„ Validation of methods in DNA diagnostics: an overview of EuroGentest activities

Milan Macek on behalf of the EuroGentest NoE Units 1 and 5

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www.eurogentest.org

Introduction and Background to Validation

(„a Procedure which demonstrates that the test is fit for the intended purpose“:

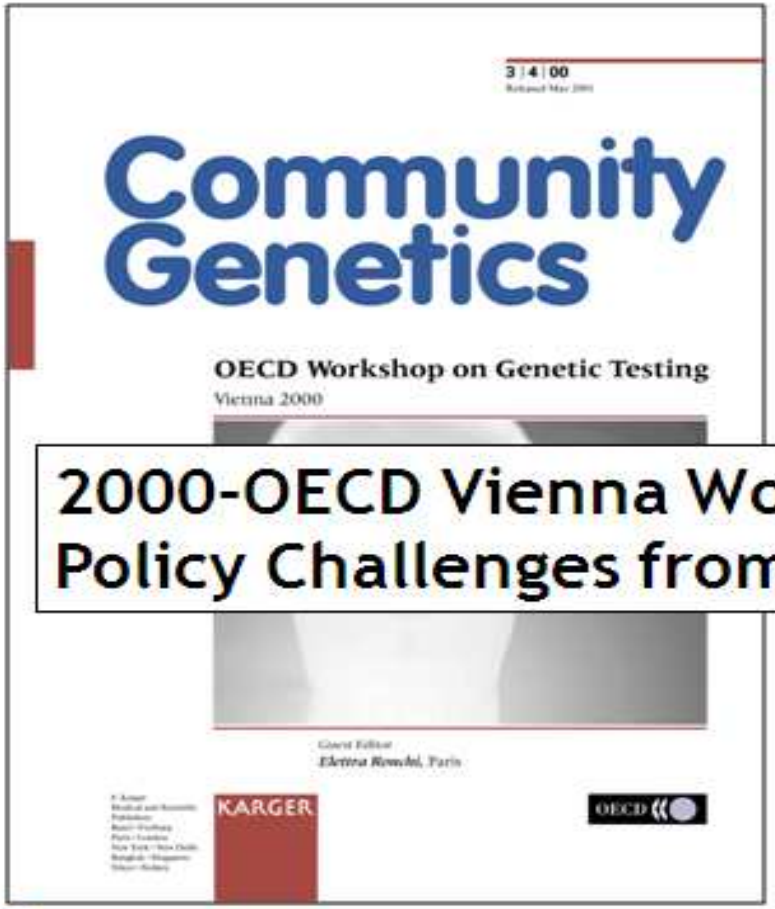
OECD / IPTS / EuroGenTest Surveys

Why guidelines for MGT?

Molecular Genetic Testing:

- An economic activity
- An international activity
- Increasing role in improving healthcare
- Potential risks of mis-application of tests with high predictive value
- Relevance of test results to other family members
- Direct access by the public

Background to the validation of laboratory tests



2000-OECD Vienna Workshop:
Policy Challenges from the New Genetics

EuroGentest

OECD.int ; provides analysis, forecasts and guidelines to members

Quality issues in Europe

More than 1000 laboratories/centers in different settings

More than 1000 rare diseases can be tested

Lack of centralized and uniform information about services

Limited networking

Lack of harmonized and standardized EQA
Lack of reference materials
Limited number of accredited labs

Insufficient counselling

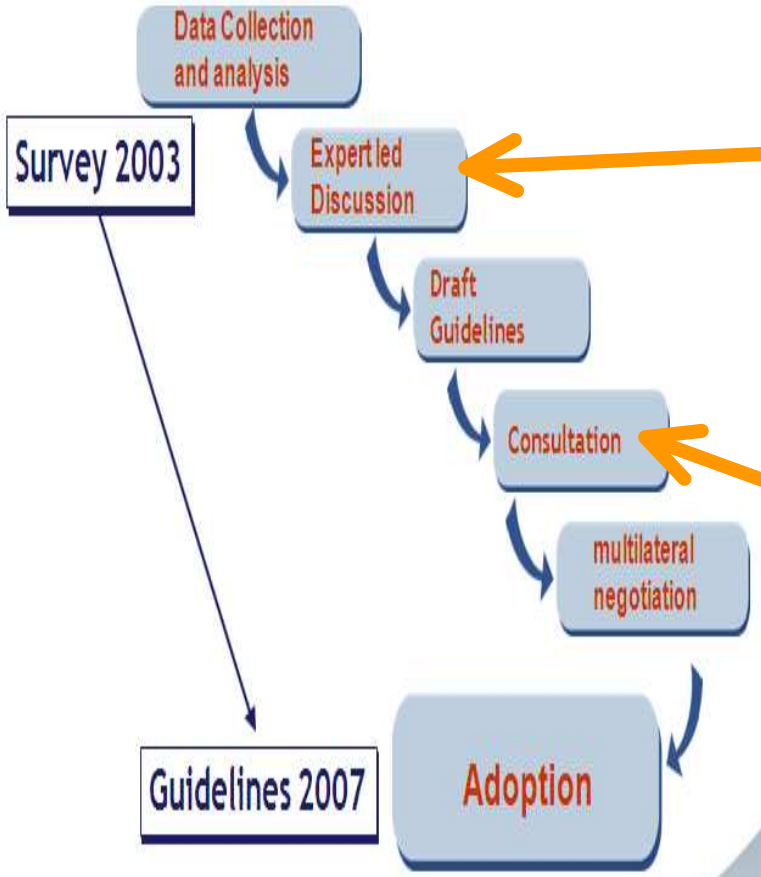
Source: IPTS/JRC-EC: Ibarreta et al. Towards quality assurance and harmonization of genetic testing services in the EU.

Report EUR20977, 2003

EuroGentest
Genetic Testing in Europe

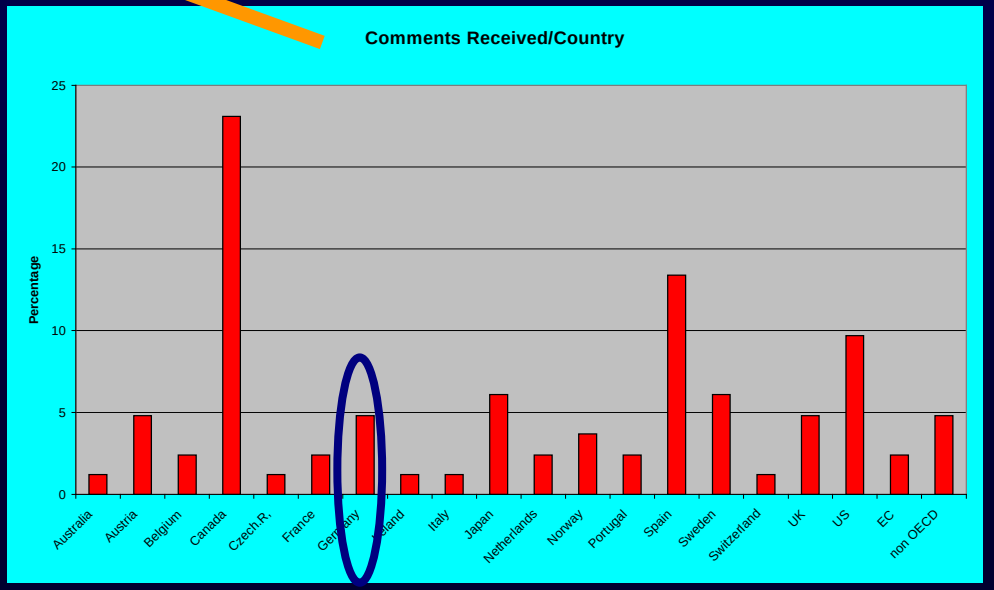
OECD Process

Biotechnology



Information Collected

- Laboratory Setting and Personnel Qualifications
- Referral Across National Boundaries
- Informed consent and confidentiality policies
- Types of Analyses
- Methods
- Standard Operating Procedures
- Reporting Practices
- Personnel qualifications
- Licensing, Accreditation and Proficiency Testing
- Patents



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Survey Results Community Genet. 10(3):123-31, 2007

- **International exchange** is a widespread feature of Molecular Genetics service provision.
  - 64% of Laboratories received specimens from other countries
  - Over 18,000 specimens crossed borders in 2002
- Variables contributing to a high Quality Score ($p<0.005$):
  - ✓ Accreditation of the laboratory
  - ✓ Participation in proficiency testing
  - ✓ Director having formal training in molecular genetics
  - ✓ Affiliation with a Genetics Unit

http://www.oecd.org/dataoecd/25/12/34779945.pdf

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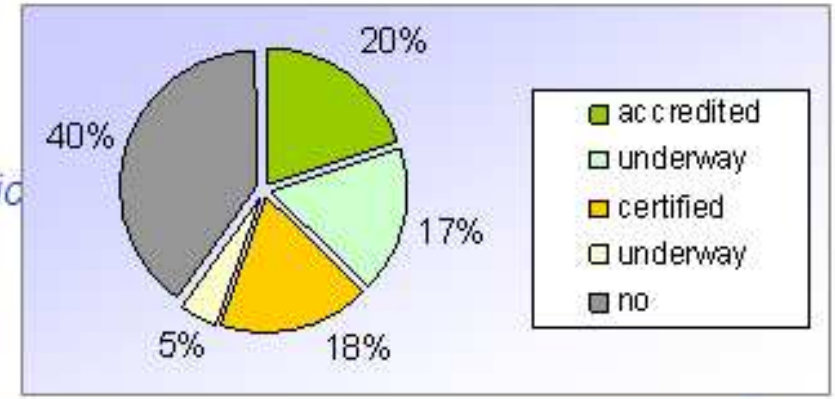
QA Survery EuroGentest (2006)

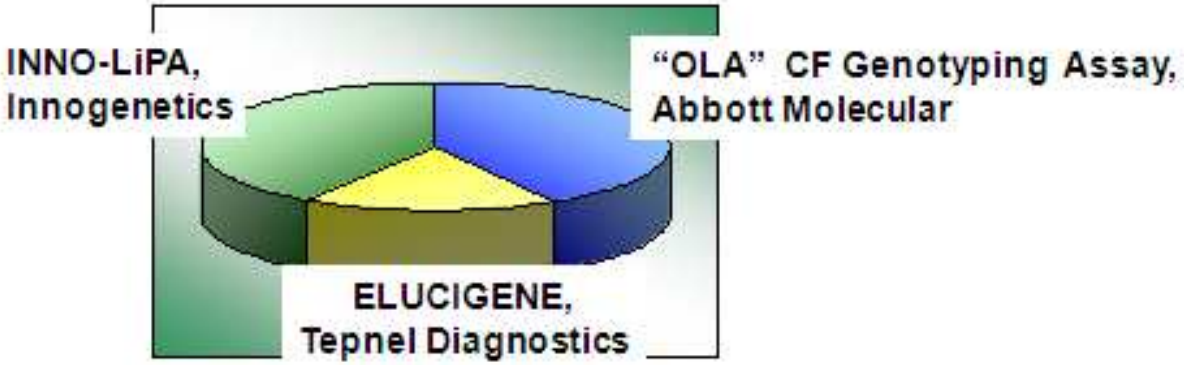
- **Answers from ~350 testing labs from 32 countries:**
  - Validation of information from questionnaire sent to 2,300 individuals
  - QAu data validated with EQA/accreditation providers
  - each lab receives unique, permanent EUGT number
  - data exchange with Orphanet
    - Orphanet completes test data
- *Key presentations:*
  - *European Society of Human Genetics, Amsterdam*
  - *International Congress of Human Genetic Brisbane*



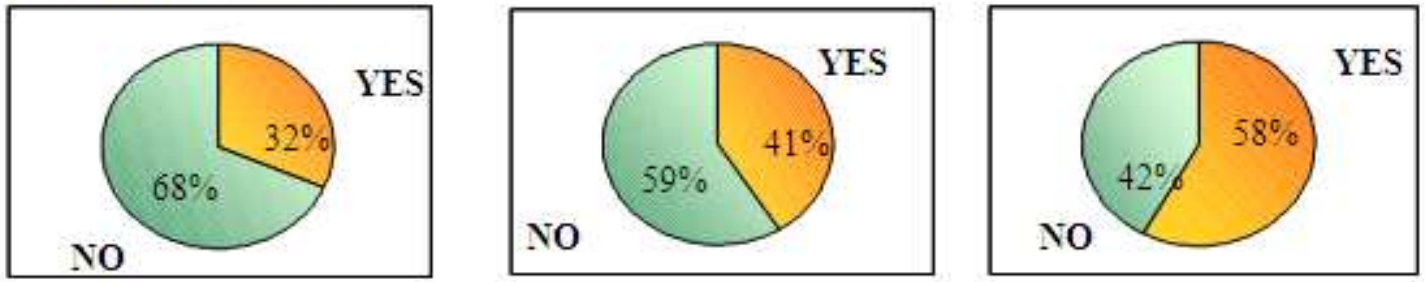
CF ThematicNetwork + EuroGentest survey (2006)

3) Diagnostic use of commercial kits for Cystic Fibrosis

- Questionnaire: variability of procedures



- Modifications



ELUCIGENE | INNO-LiPA | "OLA" CF Genotyping Assay

⇒ importance of the protocols validation!

What do the Guidelines do?

www.oecd.org/sti/biotechnology

- Set minimum standards
- Encourage best practice:
  - OECD countries are asked to implement the guidelines
  - Non-member countries invited to use them to set policies
- Progress report and review in 2011

Structure

A. General principles of MGT
B. Quality Assurance systems
C. Proficiency Testing
D. Result Reporting
E. Education and training

EuroGentest Units 1 and 5:

an brief overview of activities related to validation

Unit 1

EuroGentest — Harmonizing genetic testing across Europe

bout Us | What Can We Offer? | Databases | Lab Quality | New Technologies | Public Health | Education | Ethics & Legal | Guidelines | Events | News

QUALITY MANAGEMENT AND ACCREDITATION/CERTIFICATION OF GENETIC TESTING (UNIT 1)

Quality Management System

- Accreditation
- External Quality Assessment
  - Molecular
  - Cytogenetics
  - Biochemical
- Diagnostic Validation
- Reference Materials
- Internal audit
- IT support

Education & Training

- Workshops
- Guidelines
- Definitions
- Useful links

General Information

Our aim is to measurably improve the quality of the management and provision of genetic services for the benefit of the patient and that lab accreditation is considered the norm.

- Objectives
- Members & contact info
- Articles and reports

Events All events

Reference Materials for New Molecular Genetics Technologies - Challenges and Opportunities - 24 Apr 2008 - Geel (BE)

Molecular Genetic testing is evolving rapidly, with the introduction of new technologies such as MLPA, array CGH, ultra high throughput DNA sequencing and the possibility of genotyping over 1 million SNPs in a single experiment. More...

Training - Quality management in your laboratory: How you can get involved? (Dutch speaking) (WP1.8) - 26 May 2008 - Leuven (BE)

Practical exercises, applied to the own works situation. Sharing experiences with colleagues and experts. Topics include the

News

UK NEQAS has opened registration for the CF testing on blood spots to European laboratories

UKNEQAS UK NEQAS for Molecular Genetics has opened registration for the Cystic fibrosis testing on blood spots External Quality Assurance (EQA) 2008 scheme to European laboratories. Numbers have been limited for this year so any interested parties should contact the Scheme Organiser as soon as possible. (upd.18 Mar 2008) More...

The IVD Directive and Genetic Testing: Problems and proposals

The in-vitro Medical Devices Directive (IVD Directive) sets a framework for the regulation of in-vitro diagnostic tests in the EU. Issued in 1998, the Directive came into force in 2003 in all member states. Since then, some issues have arisen with the Directive which have particular relevance for genetic testing. (upd.12 Feb 2008) More...

Registration open for new Workshops on Quality

The registration is now open for the workshop on 'Accreditation in genetic testing services' and the workshop 'Towards accreditation - managing the human side of change' organized in parallel, just before the

Quality Assurance Database

- Access via www.orpha.net and eurogentest.org

Directory of EQA Schemes and Providers

UNIT 1 - QUALITY MANAGEMENT AND ACCREDITATION / CERTIFICATION OF GENETIC T

Qm

I. Constitutional mutations
II. Methodological EQA
Addresses of contacts
Acknowledgements

EMQN and GfH EQA scheme merger

EMQN The European Molecular Genetics Quality Network

We are very pleased to announce that on December 20th 2007 the board of the German Society of Human Genetics (GfH) approved the merger of the EMQN and GfH External Quality Assessment schemes. (upd.18 Jan 2008) More...

MOLECULAR GENETIC TESTING - EXTERNAL QUALITY ASSESSMENT SCHEME PROVISION (2005)

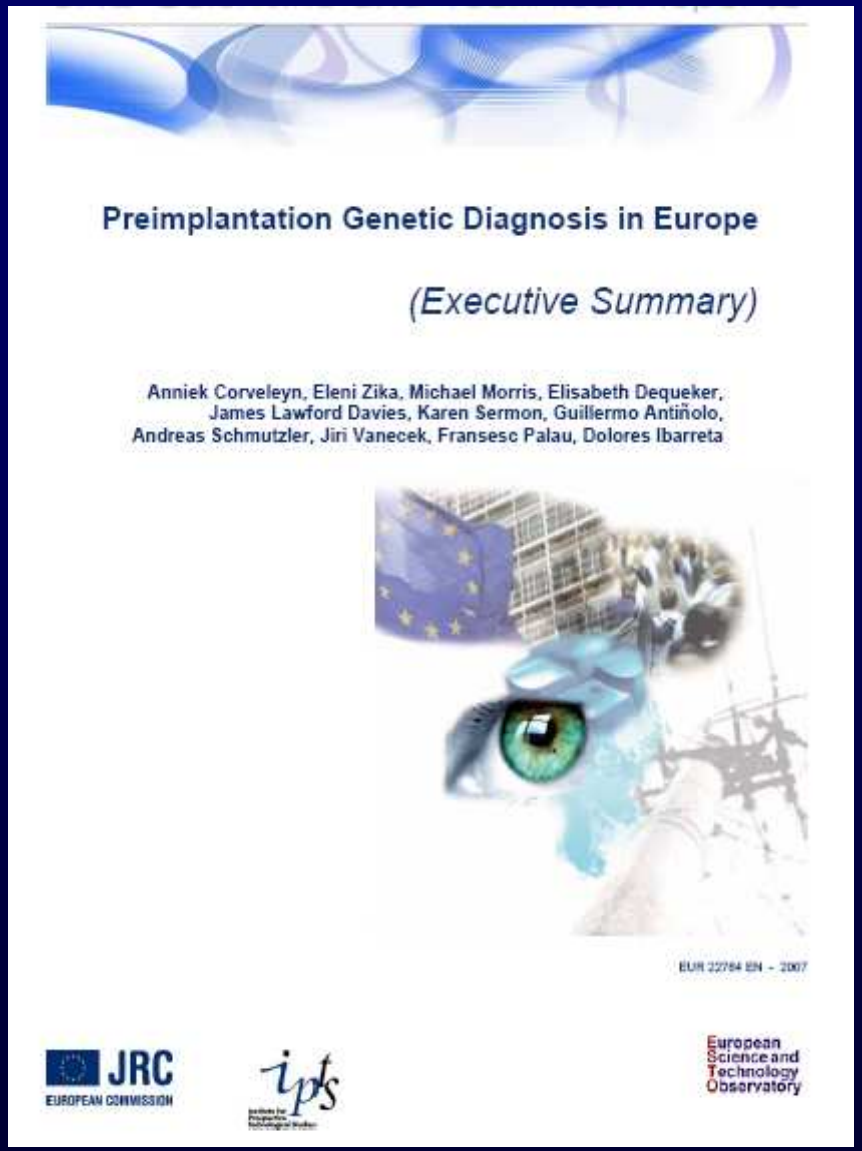
I. Constitutional mutations

Disorder	Organisation	Contact Point
1. ACE (Angiotensin I Converting Enzyme)	DGKL [2] (RtB) [12]	M. Neumaier, Mannheim
	ÖQUASTA [4] (in collaboration with DGKL)	W.-M. Halbmayer, Ch. Mannhalter, Wien
	ECAT Fdn. [7] (in collaboration with DGKL)	P. Meijer, Leiden
	LABQUALITY [16]	M. Keinanen, Helsinki
2. alpha-1-Antitrypsin (PiM, PiS, PiZ); (RV Nr.743)	INSTAND [11]	R.-R. Flörke, Düsseldorf
3. alpha-1-Proteinase-Inhibitor (M,S,Z)	DGKL [2] (RtB) [12]	M. Neumaier, Mannheim
	ÖQUASTA [4] (in collaboration with DGKL)	W.-M. Halbmayer, Ch. Mannhalter, Wien
	ECAT Fdn. [7] (in collaboration with DGKL)	P. Meijer, Leiden
4. Apolipoprotein B100 (ApoB100)	DGKL [2] (RtB) [12]	M. Neumaier, Mannheim
	ÖQUASTA [4] (in collaboration with DGKL)	W.-M. Halbmayer, Ch. Mannhalter, Wien
	ECAT Fdn. [7] (in collaboration with DGKL)	P. Meijer, Leiden
5. Apolipoprotein E (ApoE); E2/E3/E4	DGKL [2] (RtB) [12]	M. Neumaier, Mannheim
	ÖQUASTA [4] (in collaboration with DGKL)	W.-M. Halbmayer, Ch. Mannhalter, Wien
	ECAT Fdn. [7] (in collaboration with DGKL)	. Meijer, Leiden
	LABQUALITY [16]	M. Keinanen, Helsinki
6. Apolipoprotein E (E2, E3, E4); (RV Nr.744)	INSTAND [11]	R.-R. Flörke, Düsseldorf
7. Azoospermia (AZF/DAZ)	EAA [6]/EMQN [10]	M. Simoni, Münster
8. Breast/ovarian cancer familial (BRCA1, BRCA2)	EMQN [10]	S. Patton, Manchester

EuroGenTest
Genetic [illegible] Europe

Disease specific best practice guidelines

www.cfnetwork.be



www.eurogentest.org

Types of workshops

Accreditation and quality management

General workshop for those who would like to start up or improve their quality system.

Internal audit

Specific workshop on the preparation, execution and evaluation of an internal audit.

Towards accreditation - Managing the human side of change

This workshop will give insight on the behavioral side of change and how to apply this in real life.

IT support

Learn what is on the market and which criteria are important when implementing electronic support for your QMS.

Fulfilling the requirements of ISO 15189

Workshop tackling specific topics of the ISO standard such as management review.

Diagnostic validation

Workshop on the requirements for validation regarding ISO 15189 and related issues.

Round table sessions ESHG - Case studies on quality assurance and quality control issues in genetic testing laboratories

Short session to discuss on and share experiences with quality management and

November 2008

19	Train The Trainer Workshop (WP1.8)	Belgium

October 2008

9 - 10	Workshop - Fulfilling the requirements of ISO 15189 for management review, internal quality control and external quality assessment (WP1.8) Want to learn more on how to do a management review, on how to introduce internal quality controls in the lab or what participating in EQA means?	Germany

May 2008

31	Round table sessions - Case studies on quality assurance and quality control issues in genetic testing laboratories (WP1.8) Discuss in small groups about concrete situations related to quality processes in genetic testing labs. Meet colleagues faced with similar challenges and problems.	Spain
30 - 31	Workshop - Towards accreditation: Managing the human side of change (WP1.8) This workshop will tackle the human side of change. How do you motivate the lab to implement a new quality system? How do you manage the human side of this process of change?	Spain
30 - 31	Workshop - Accreditation in genetic testing laboratories (WP1.8) Compare and share experiences of implementing and living with quality systems. Examine cases of concrete situations related to quality processes like non-conformities, reporting and training.	Spain
26	Training - Quality management in your laboratory: How you can get involved? (Dutch speaking) (WP1.8) Practical exercises, applied to the own works situation. Sharing experiences with colleagues and experts. Topics include the elements of a quality management system, aspects of ISO 15189 standard, non-conformities, ...	Belgium

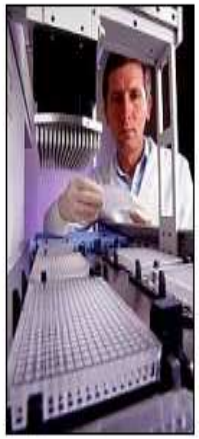
February 2008

7 - 8	Workshop - Towards accreditation: Managing the human side of change (WP1.8) This new topic in a series of workshops related to quality management will tackle the human side of change. How do you motivate the laboratory to implement a new quality system? How do you manage the human side of this process of change?	France

Unit 5

Research And Emerging Technologies (Unit 5)

General aim



Thus far the introduction of molecular genetic tests into diagnostic service has mainly been at an ad hoc and case-to-case basis. Quality systems, proper evaluation and general validation procedures were often lacking in the implementation process of the novel technology. This has seriously delayed and hampered the proper introduction of new genetic tests and has also caused increased financial expanses for many laboratories and research partners.

The aim of EuroGentest Unit 5 is to support and to guide the implementation of emerging technologies into diagnostic application. A rigorous test evaluation program, including Beta testing in accredited laboratories and on selected clinical samples, will be used to introduce new technologies into the European diagnostic labs.

Unit Information

- Meet The Team
- Objectives, Deliverable, Milestones

Resources

Technology Database (WP5.2)

A database tool to help you find information on new and innovative techniques in genome diagnostics in the world of genetics

Call for Technology (WP5.2)

Unit 5 is currently looking for Innovative Techniques in Genome Diagnostics, for more information please click on the link above.

Bioinformatics (WP5.4)

- Description of Quality Assessment Tool
- Quality Assessment of Bioinformatics Tools

Current Test Trials (WP5.1)

- The MLPA validation study
- Update on the HR-MCA evaluation study
- DNA Extraction Methods For Large Blood Volumes
- Confirmation Sensitive Capillary Electrophoresis
- High-throughput BRCA1 mutation scanning using HR-MCA

Intellectual Property (WP5.3)

- Survey Patent Licensing in Medical Biotechnology in Europe

Articles

News

Technology Assessment report on Fragile X testing by use of the "Abbott kit"

A multi-centre assessment of the Abbott Molecular Fragile X analyte specific reagent (ASR) kit. (upd.14 Feb 2008) More...

Survey Patent Licensing in Medical Biotechnology in Europe

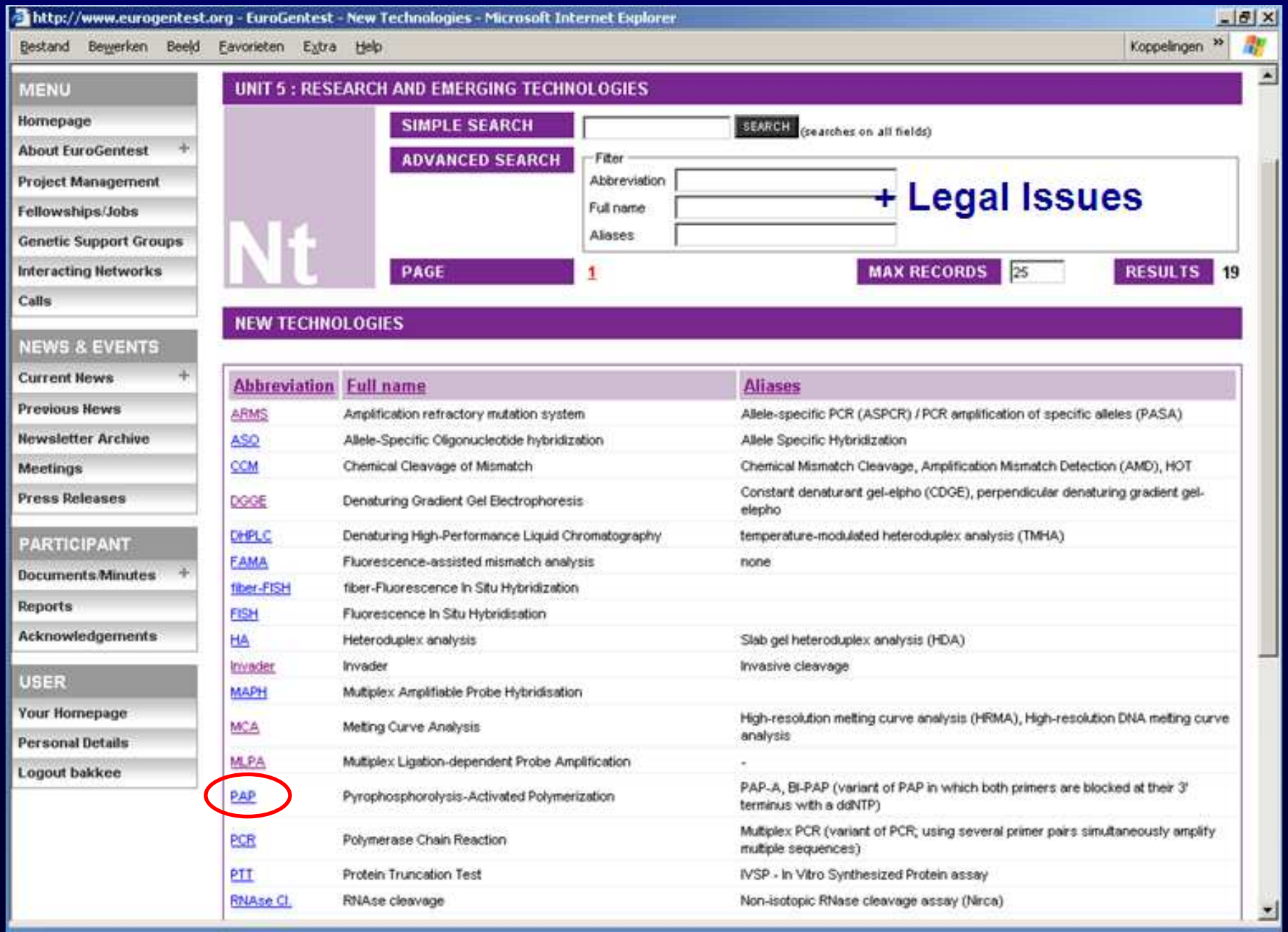
The invitation for the survey has in particular been sent to professionals dealing with patenting and licensing in medical biotechnology. Eurogentest strongly encourages its Members to take part in this survey, so as to ensure that its results will accurately reflect the current situation and the needs of the genetic community. (upd.06 Feb 2008) More...

Call for new technologies

EuroGentest offers industry a unique network of accredited laboratories to evaluate new technologies. The next call for submissions is now underway. (upd.16 Oct 2007) More...

EUGT Satellite Meeting: "Innovative Techniques in Genome diagnostics"

EUGT Unit 5 announce a satelite meeting to be held at ESHG on the subject of "Innovative Techniques in Genome diagnostics". (upd.18 May 2007) More...



Abbreviation	Full name	Aliases
ARMS	Amplification refractory mutation system	Allele-specific PCR (ASPCR) / PCR amplification of specific alleles (PASA)
ASO	Allele-Specific Oligonucleotide hybridization	Allele Specific Hybridization
CCM	Chemical Cleavage of Mismatch	Chemical Mismatch Cleavage, Amplification Mismatch Detection (AMD), HOT
DGGE	Denaturing Gradient Gel Electrophoresis	Constant denaturant gel-elpho (CDGE), perpendicular denaturing gradient gel-elepho
DHPLC	Denaturing High-Performance Liquid Chromatography	temperature-modulated heteroduplex analysis (TMHA)
FAMA	Fluorescence-assisted mismatch analysis	none
fiber-FISH	fiber-Fluorescence In Situ Hybridization	
FISH	Fluorescence In Situ Hybridisation	
HA	Heteroduplex analysis	Slab gel heteroduplex analysis (HDA)
Invader	Invader	Invasive cleavage
MAPH	Multiplex Amplifiable Probe Hybridisation	
MCA	Melting Curve Analysis	High-resolution melting curve analysis (HRMA), High-resolution DNA melting curve analysis
MLPA	Multiplex Ligation-dependent Probe Amplification	-
PAP	Pyrophosphorolysis-Activated Polymerization	PAP-A, BI-PAP (variant of PAP in which both primers are blocked at their 3' terminus with a ddNTP)
PCR	Polymerase Chain Reaction	Multiplex PCR (variant of PCR; using several primer pairs simultaneously amplify multiple sequences)
PTT	Protein Truncation Test	IVSP - In Vitro Synthesized Protein assay
RNAse CL	RNAse cleavage	Non-isotopic RNase cleavage assay (Nirca)

Evaluation of novel techniques

-PAP in Non-invasive prenatal diagnosis (Bert B, Rob E. and Maj H. -SAFE-)

First results were presented at the ESHG meeting in Nice, **two oral presentations**

Article published in Prenat Diagn. 2007 Oct;27(10):932-7.

Y chromosome detection by Real Time PCR and pyrophosphorolysis-activated polymerisation using free fetal DNA isolated from maternal plasma.

Boon EM, Schlecht HB, Martin P, Daniels G, Vossen RH, den Dunnen JT, Bakker B, Elles R.

Results foetal sexing on ffDNA using PAP

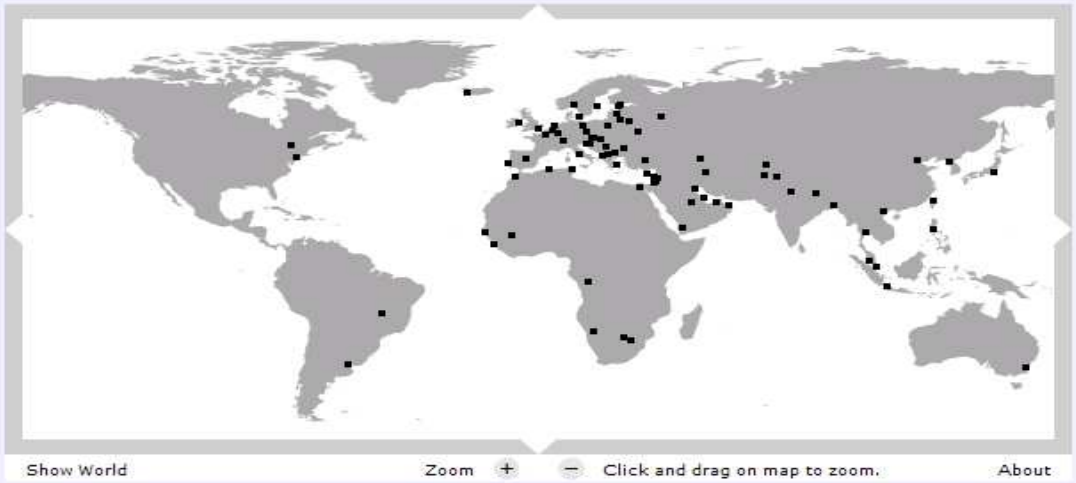
3% TBE gel

	PAP result	Confirmed result
M= 100 bp marker		
1= sample1	+ve	male
2= sample 2	+ve	male
3= sample 3	+ve	male
4= sample 4	+ve	male
5= sample 5	-ve	female
6= sample 6	+ve	male
7= female control		
8= male control		
9= female + male control		
10= H_2O		

Future:

Training labs

FindBase – www.findbase.org



You may start a search by clicking on the map or selecting from the pull-down lists below.

Search by population

Search by disorder

he initial data came from previously published reports as well as from unpublished information contributed from indiv
esearchers prior of publication. This information was converted to a database, and now new entries are added and o
re corrected by our expert advisors and collaborators.

Encouraged by

HGV
HUMAN GENOME VARIATION SOCIETY

Golden Helix.org

Supported by

ithanet
Electronic Infrastructure For Thalassaemia Research Network

FP6 Collaboration action

EuroGentest

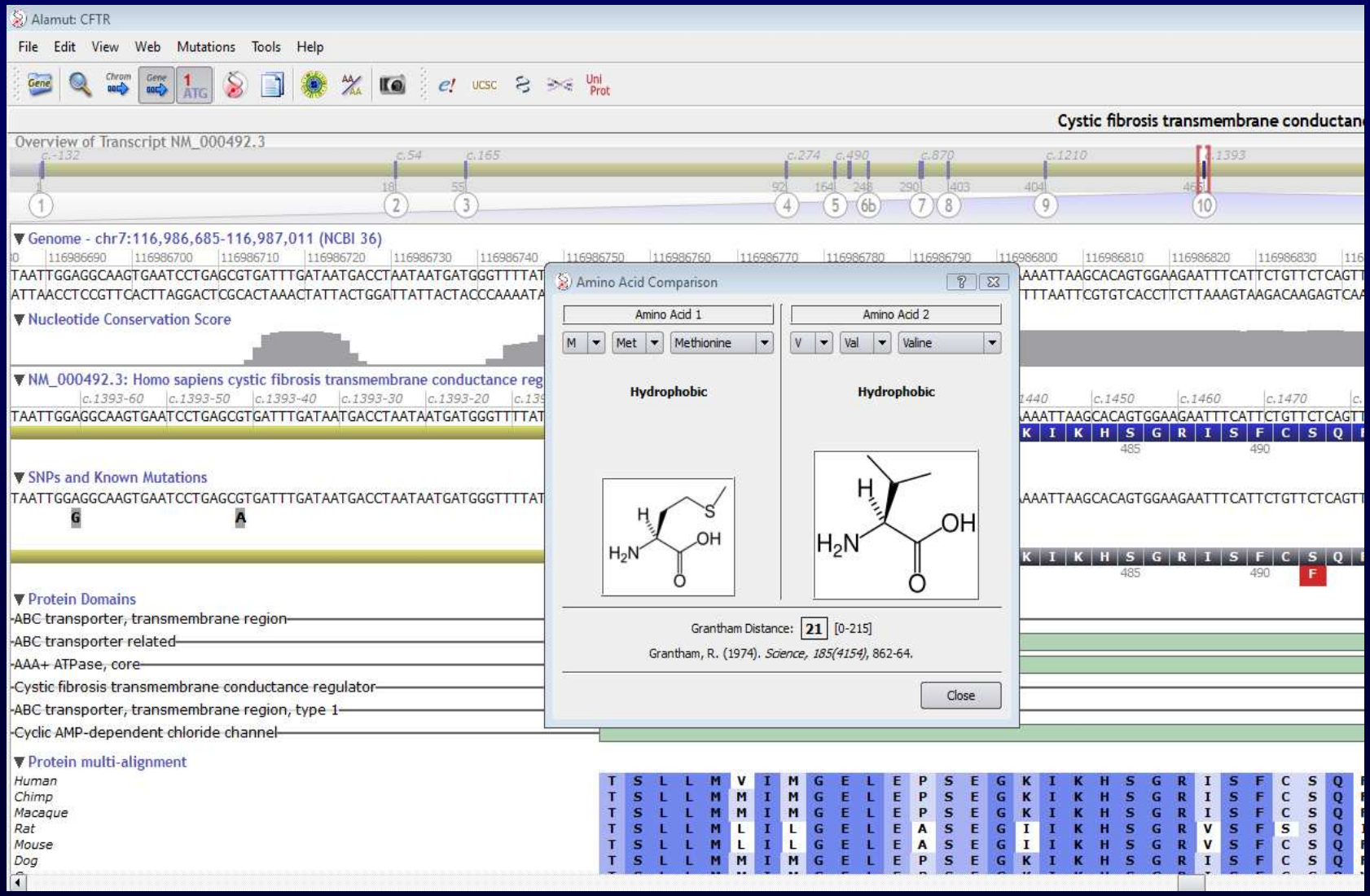
Member of

EuroGentest

FP6 Network of excellence

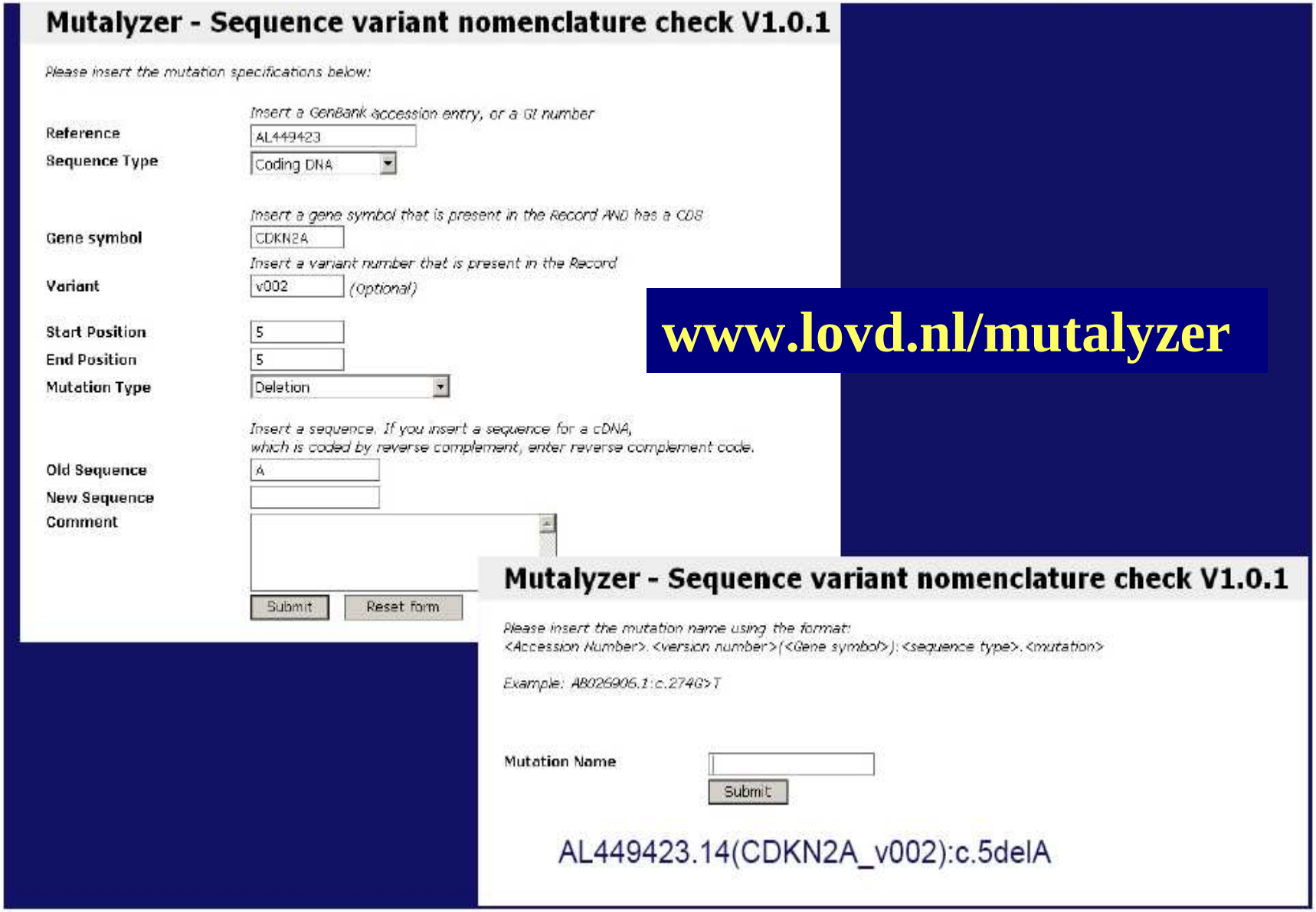
LSDB

Validation on CF Mutation Database in Toronto



disappointment…www.europeancfconference.org

Mutalyzer – Name Generator & Checker input



Validation overview

Definitions

- **"Demonstrate that the test is fit for the intended purpose"**
- ISO/CEI 17025 and 15189
- "
  - "The confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled"
  - "the validation shall be as extensive as is necessary to meet the needs of the given application"
  - "The methods and procedures selected for use shall be evaluated and found to give satisfactory results before being used for medical examinations".

Preexisting guidelines

- EAL/Eurolab EAL-P11 (1997)
  - "Validation of test methods – General principles and concepts "
- S.S. Ehrmayer 2000 (www.westgard.com)
  - " Method validation: the regulations"
- US FDA & CVM, 2001
  - " Guidance for Industry – Bioanalytical method validation "
- IUPAC 2002
  - " Harmonized Guidelines for single-laboratory validation of methods of analysis"
- NCCLS/CLSI
  - MM1-A, 2000 "Molecular diagnostic methods for genetic diseases; approved guideline"
- COFRAC
  - LAB GTA04, juin 2004 "Guide de validation des méthodes en biologie médicale"

However, these guidelines are „user hostile"…

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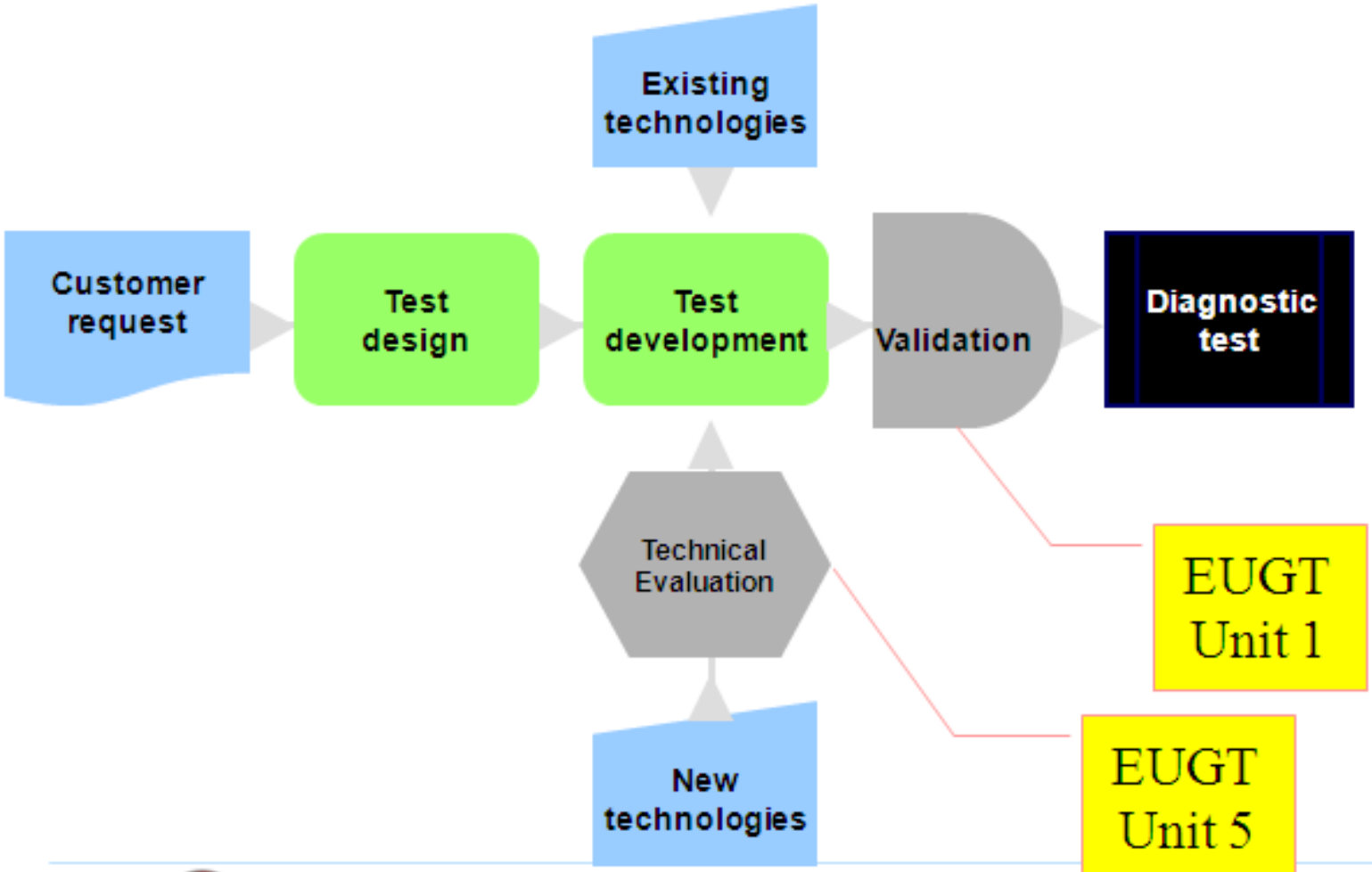
What is validating a test ?



What to validate?

- **Precision**
  - Repeatability
    - Ability to provide closely similar results, for repeated tests under the same conditions
  - Reproducibility
    - Ability to provide closely similar results, for repeated tests under different conditions
- **Ruggedness**
  - If relevant
  - Resistance to changed conditions
- **Uncertainty of measurement (mol. genetics becomes quantitative !)**
  - Required if the client requests it;
    when it may be relevant to the validity or application of the results
    when it may affect compliance to a specification limit
  - 'Estimation' not 'Determination' or 'Quantitation'

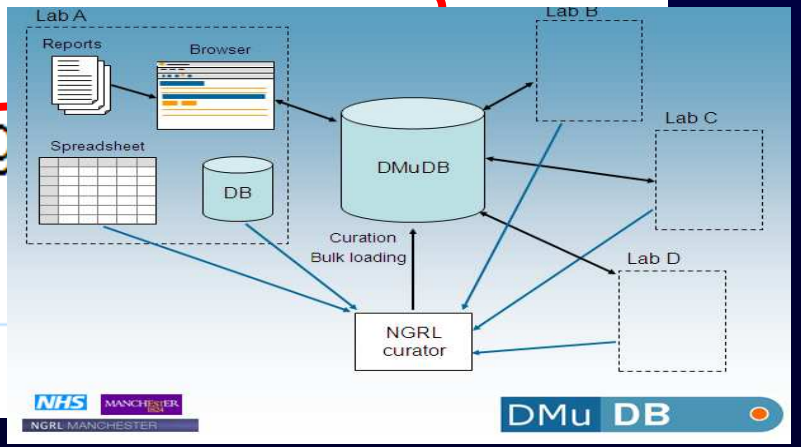
Practically: "Validation"

- Repeatability
  - 10 different samples (normal + mutant)
  - 2 samples, tested 10 times
- Reproducibility
  - 2 samples, 10 times, on 3 different days
  - Same 2 samples, 10 times, by a second person
- Ruggedness
  - Adjust to the test
  - Different DNA concentrations?
  - Include "difficult" samples (uncultured amniocytes…)?
  - Different types of PCR machine and/or genescanner
  - Prealiquotted or diluted primers?
  - Frozen aliquots?

The devil is in the detail and… statistics

"Verification"

- Applied to lesser modifications
- e.g. PCR-"cycle" sequencing
  - Amplification is of sufficient quality
  - Product is the right size
  - Sequence of sufficient quality
  - Region of interest covered in both directions
  - Normal sequence of frag
    predicted

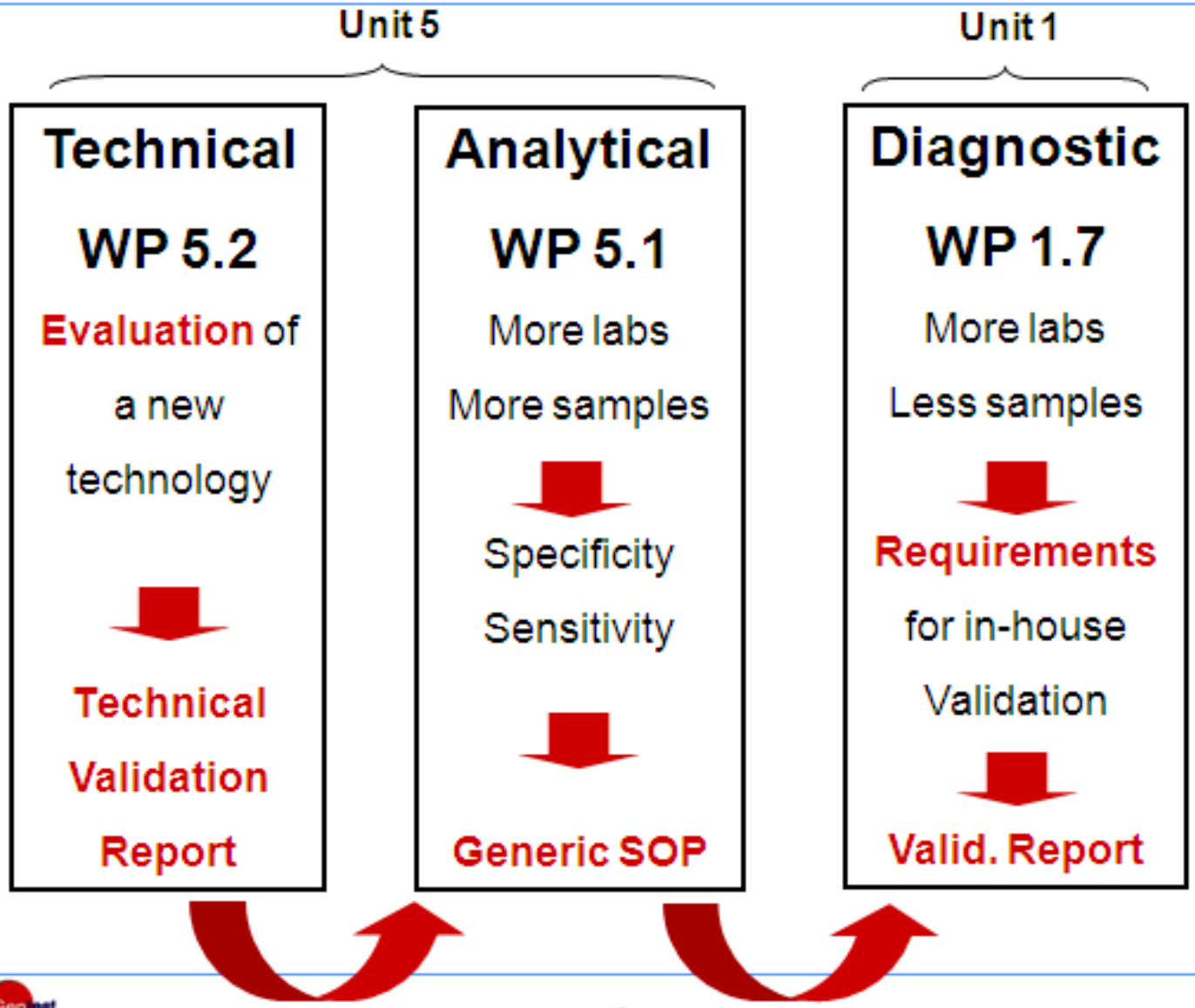


EuroGentest
Genetic Testing in Europe

Phenosystems

Practical examples:
MLPA and HRMCA

Validation Flow Chart



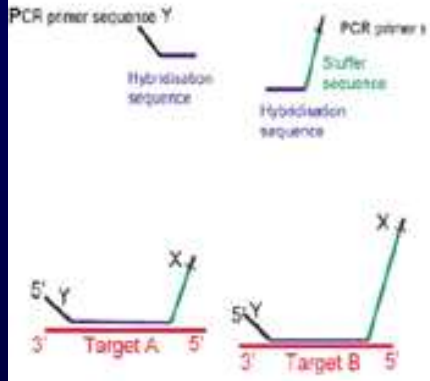
Genetic Testing in Europe - FP6 Network of Excellence

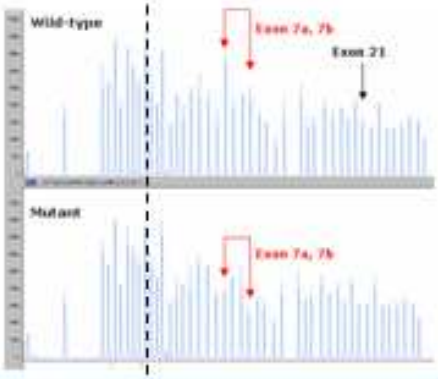
Network for test development harmonization, validation and standardization of services

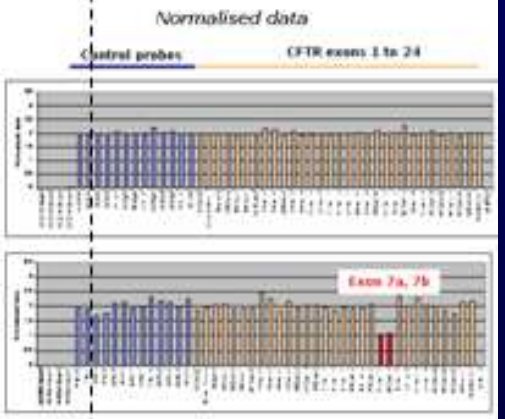
EuroGentest

www.eurogentest.org

MLPA reaction → Separation of MLPA products → Data analysis → Normalisation of the results







EuroGentest


Relationship between WP 1.7 and WP 5.1

Technologies utilised

	Laboratories	
	n	(%)
System for the separation of MLPA products		
ABI-310	3	19
ABI-3100	9	56
ABI-3130	1	6
ABI-3700	0	0
ABI-3730	2	13
Beckman CEQ-2000	1	6
Beckman-CEQ-8000	2	13
Spectrumedix 96	1	6
Software for analysis of the capillary peak profiles		
GeneScan version 3,7	9	56
Genotyper version 3,7	6	38
GeneMapper	3	19
Beckman analysis Software	3	19
Genemarker from Softgenetics	2	13
IT tools for the normalisation of MLPA results		
Coffalyser	0	0
SequencePilot from JSI Medicals Systems	1	6
Genemarker from Softgenetics	3	19
NGRL, Manchester analysis sheets	3	19
CMGL LEEDS analysis sheets	1	6
Own excel sheets	10	63

MLPA validation kit

BRCA1 P002 MLPA kit (MRC-Holland, Holland)
10 "negative" DNA samples (NGRL, Manchester)

Precision test report
Assessment of performance of laboratories
ISO 5725-6

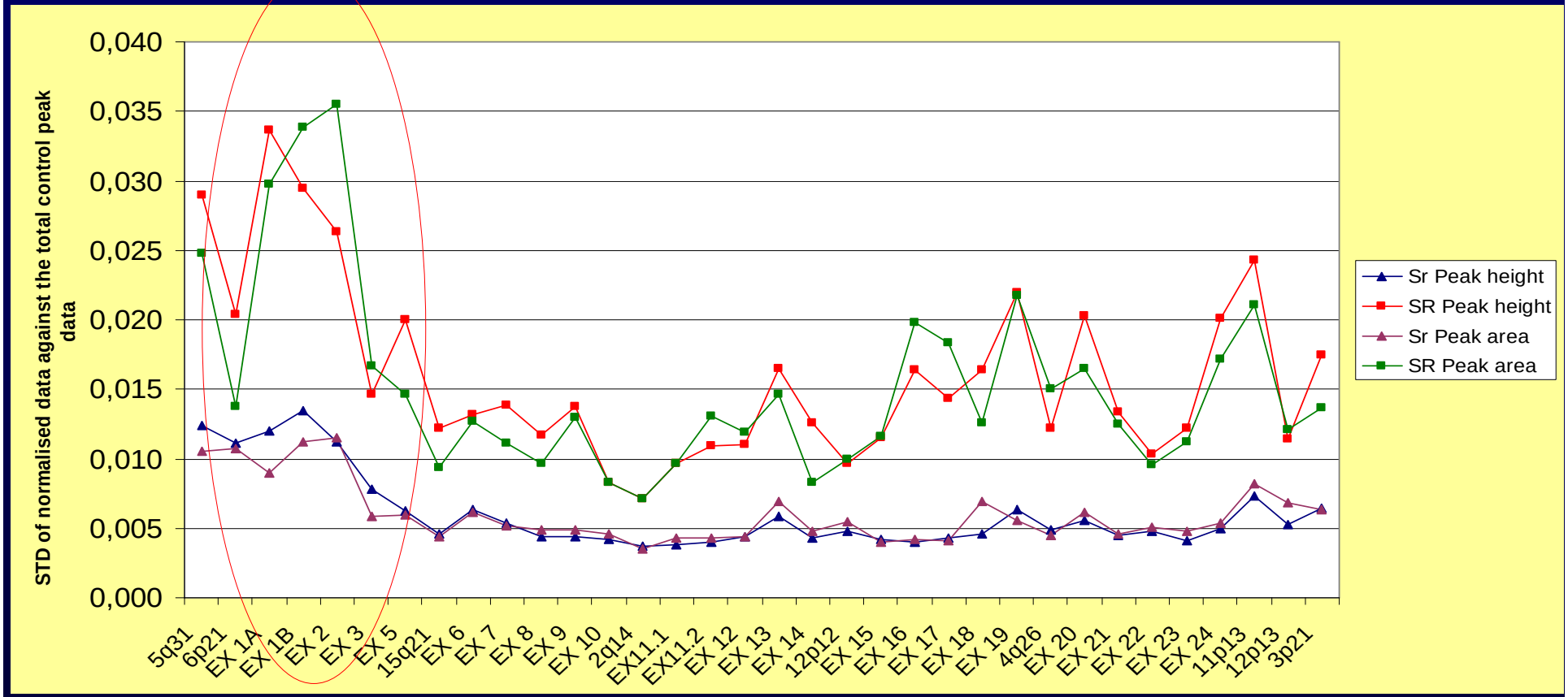
$$Z_i = \frac{x_i - X}{s} = \frac{\bar{y}_i - \hat{m}}{\sqrt{s_R^2 - \left(1 - \frac{1}{n}\right)s_r^2}}$$

where x_i is the test result from the i-th laboratory
X is the group average;
s is the group standard deviation.

- if $Z \leq 2$, the performance is satisfactory;
- if $2 < Z \leq 3$, the performance is questionable;
- if $Z > 3$, the performance is unsatisfactory.

EuroGenTest

Pooled data from collaborators



Repeatability Std (Sr) and Reproducibility Std (SR)
1 sample 10 replicates, 34 probes (P002 BRCA1 kit)

Správa vzorků › Najít měření › Filtrace měření

Označení měření (ID): *KM 1_P095_23-11-06 (69)*
Datum a čas: *27.06.2007 11:00*
Šarže: *P095_1 - P095 Aneuploidy*
Naměřená data: *Zobrazit*

eMLPA

FILTROVAT VZOREK

Adaptivní shluky - největší peak ve shluku;úprava odchylek prób - analýza výšek

FILTROVAT

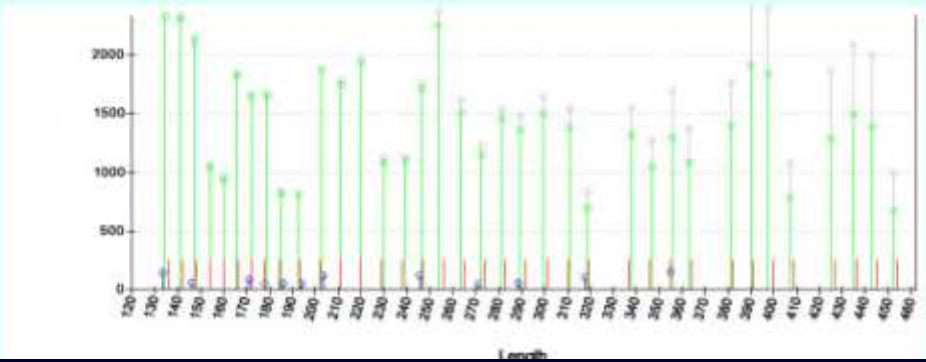
Chci vložit již filtrovaná data (externím filtrem)

ZOBRAZIT FORMULÁŘ

filtrovaná data:

Length	Height	Area	Salsa Probe#	Chrom. Position
134.4	2335	13591	0815-L0333	21q22.2
141.25	2326	13437	2127-L1638	18q21.1
147.2	2140	12047	0798-L0316	13q32
154.06	1046	6168	0652-L0637	Xq11.2
160	951	5386	2153-L0596	Yp11.3
165.79	1834	10636	0813-L0636	21q21.1
172.01	1659	9469	0808-L0326	18q21
178.93	1666	9502	0799-L0317	13q12.3
185.09	830	4783	2155-L1607	Xq23
192.88	813	4733	2152-L0592	Yp11.3

ULOŽIT



emlpa.lf2.cuni.cz

High Resolution Melting Curve Analysis

Purpose: - Technical evaluation of HR-MCA using BRCA1 as target gene

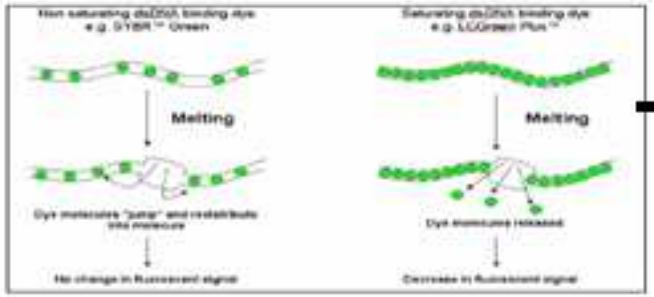
Setup: - Equipment - Lightscanner 96 well (Idaho)- Rotorgene 6000, (Corbett), 480 Lightcycler (Roche)

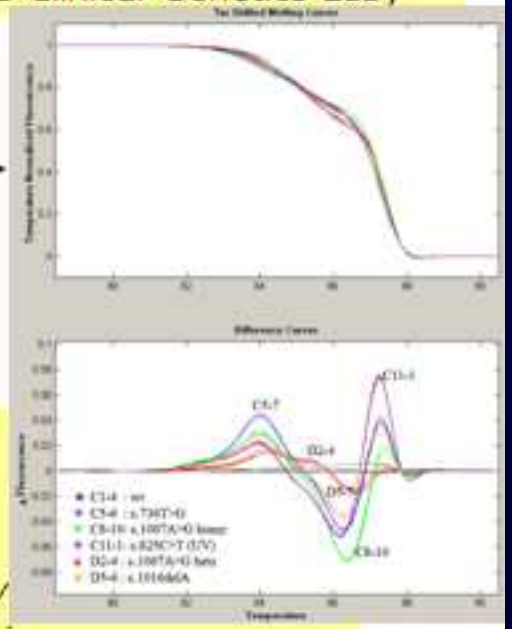
- Dyes: - LC Green plus in mastermix,- LC Green plus, - Syto-9

# of amplicons - ± 45

- Software, - 'Call IT 1.1' - new 'Call IT' beta version (genotype option)

- Control DNA samples - clinical DNA samples (LUMC Clinical Genetics Lab)





Work flow:

-Technical MCA evaluation program encompasses 3 phases

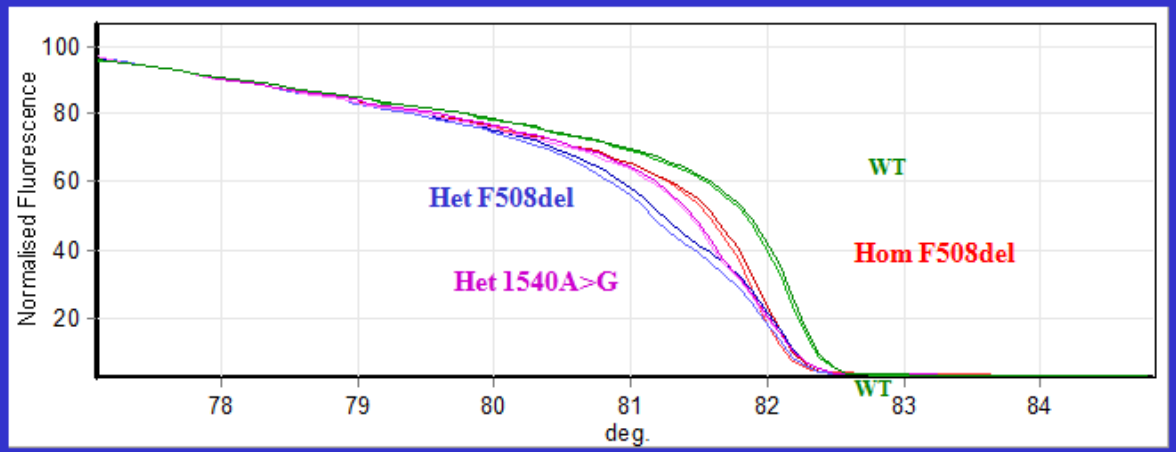
I. set up and evaluate technique in first laboratory (Leiden)

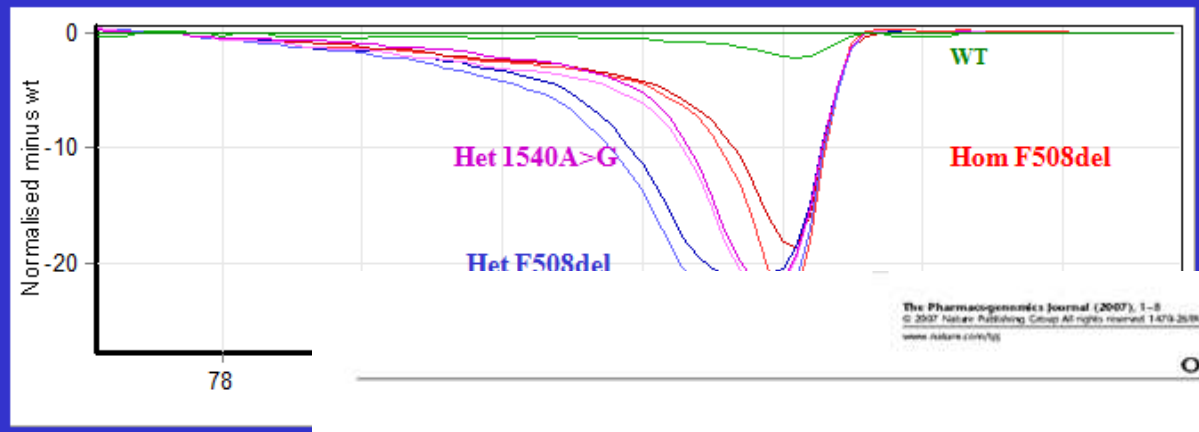
II. evaluate the MCA performance in a second/third Lab (Prague/Leuv

III. perform blind studies with known samples + scan unknown samples…

HRMCA

gene *CFTR*, exon 10,
mutation F508del, sekv. var. 1540G>A and WT
Rotor Gene 6000





FRET artefacts:


The Pharmacogenomics Journal (2007), 1–8

ORIGINAL ARTICLE

UGT1A7 polymorphisms in chronic pancreatitis: an example of genotyping pitfalls

The team

EuroGentest

Thank you for your attention !

milan.macek.jr@LFmotol.cuni.cz