Joint Research Centre (JRC)

David Gancberg
Reference Materials Unit

IRMM - Institute for Reference Materials and Measurements

Geel - Belgium

http://irmm.jrc.ec.europa.eu/

http://www.jrc.ec.europa.eu/

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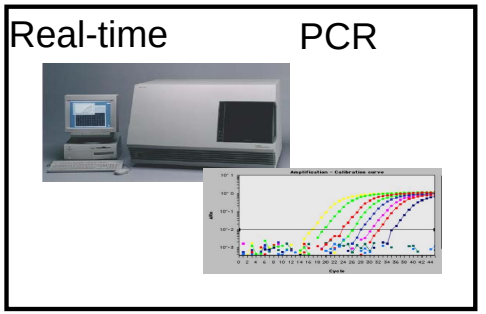
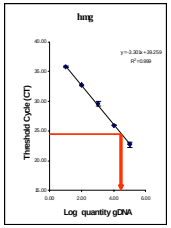
1. Reference Materials needs for QC in genetic testing

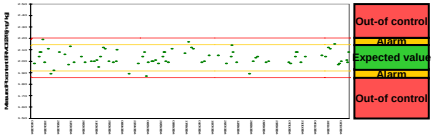
- **Method development and validation**



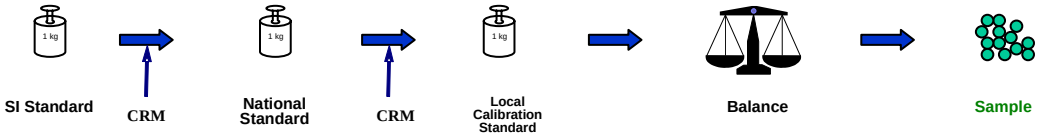
- **Calibration**



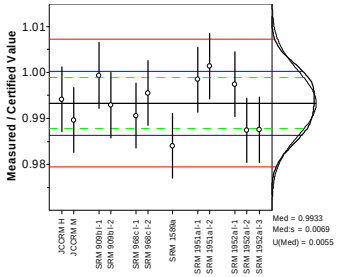
- **Quality Control (internal)**



- **Metrological traceability**



- **Proficiency testing (external)**



Guidance document on the Use of RMs for Genetic Testing

IRMM, irmm.jrc.ec.europa.eu and www.eurogentest.org

- **State of the art**
- **Selection criteria of RMs according to their use**
- **Application guidance**

Certification of reference materials for detection of the human prothrombin gene G20210A sequence variant.

Clin Chem Lab Med 2008, 46: 463-469.

Steps to be followed for certification:

- Planning
- (Feasibility)
- Homogeneity
- Stability
- Fitness for purpose/applicability

Quality (International Standards)

- ISO Guides 34 (RM producers) and 35 (2006, statistical principles for certification)
- ISO 17511 (traceability), 15189 (2007, quality and competence)
- ISO 15193 (reference measurement procedures), 15194 (description of reference materials)
- ISO 25680.4 (calculation and expression of uncertainty)
- ISO 17025 (testing and calibration labs)

Goal: Reference measurement systems (procedures and materials)

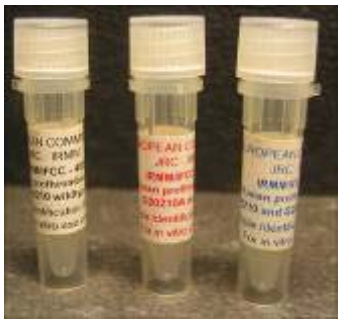
WHO (International Agreement)

Regulation (National/International)

- FDA clearance (USA)
- CE-marking (EU)

Essential requirements

- Description of procedures for production and characterization
- Proven homogeneity
- Proven stability (short- and long-term)
- Batch characterization (identity check)
- Proven fitness for intended purpose (validation)
- Value assignment and uncertainty (or probability)



Additional information

- Traceability of the material
- Instruction for use (minimum sample intake), storage conditions, shelf life, description of the sample, analytical method used, participants involved, safety issues, legal issues

2. Elements describing a higher order RM (ISO 15194)

Element	Mandatory	Optional
Title page	X	
Content list		X
Foreword	X	
Warning and safety precautions	X	
Introduction		X
Title of report	X	
Scope	X	
Definitions		X
Symbols and abbreviations		X
Terminology		X
Justification for choice of reference material	X	
General Characteristics	X	
Specific Characteristics	X	
Validation	X	
Intended function	X	
Instructions for use	X	
Supplier	X	
Bibliography		X
Annexes		X
Dates	X	

3. Examples of materials available for genetic testing

CRMs	RMs	Other			
		FDA	CE-marked	WHO	RUO
Factor II: IRMM/IFCC-490-492	Coriell cell lines	MMQCI-CFTR	Roche- Factor II	Factor V	MMQCI-FV,FII,MTHFR
Fragile X: NIST 2399	ATCC cell lines	ParagonDx-CYP450	Greiner-Bioscience-CFTR	Factor II	Acrometrix- CFTR
Paternity: NIST 2390,2391	CFTR	Roche CYP-450	Roche CYP-450		Genelex-paternity,etc
Sequencing and PCR: NIST	Fragile X		Roche- Factor V		
Mitochondrial DNA: NIST	Huntington		Roche- MTHFR		
	Pharmacogenetic loci		GeneXpert-BCR/ABL		

- Few CRMs
- Quality more important than regulation?
- RMs into CRMs?
- Other materials: quality to be (re)-assessed?

Hereditary disorders

Rank	Disease	Being addressed by
1	FRAX	NIBSC/CRMGEN/NGRL, CDC
2	CF	CDC/Coriell, MMQCI, others
3	FVL/FII	NIBSC, CDC/ IRMM /MMQCI
4	BRCA	NGRL Wessex
5	HH	NIBSC, CDC/Coriell
6	HNPCC	NIBSC/CRMGEN/NGRL
7	PWS/AS	NIBSC/EuroGentest
8	DM	NGRL Wessex
9	HD	CDC/Coriell, NIST
10	DMD/BMD	
11	SMA	
12	Haemoglobinopathies	CDC/Coriell (HbS)/NIBSC

Other genetic testing disciplines

- Pharmacogenetics (Herceptin, Warfarin)
- Pre-natal diagnosis
- Paternity testing
- Biogeographical ancestry
- Molecular typing
- Pathology (Oncology, Virology, Microbiology, Cytogenetics)
- Genetically modified organisms (plants, animals, bacteria)
- Forensics

New technologies

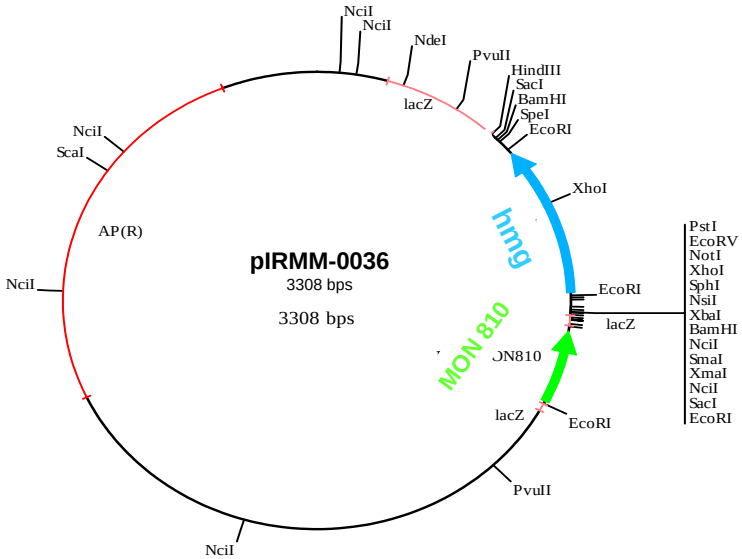
- MLPA (multiplex ligation-dependent probe amplification),
- CSCE (conformation sensitive capillary electrophoresis),
- PAP (pyrophosphorolysis-activated polymerisation),
- CGH (comparative genomic hybridization),
- HR-MCA (high resolution melting curve analysis),
- sequencing,
- micro-arrays,
- WGA (whole genome amplification)
- Point-of-care methods

….

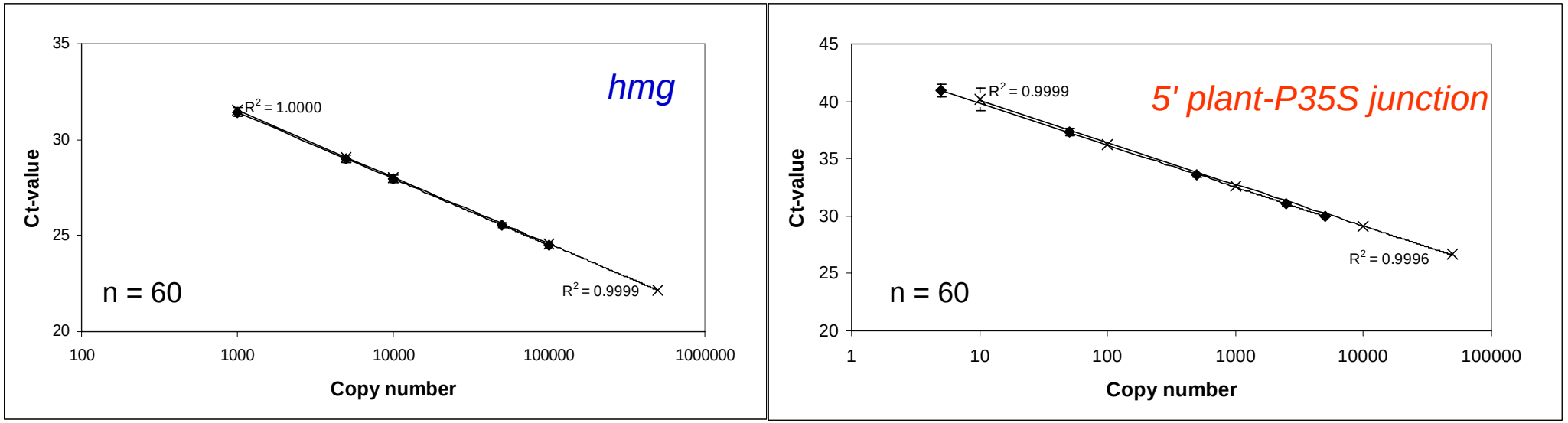
New materials

- “real sample”: gDNA
- synthetic: Plasmid, PCR product, BAC, YAC
- generic RM? Synthetic, with particular features (GC content, type of bases substitution, location of the mutation in the fragment), to be used for assessment of sensitivity and specificity of mutation detection techniques (existing or new)
- format (liquid, freeze-dried, frozen, with or without additive?...)

Plasmidic calibrant solution certified to contain the same amount of MON810 fragment as hmg fragment: ERM-AD413



Commutability of ERM-AD413



Alignment of pDNA (x) and gDNA (♦) calibration curves specific for the endogenous and transgenic target sequences. (*n* indicates the number of calibration curves aligned)

Conclusion: The commutability of the plasmidic material could be demonstrated

Adapted from Charels *et al,* 2007, *J. Agriculture Food Chemistry*

Reference Measurement Systems (for genetic testing):

Reference Material	+	Reference Method
several possibilities	+	sequencing? (for SNPs) (forward and backward)

5. Perspectives

- A reference method should be recognized by the genetic community
- Reference materials and new methods can then be developed and validated, establishing a traceability chain
- Generic reference materials can be used to test the method performances
- Guidance documents are ongoing for development, harmonization, validation and use of reference materials and new methods