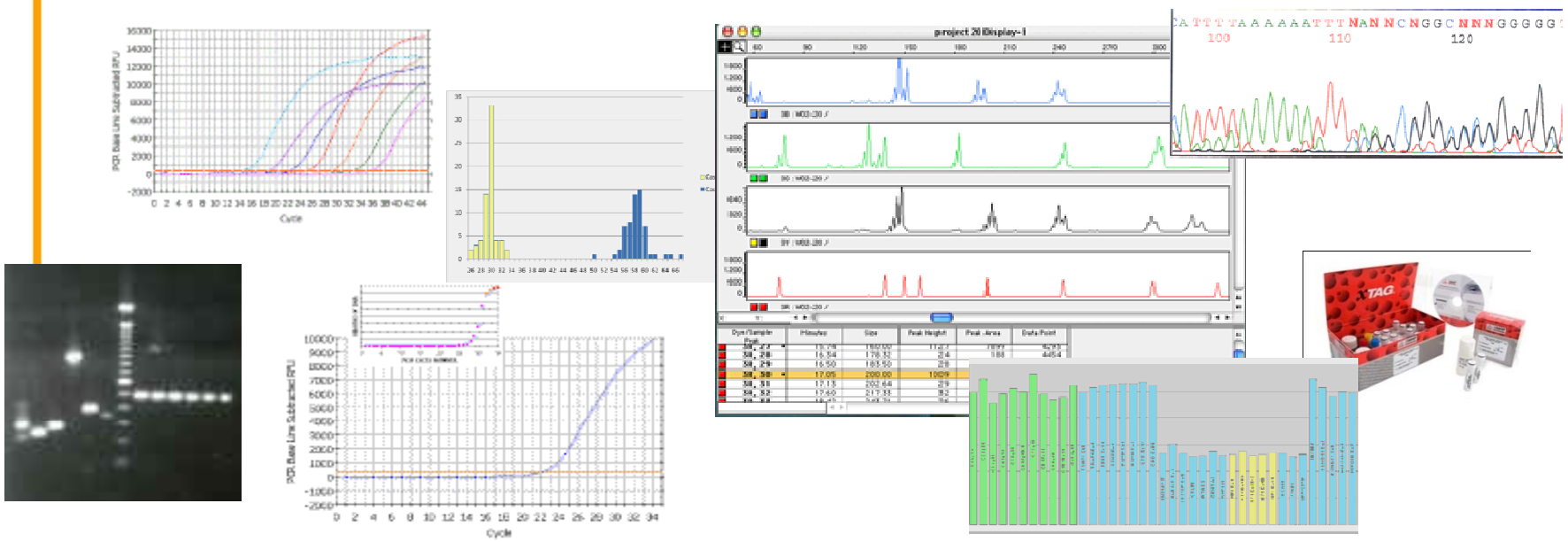
EMQN
The European Molecular Genetics Quality Network

Evaluating the interpretation of clinical molecular genetic results

Outi Kämäräinen

Molecular genetic testing

- Examine changes in DNA sequence
  - Point mutations, deletions, duplications, insertions…
- Wide range of technologies used
  - One or combination used depending on application



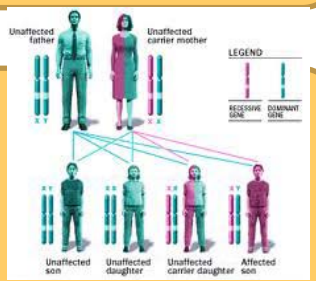
Referrals and interpretation

- Rapidly evolving field
- TAT long

Diagnostic testing

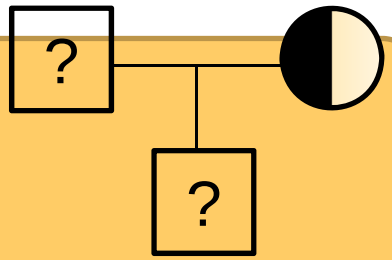
Mutation scanning

Predictive testing



Screening for known mutation

Carrier testing



A priori / a posteri risk
- Affected / carrier
- Risk calculations

EMQN – network at a glance

- Network started in 1997
- Accredited to ISO 17043
- Over 900 member labs world wide
- 28 disease specific EQA schemes
- 2 technique specific EQA schemes

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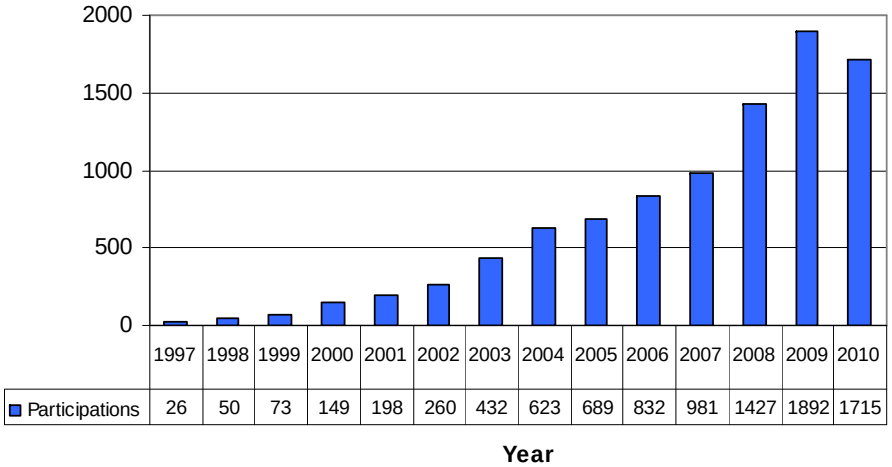
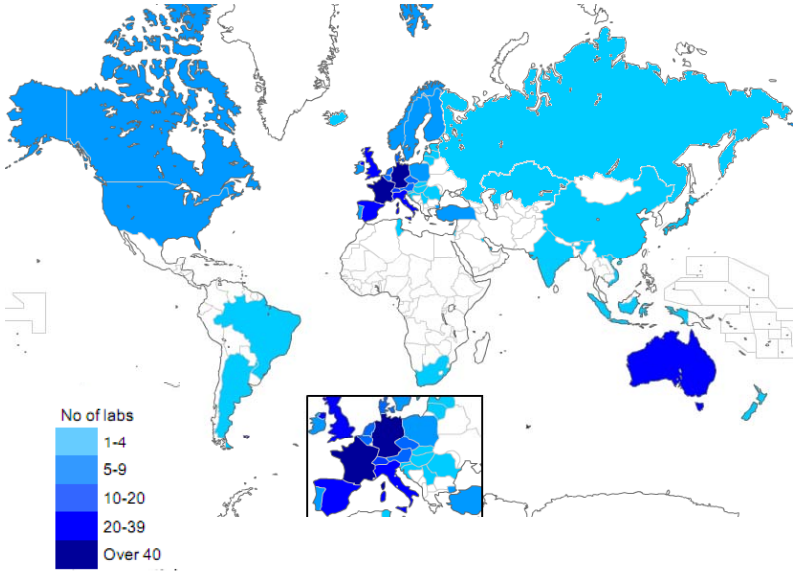
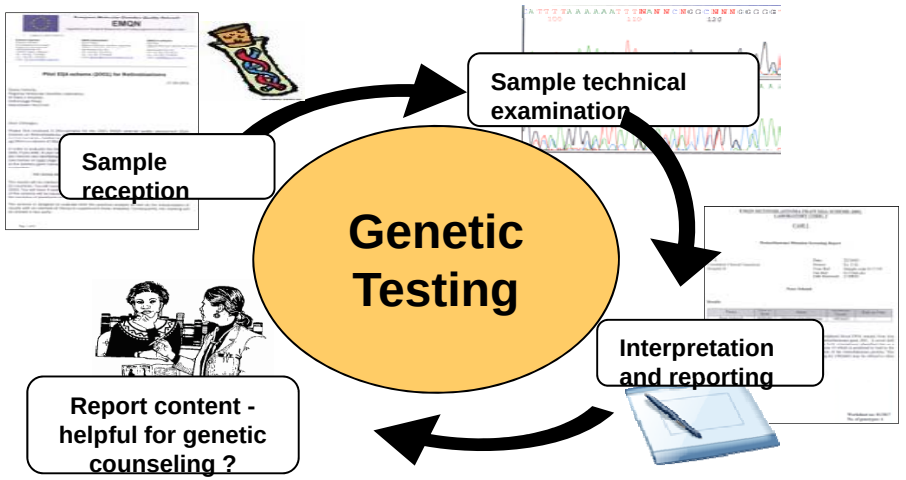




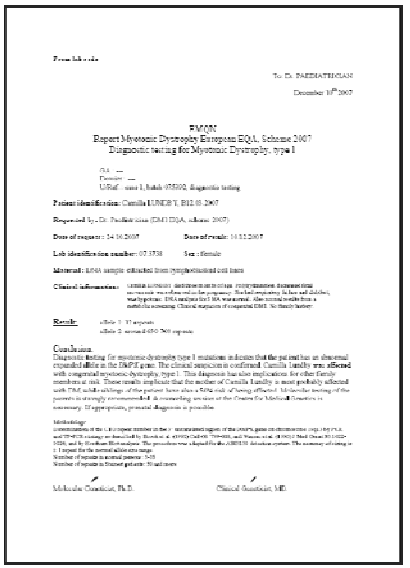
EMQN schemes

- Diseases specific EQA schemes
  - 3 samples once a year
  - Assessment of analytical accuracy and interpretation of results
  - Labs return results via website



Assessment of returns

- Assessment done by expert groups
  - Pre agreed marking criteria
    - Standard scoring of 2
    - Points are scheme / case specific
  - Yearly meeting to harmonise marking



Genotype

Interpretation of result

Report style

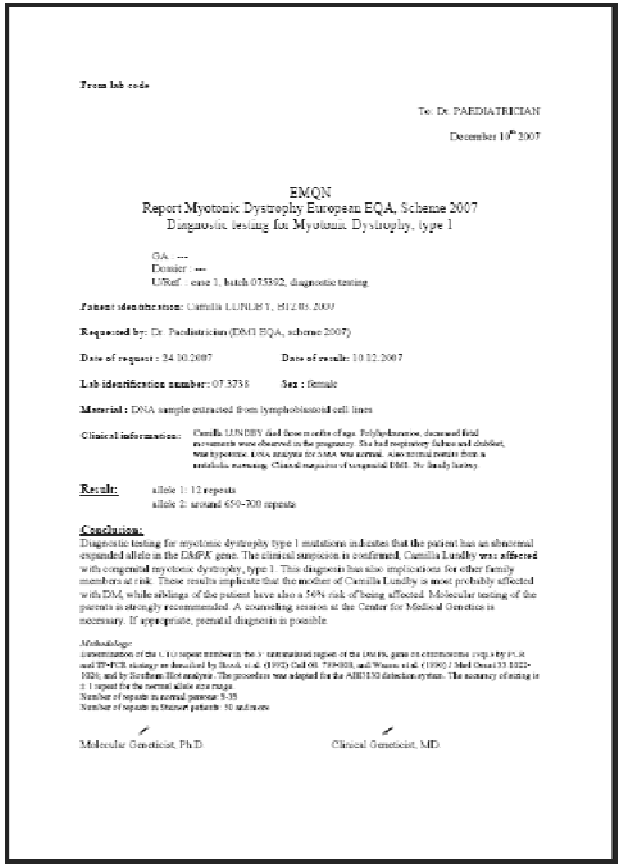
Assessment of genotyping

- Scored out of 2 marks
- Most data qualitative - genotype correct or not?
  - *BRCA2*: c.9117G>A, p.Pro3039Pro

- Potential deductions
  - Correct nomenclature used?
    - IVS2-13A/C.bp656
    - IVS2-13a>g
    - IVS2-13C>G
    - c.289-13C>G
    - c103-13A/C>G
    - c.293-13C/A>G
    - c.290-13A/C>G
    - 655A/C>G
    - g656C>G
    - g.656a>g
    - g.659A/C>G
    - CYP21A2*9
    - I2Gnt656
    - c.97-13A,C>G
  - Quantitative data – error limits

Assessment of interpretation

- Scored out of 2 marks
- Pre-agreed criteria of elements:
  - Answer to clinical question
  - References
  - Risk calculations...



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 : ---
URef : case 1, batch 015302, diagnostic testing

Patient identification: Camilla LUNDBY, 01.05.2000

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

Date of request : 24.10.2007 **Date of results:** 10.12.2007

Lab identification number: 07.5238 **Sex :** female

Material : DNA sample extracted from lymphoblastoid cell lines

Clinical information on: Camilla LUNDBY third born of the pregnancy. Polyhydramnios, decreased fetal movements were observed in the pregnancy. She had respiratory failure and cyanosis, was hypotonic. DNA analysis for SMA was normal. Also normal karyotype in amniotic sampling. Clinical diagnosis of congenital DM1. No family history

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

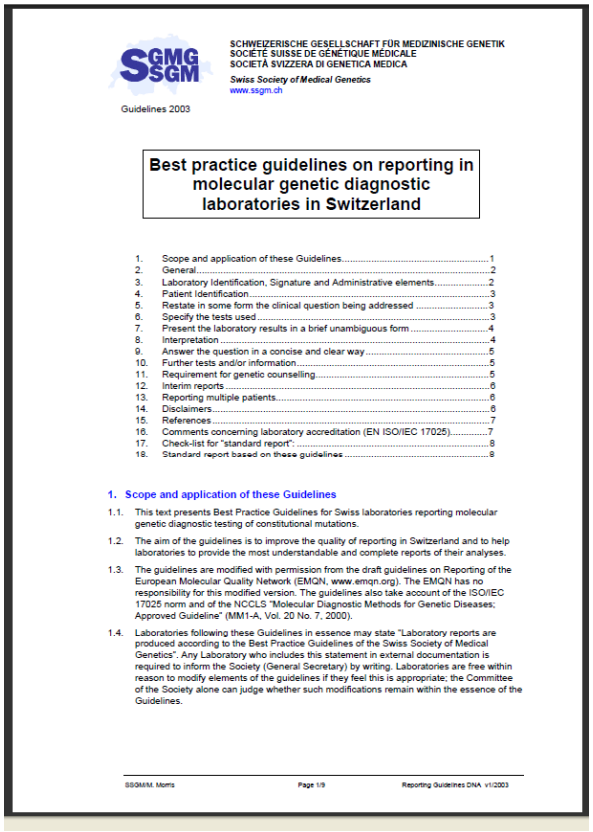
Conclusion:
Diagnostic testing for myotonic dystrophy type 1 mutations indicates that the patient has an abnormal expanded allele in the DMPK gene. The clinical suspicion is confirmed. Camilla Lundby was affected with congenital myotonic dystrophy, type 1. This diagnosis has also implications for other 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 counselling 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 19q13.3 by PCR and TP-PCR strategy as described by Reddi et al. (1992) Cell 69: 759-803, and Warner et al. (1996) J Med Genet 33: 1022-1026, and by Southern Blot analysis. The procedure was adapted for the ABI3130 detection system. The normality of range is 5-37 repeat for the normal allele size range.
Number of repeats in normal persons: 5-35
Number of repeats in disease patients: 50 and more

Molecular Geneticist, Ph.D. Clinical Geneticist, MD

Report style

- Comments rather than scores
  - Accuracy and consistency
  - Layout
  - Spelling errors
  - Methods referenced
- International guidelines
  - Local practice may differ



SCHWEIZERISCHE GESELLSCHAFT FÜR MEDIZINISCHE GENETIK
SOCIÉTÉ SUISSE DE GÉNÉTIQUE MÉDICALE
SOCIETÀ SVIZZERA DI GENETICA MEDICA
Swiss Society of Medical Genetics
www.ssgm.ch

Guidelines 2003

Best practice guidelines on reporting in molecular genetic diagnostic laboratories in Switzerland

1. Scope and application of these Guidelines

1.1. This text presents Best Practice Guidelines for Swiss laboratories reporting molecular genetic diagnostic testing of constitutional mutations.

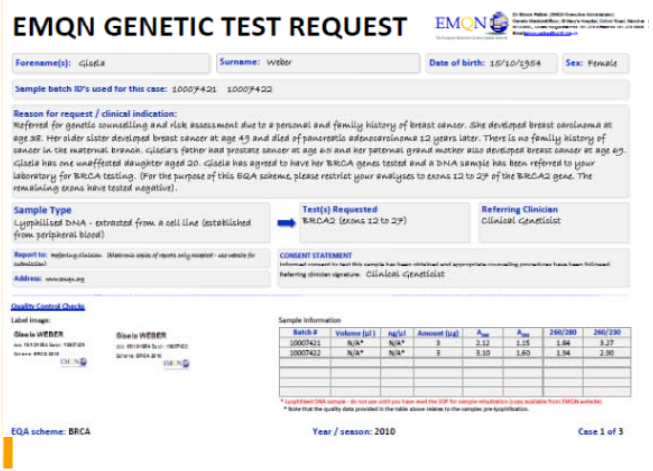
1.2. The aim of the guidelines is to improve the quality of reporting in Switzerland and to help laboratories to provide the most understandable and complete reports of their analyses.

1.3. The guidelines are modified with permission from the draft guidelines on Reporting of the European Molecular Quality Network (EMQN, www.emqn.org). The EMQN has no responsibility for this modified version. The guidelines also take account of the ISO/IEC 17025 norm and of the NCCLS "Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline" (MM1-A, Vol. 20 No. 7, 2000).

1.4. Laboratories following these Guidelines in essence may state "Laboratory reports are produced according to the Best Practice Guidelines of the Swiss Society of Medical Genetics". Any Laboratory who includes this statement in external documentation is required to inform the Society (General Secretary) by writing. Laboratories are free within reason to modify elements of the guidelines if they feel this is appropriate; the Committee of the Society alone can judge whether such modifications remain within the essence of the Guidelines.

Example from Duchenne and Becker Muscular Dystrophy scheme

- X-linked muscle wasting disease
  - Deletions and duplications in dystrophin (65% known mutations)
  - Severity of disease depends on mutation



EMQN GENETIC TEST REQUEST

Forename(s): Gisela | Surname: Weber | Date of birth: 15/10/1964 | Sex: Female

Sample batch ID's used for this case: 10007421 10007422

Reason for request / clinical indication:
Referred for genetic counselling and risk assessment due to a personal and family history of breast cancer. She developed breast carcinoma at age 38. Her older sister developed breast cancer at age 49 and died of pancreatic adenocarcinoma 12 years later. There is no family history of cancer in the maternal branch. Gisela's father had prostate cancer at age 60 and her paternal grand mother also developed breast cancer at age 69. Gisela has one unaffected daughter aged 20. Gisela has agreed to have her BRCA genes tested and a DNA sample has been referred to your laboratory for BRCA testing. (For the purpose of this EQA scheme, please restrict your analyses to exons 12 to 27 of the BRCA2 gene. The remaining exons have tested negative).

Sample Type: Lyophilised DNA - extracted from a cell line (established from peripheral blood)

Test(s) Requested: BRCA2 (exons 12 to 27)

Referring Clinician: Clinical Geneticist

CONSENT STATEMENT

Referring clinician signature: Clinical Geneticist

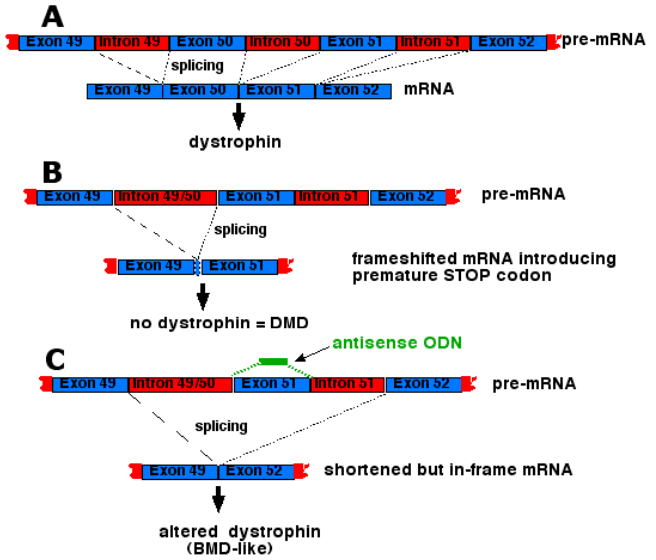
Quality Control Checks

Label image:

Sample information

Batch #	Volume (µl)	ng/µl	Amount (µg)	A_{260}	A_{280}	260/280	260/230
10007421	N/A*	N/A*	3	2.12	1.15	1.84	3.27
10007422	N/A*	N/A*	3	3.10	1.60	1.94	2.90

EQA scheme: BRCA | Year / season: 2010 | Case 1 of 3



Example of case

- Clinically diagnosed DMD patient. Genetic testing is requested for carrier testing for female relatives.

EMQN GENETIC TEST REQUEST

Dr Simon Patton (EMQN Executive Administrator)
Genetic Medicine, Room, St Mary's Hospital, Oxford Road, Manchester
M13 9WL, United Kingdom Tel +44 161 276 8 Fax +44 161 276 8606
Email simon.patton@cmft.nhs.uk

Forename(s): Jarrell | **Surname:** Hanssen | **Date of birth:** 10/01/2007 | **Sex:** Male

Sample batch ID's used for this case: 10007290 10007907 10007906

Reason for request / clinical indication:
Clinically diagnosed as a Duchenne Muscular Dystrophy patient (muscle biopsy shows absence of dystrophin). Genetic testing is requested for future carrier testing for female relatives of Jarrell.

Sample Type
Lyophilised DNA - extracted from a cell line (established from peripheral blood)

Test(s) Requested
DMD/BMD testing

Referring Clinician
Consultant Clinical Geneticist

Report to: Referring clinician (Electronic copies of reports only accepted - use website for submission)

Address: www.emqn.org

CONSENT STATEMENT
Informed consent to test this sample has been obtained and appropriate counseling procedures have been followed.
Referring clinician signature: Consultant Clinical Geneticist

Quality Control Checks

Label Image:

Sample Information

Batch #	Volume (µl)	ng/µl	Amount (µg)	A_{260}	A_{280}	260/280	260/230
10007290	N/A*	N/A*	4	2.82	1.43	1.97	2.97
10007907	N/A*	N/A*	4	2.44	1.27	1.92	3.16
10007906	N/A*	N/A*	4	1.96	1.02	1.93	3.72

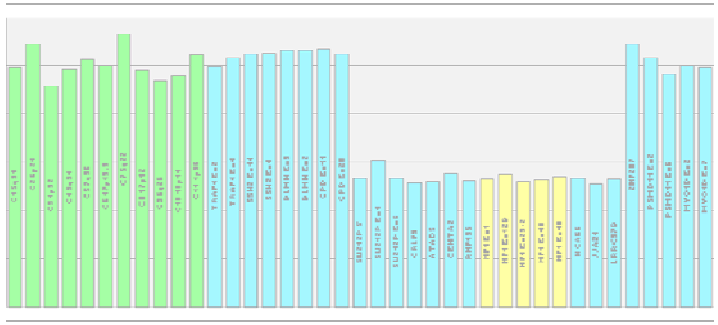
* Lyophilised DNA sample - do not use until you have read the SOP for sample rehydration (copy available from EMQN website)
* Note that the quality data provided in the table above relates to the samples pre-lyophilisation.

EQA scheme: DMD / BMD **Year / season:** 2010 **Case** 2 of 3

Marking

- GENOTYPE:
  - Deletion of exons 64-67

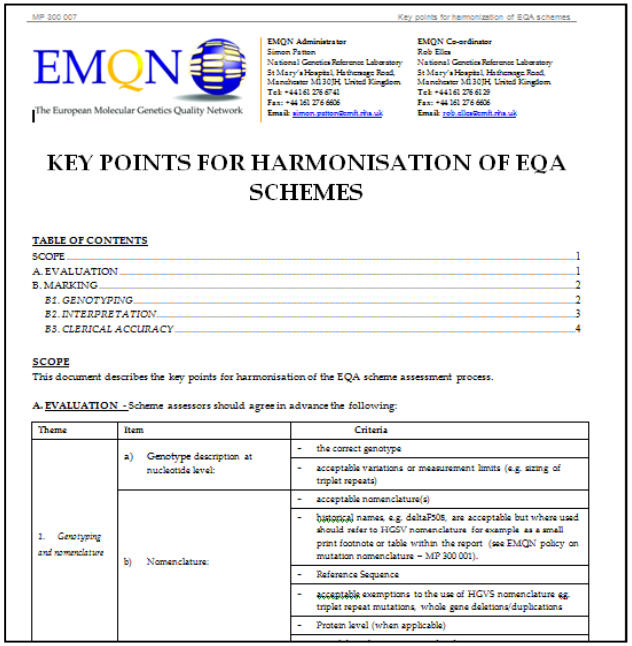
    (2.0 points)



- INTERPRETATION
  - The deletion found is out of frame – no functional protein > confirms diagnosis *(1.5 points)*
  - Female relatives maybe carriers and have risk for affected children – testing available *(0.5 points)*

Assessment and ISO17043

- Assessment against pre-agreed criteria
- Technical experts used for assessment
  - Training
  - Competency and confidentiality
  - Harmonisation
    - between assessors
    - between schemes



MP 300 007 Key points for harmonization of EQA schemes

EMQN

The European Molecular Genetics Quality Network

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KEY POINTS FOR HARMONISATION OF EQA SCHEMES

TABLE OF CONTENTS

SCOPE

This document describes the key points for harmonisation of the EQA scheme assessment process.

A. **EVALUATION** - Scheme assessors should agree in advance the following:

Theme	Item	Criteria
1. *Genotyping and nomenclature*	a) Genotype description at nucleotide level:	- the correct genotype
		- acceptable variations or measurement limits (e.g. sizing of triplet repeats)
	b) Nomenclature:	- acceptable nomenclature(s)
		- historical names, e.g. deltaF508, are acceptable but where used should refer to HGSV nomenclature for example as a small print footnote or table within the report (see EMQN policy on mutation nomenclature – MP 300 001).
		- Reference Sequence
		- acceptable exemptions to the use of HGVS nomenclature eg. triplet repeat mutations, whole gene deletions/duplications
		- Protein level (when applicable)

Genotyping and interpretation scores from HD scheme

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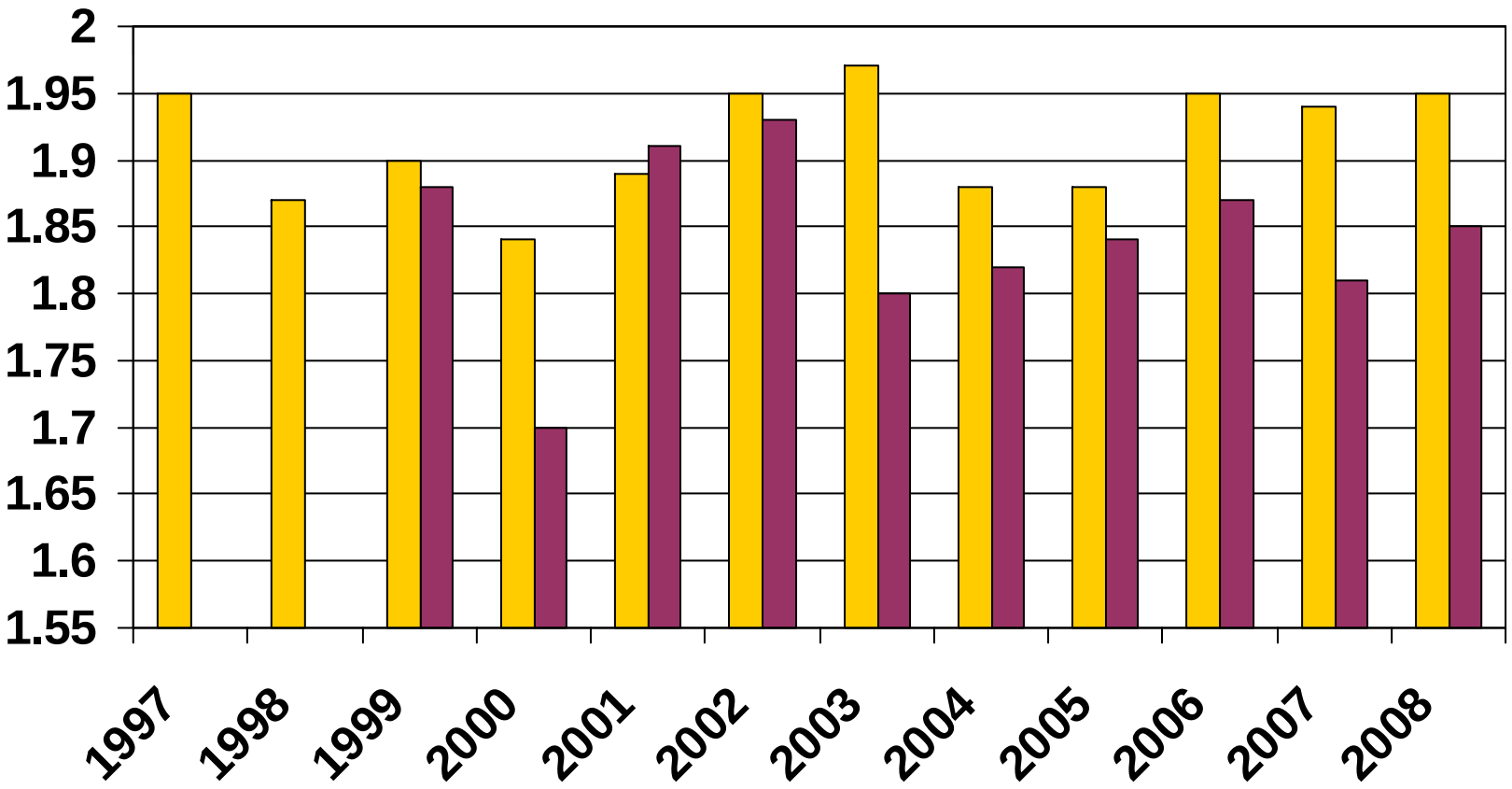

Average genotyping (yellow) and interpretation (purple) scores for Huntington disease

Future directions

EMQN
The European Molecular Genetics Quality Network

Free fetal DNA analysis for aneuploidy

Next generation sequencing multi-gene disorders

Pharmacogenetics / Molecular Pathology

Acknowledgements

Scheme participants and organisers

More information:

- Website – www.emqn.org
- EMQN OFFICE
  - support@emqn.org