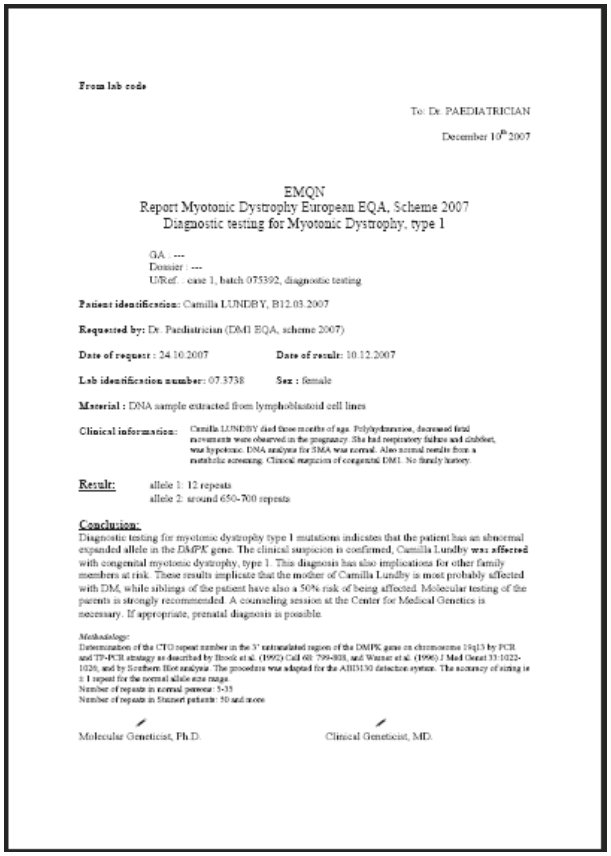
Disease specific EQA schemes

- Participants receive 3 samples once a year with mock clinical referral
- Laboratories carry out analysis and report findings
- Assessment for genotyping, interpretation and reporting
- Participants receive their marks and a final report



Scheme assessment

- Genotyping – correct?
- Reporting
  - Accuracy and consistency
  - Clear ‘take home’ message
  - Methods referenced
  - Authorised / audit trail
- Interpretation
  - Can be scored
  - Language can be accommodated
  - Reporting policies differ



From lab code

To: Dr. PAEDIATRICIAN

December 10th 2007

EMQN
Report Myotonic Dystrophy European EQA, Scheme 2007
Diagnostic testing for Myotonic Dystrophy, type 1

GA : ---
Dossier : ---
U/Ref. : case 1, batch 075392, diagnostic testing

Patient identification: Camilla LUNDBY, B12.03.2007

Requested by: Dr. Paediatrician (DM1 EQA, scheme 2007)

Date of request : 24.10.2007 **Date of result:** 10.12.2007

Lab identification number: 07.3738 **Sex :** female

Material : DNA sample extracted from lymphoblastoid cell lines

Clinical information: Camilla LUNDBY died three months of age. Polyhydramnios, decreased fetal movements were observed in the pregnancy. She had respiratory failure and clubfoot, was hypotonic. DNA analysis for SMA was normal. Also normal results from a metabolic screening. Clinical suspicion of congenital DM1. No family history.

Result: allele 1: 12 repeats
allele 2: around 650-700 repeats

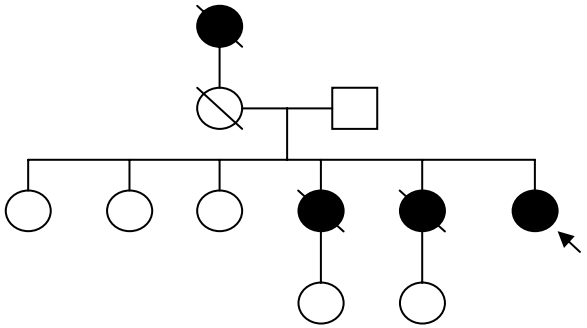
Conclusion:
Diagnostic testing for myotonic dystrophy type 1 mutations indicates that the patient has an abnormal expanded allele in the *DMPK* gene. The clinical suspicion is confirmed, Camilla Lundby was affected with congenital myotonic dystrophy, type 1. This diagnosis has also implications for other family members at risk. These results implicate that the mother of Camilla Lundby is most probably affected with DM, while siblings of the patient have also a 50% risk of being affected. Molecular testing of the parents is strongly recommended. A counseling session at the Center for Medical Genetics is necessary. If appropriate, prenatal diagnosis is possible.

Methodology:
Determination of the CTG repeat number in the 3' untranslated region of the DMPK gene on chromosome 19q13 by PCR and TP-PCR strategy as described by Brook et al. (1992) Cell 68: 799-808, and Warner et al. (1996) J Med Genet 33:1022-1026, and by Southern Blot analysis. The procedure was adapted for the ABI310 detection system. The accuracy of sizing is ± 1 repeat for the normal allele size range.
Number of repeats in normal persons: 5-35
Number of repeats in Steinert patients: 50 and more

Molecular Geneticist, Ph.D. Clinical Geneticist, MD.

Swiss Society for Medical Genetics
http://www.ssgm.ch/sections/Documents/Statements/publications.htm

EMQN BRCA scheme case 2007



Claudia CAPABLANCA (dob 21/08/1976) is the youngest of 6 sisters. Two of her older sisters and her grand-mother all died before menopause from breast or ovarian cancers. Her mother had died in an accident at age 49. Mrs. Capablanca's gynaecologist recently suspected an ovarian cancer and referred her to an oncology centre. There, the diagnosis was confirmed and treatment initiated. Because of the high genetic risk for her nieces, Mrs. Capablanca decided to have a genetic screening done.

Please analyse exon 18 of the BRCA1 gene. Report back to the oncology centre in your standard reporting format.

EQA evaluation of results

Name	Sex	Date of Birth	Results
Claudia CAPABLANCA	FEMALE	21/08/1976	U14680.1: c.5075-2A>C

Name	Criteria	Marks
Genotyping	**Genotype correct**, deduction of marks only if genotype description is misleading.	2.0
Interpretation and reporting	**Mutation affects 5'-splice site of exon 18**. Listed once in BIC as 'clinically relevant'. Most likely **compatible with a hereditary predisposition to breast/ovarian cancer**. Patient remains at **high risk for secondary tumours** and should join a monitoring programme. **Predictive testing** of relatives is possible after genetic counselling. Deductions: missing/incomplete biological interpretation; lack of suggestion of monitoring programme (0.4 marks); lack of suggestion of predictive testing and/or counselling (0.2 marks).	2.0
	Personal data of patient (name & DOB or name & lab no) Brief recapitulation of the **patient's personal & family history** Clear presentation of the results *Minor points (not leading to deduction of marks):* Arrival and reporting dates, Signature of the report by two authorised persons, Indication of the reference sequence used. Deductions: (0.2 marks each)	
Comments	BIC-nomenclature: c.5194-2A>C; IVS17-2A>C	

Technique specific EQA

- EQUAL – qual
  - EQA for DNA extraction and PCR
- EQUAL – quant
  - EQA for Real Time PCR
- EMQN schemes
  - Sequencing
  - Mutation scanning
  - CNVs/array CGH

EQUAL - qual

- EQA for DNA extraction and PCR
  - Participants received blood, primers and DNA
  - Labs asked to extract DNA and set up PCR reactions
  - Submit DNA concentration, quality, quantity data as well as aliquots of DNA and PCR products

Orlando *et al (2007) Clinical Chemistry* **53**:**7**, 1349-1357

EQUAL – qual results

- Assessment:
  - Look at data returned by participants
  - Reevaluation of returned material by reference laboratory
  - Assessment of photometric measurements of DNA quantity / quality
  - Performance of blood extraction procedure

EQUAL - qual

EMQN
The European Molecular Genetics Quality Network

Table 1. Simple data description of DNA quantity and quality in blood and pre-extracted samples (preamplification phase).[a]

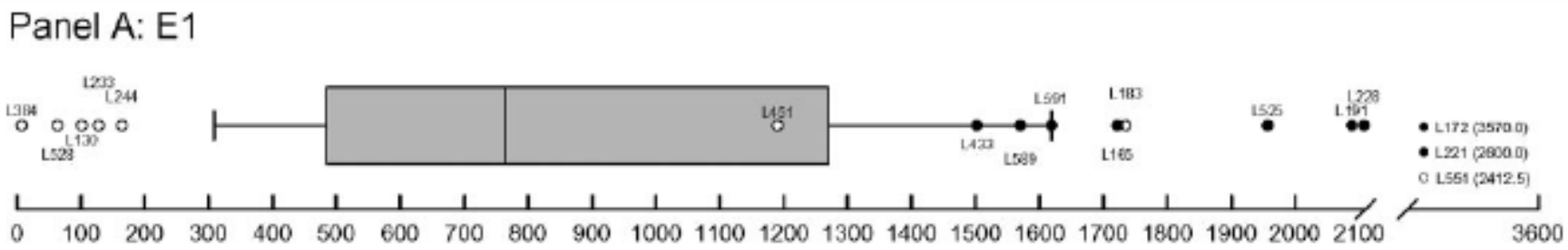
Variable	n	Minimum	25th centile	Median	75th centile	Maximum	IQR
*R*1	166	0.36	1.65	1.78	1.89	5.14	0.23
*R*2	166	0.50	1.65	1.78	1.90	8.04	0.25
*R*3	164	0.29	1.66	1.81	1.95	18.00	0.29
*R*4	165	0.91	1.33	1.38	1.46	5.00	0.13
*Q*1	150	1.67	11.50	17.00	22.33	187.50	10.83
*Q*2	152	1.67	13.32	19.69	25.29	200.00	11.97
*Q*3	148	5.50	17.86	20.13	30.00	200.00	12.14
*Q*4	148	20.00	49.98	54.25	65.65	190.00	15.68
*Re*1	166	0.90	1.69	1.84	1.90	13.71	0.21
*Re*2	166	0.52	1.64	1.83	1.90	2.94	0.25
*Qe*1	149	1.42	9.13	14.41	19.95	127.63	10.82
*Qe*2	151	0.48	11.52	17.85	25.25	361.10	13.73
Δ*R*1	166	−3.29	−0.15	0.03	0.19	13.21	0.34
Δ*R*2	166	−7.04	−0.21	0.03	0.19	1.43	0.39
Δ*Q*1	152	−86.00	−6.38	−1.22	0.94	51.35	7.32
Δ*Q*2	148	−105.10	−5.25	−0.70	1.87	94.10	7.12

[a] *R*1, *R*2, *R*3, and *R*4 are the (260 nm − 320 nm)/(280 nm − 320 nm) or 260 nm/280 nm ratio for samples 1, 2, 3, and 4, respectively. *Q*1, *Q*2, *Q*3, and *Q*4 are the quantity of DNA extracted from blood samples 1 and 2 (mg/L) obtained from [(260 nm − 320 nm) × 50 × dilution factor × DNA reconstitution volume)/extracted blood volume] or directly measured in pre-extracted samples 3 and 4, respectively. *Re*1 and *Re*2 are the (260 nm − 320 nm)/(280 nm − 320 nm) ratios measured in the EQUAL-Laboratory by Nanodrop in blood samples 1 and 2, respectively, as provided by participants. *Qe*1 and *Qe*2 are the DNA quantities measured in the EQUAL-Laboratory with Nanodrop in blood samples 1 and 2, respectively, as provided by participants. Δ *R*1 = *Re*1 − *R*1. Δ *R*2 = *Re*2 − *R*2. Δ *Q*1 = *Qe*1 − *Q*1. Δ *Q*2 = *Qe*2 − *Q*2.

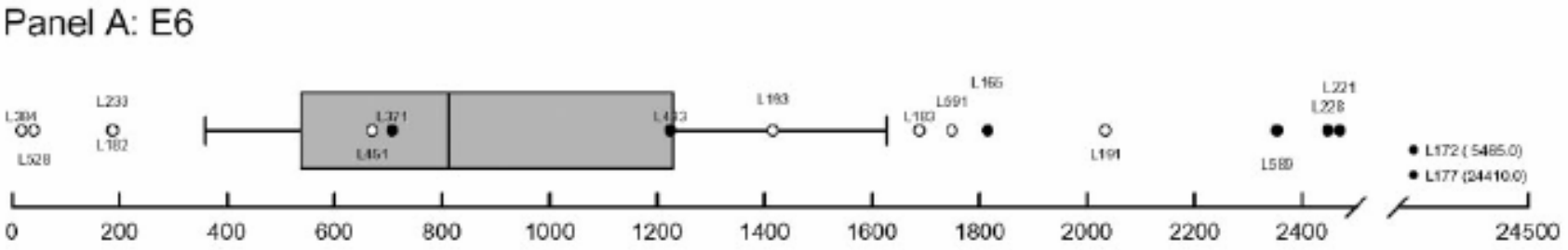
- 25 % of laboratories (42/165) gave out of limits readings for pre-extracted DNA

EQUAL - qual

PCR from DNA extracted by the participants



PCR from DNA extracted by the organiser



- High variability of PCR efficiency
- Differences in sizes
- Contamination issues

EQUAL-quant

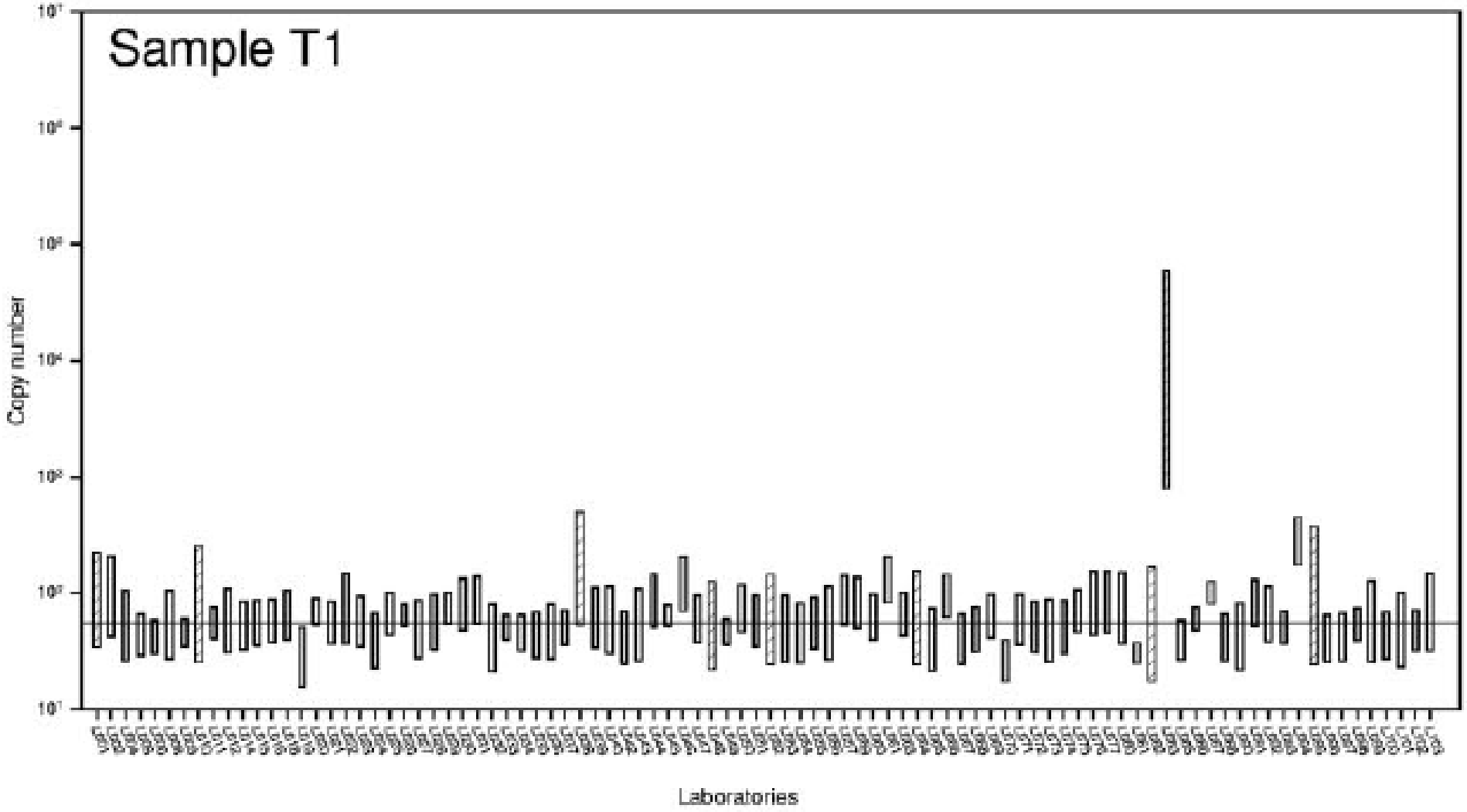
EMQN
The European Molecular Genetics Quality Network

- EQA testing Real Time PCR
  - Used *ABL* proto oncogene
  - Participants given:
    - Primers and fluorescent probes
    - Plasmid standards (10, 10^2, 10^3, 10^4, 10^5 copies/5µl)
    - 3 unknown test samples (cloned cDNAs)
  - Laboratories asked to:
    - Construct a calibration curve
    - Estimate cDNA copy numbers

Ramsden *et al (2006) Clinical Chemistry* **52**:**8**, 1584-1591

EQUAL-quant

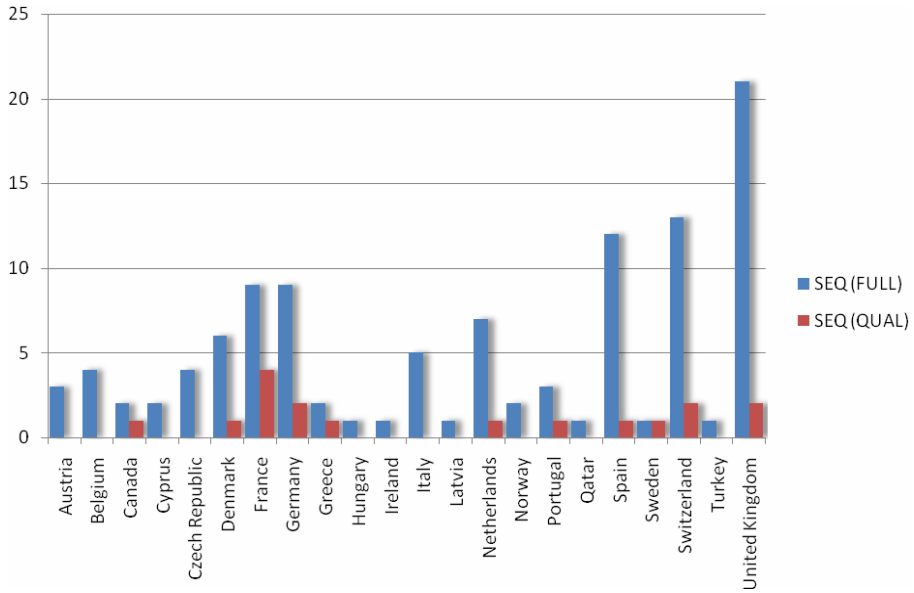
- 93 labs returned results and 74 met performance criteria



Generic Technical EQA schemes

EMQN
The European Molecular Genetics Quality Network

- **DNA Sequencing**
  - 6 years : 2002 - 2008



EMQN
The European Molecular Genetics Quality Network

FINAL REPORT

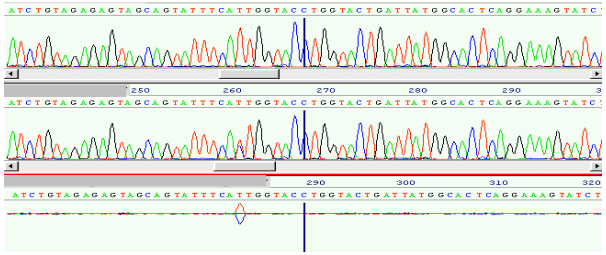
EMQN External Quality Assessment scheme for DNA Sequencing (2007)

DNA Sequencing EQA scheme

EMQN
The European Molecular Genetics Quality Network

- **Materials**
  - Unpurified PCR products
    - CFTR, BRCA, OCRL-1 genes
    - Normal control, Normal, Heterozygote, Homozygote & a deletion Heterozygote
  - Sequencing primers provided

- **Validation**:
  - 2 independent laboratories



Assessment of Genotyping & Interpretation

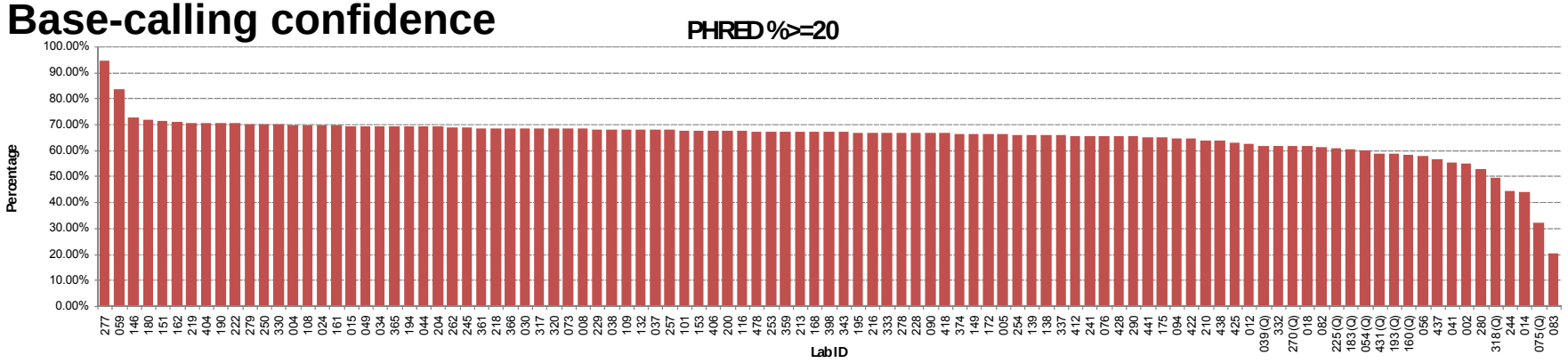
EMQN
The European Molecular Genetics Quality Network

SAMPLE	GENOTYPE	SCORE	HGVS INTERPRETATION	ACCEPTABLE VARIATIONS	REF SEQ
SEQ07_01	**Mutation present** c.[733G>A] + [=]	2.00	c.[733G>A] + [=]	c.[733G>A] c.733G>A	NM_000276.3
			p.[Gly245Arg] + [=]	p.[Gly245Arg] p.Gly245Arg p.[G245R] p. G245R	NM_000276.3
SEQ07_02	**Mutation present** c.[729dupT]	2.00	c.[729dupT]	None	NM_000276.3
			p.[Val244CysfsX13]	p.[V244CfsX13]+p.[V244CfsX13] p.[Val244CysfsX13] p.[V244CfsX13] p.[Val244fs] p.[V244fs] p.Val244fs p.V244fs	NM_000276.3
SEQ07_03	**Mutation absent** c.[=] + [=]	2.00	c.[=] + [=]	c.[=] c.= Wild type WT	NM_000276.3
			p.[=] + [=]	p.[=] p.=	NM_000276.3

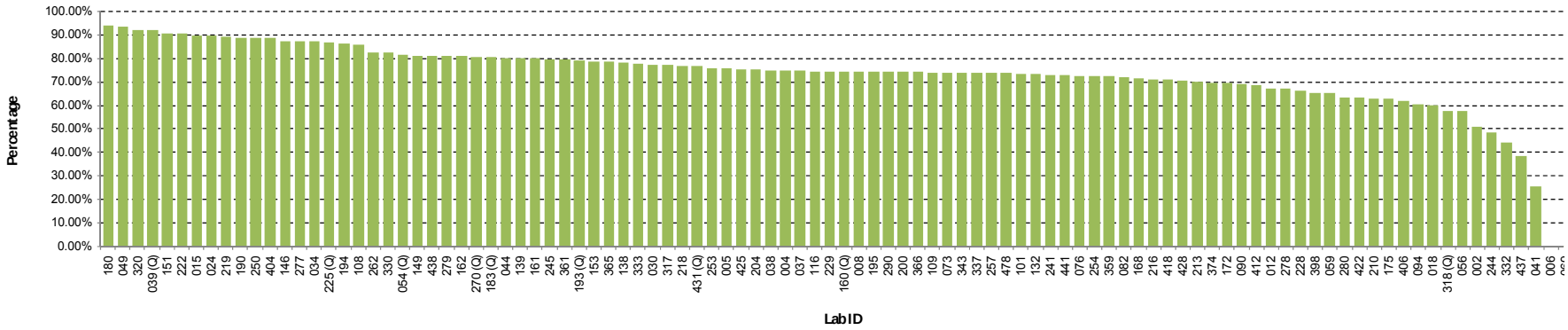
Quality of raw data

- 5 different parameters
  - PHRED scores (20,30,40), Quality Read Length (QRL), Quality Read Overlap (QRO)

Base-calling confidence

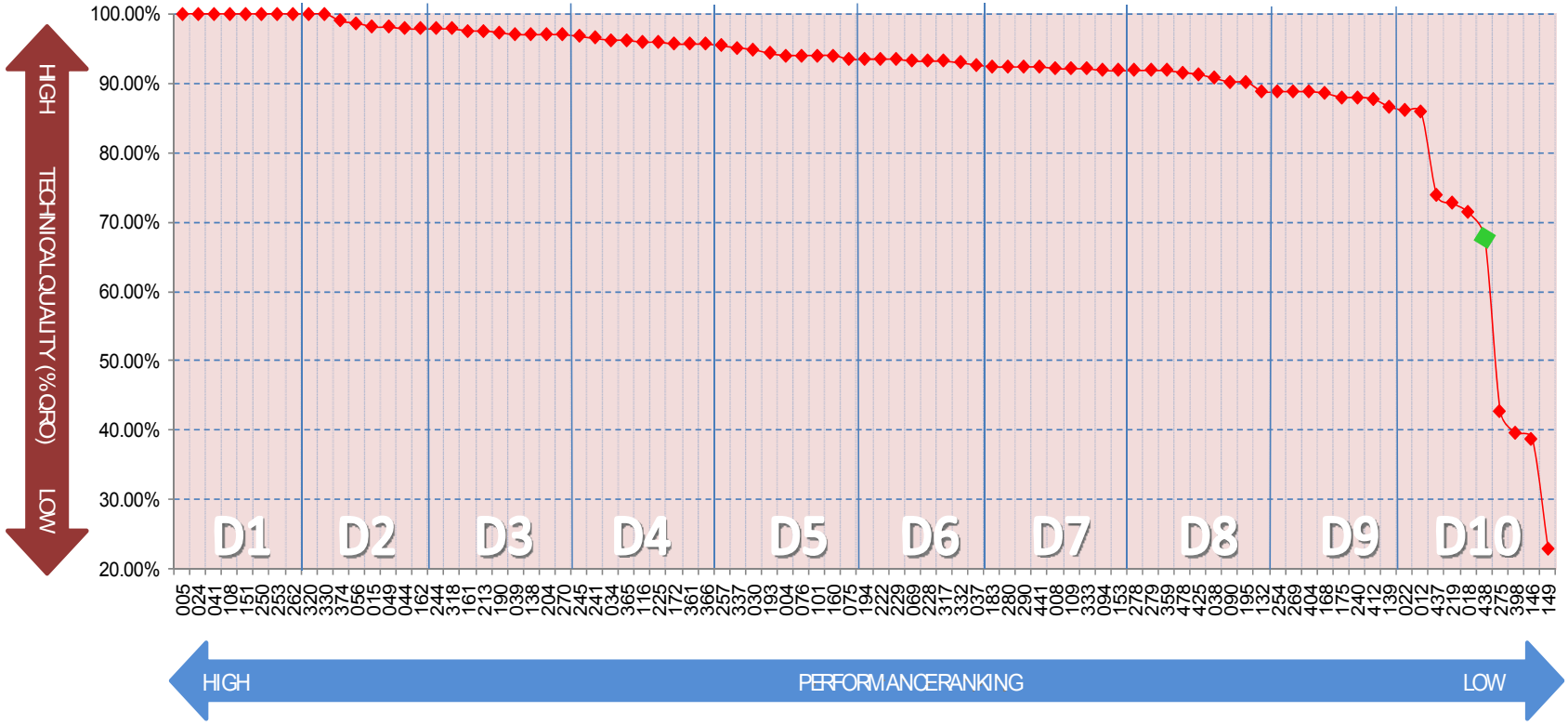


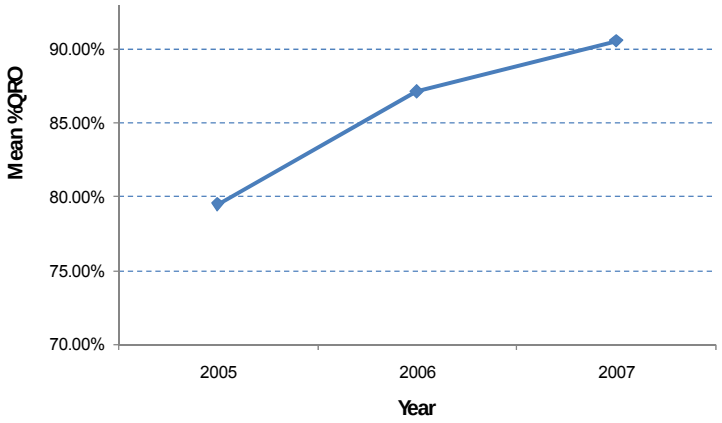
Diagnostic data quality (QRO)



Benchmarking data quality

EMQN
The European Molecular Genetics Quality Network





Benchmarking DNA sequence quality
Patton et al., Clinical Chemistry. 2006;52:728-736

Learning from EQA?
2007 scheme scores

EMQN
The European Molecular Genetics Quality Network

Scheme	No of reports	Av. genotyping score	Av. interpretation score	No of errors leading to misdiagnosis
Total	788	1.91 (max 2.00)	1.78 (max 2.00)	45 (5.7%)
DMD	45	1.87	1.83	7
FRAX	73	1.94	1.88	3
FRDA	34	1.96	1.78	1
HFE	60	1.96	1.82	2
HNPCC	60	1.93	1.8	3
HD	55	1.94	1.81	3
SMA	34	1.94	1.9	0

Error rate = number of genotyping errors over all returns

Common types of errors

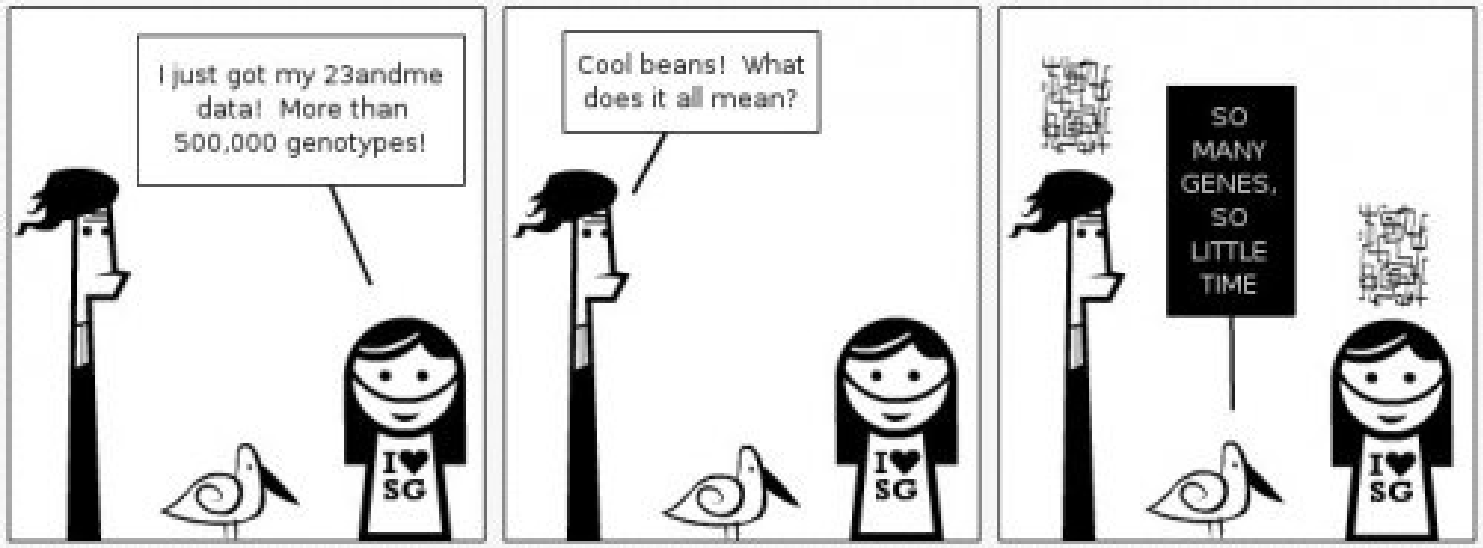
EMQN
The European Molecular Genetics Quality Network

- Wrong name / date of birth
- Sample mix up
  - 10 incidences in 2008
- Incorrect genotype
- Incorrect interpretation
- Incorrect nomenclature

Summary

EMQN
The European Molecular Genetics Quality Network

- Best practice issues
  - What quality needed for reporting
  - SNP
  - Unclassified variants
- EQA
  - Single disease specific schemes
    - Issues specific for disorder (nomenclature etc)
    - Reporting and interpretation also assessed
  - Technique specific EQA
  - Errors are still being made


I just got my 23andme data! More than 500,000 genotypes!
I ♥ SG
Cool beans! What does it all mean?
I ♥ SG
SO MANY GENES, SO LITTLE TIME
I ♥ SG

Acknowledgments

- Rob Elles, Simon Patton, Simon Ramsden
- EMQN management team
- EMQN scheme organisers and assessors
- Participating laboratories

- EuroGentest