XXV Congreso de la Sociedad Española de Anatomía Patológica y
División Española de la International Academy of Pathology

Estado actual y perspectivas del estudio de Biomarcadores

Desde el ámbito europeo

Fátima Carneiro
President-Elect of the European Society of Pathology
IPATIMUP & Medical Faculty/Hospital SJoão
Porto, Portugal

European Society of Pathology

Several initiatives, both diagnostic and research driven and both private and public have been established under the auspices or with the collaboration of the European Society of Pathology

EMQN

The European Molecular Genetics Quality Network

EuroGentest

HARMONIZING GENETIC TESTING ACROSS EUROPE

KRAS-ESP External Quality Assurance (EQA) program

Virchows Arch (2008) 453:417–431
DOI 10.1007/s00428-008-0665-y

REVIEW AND PERSPECTIVE

KRAS mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma: proposal for an European quality assurance program

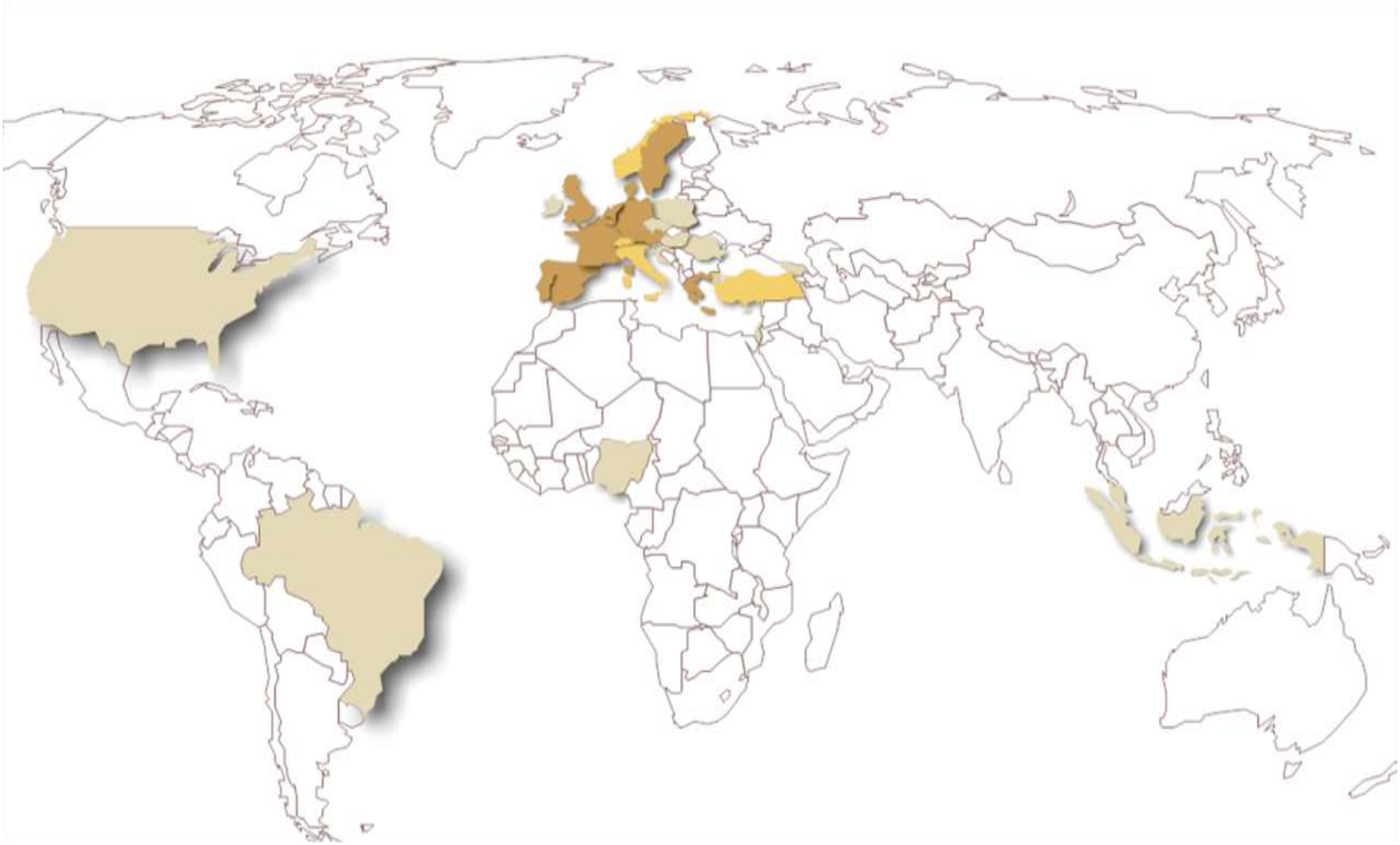
J. H. J. M. van Krieken · A. Jung · T. Kirchner · F. Carneiro · R. Seruca · F. T. Bosman · P. Quirke · J. F. Fléjou · T. Plato Hansen · G. de Hertogh · P. Jares · C. Langner · G. Hoefler · M. Ligtenberg · D. Tiniakos · S. Tejpar · G. Bevilacqua · A. Ensari

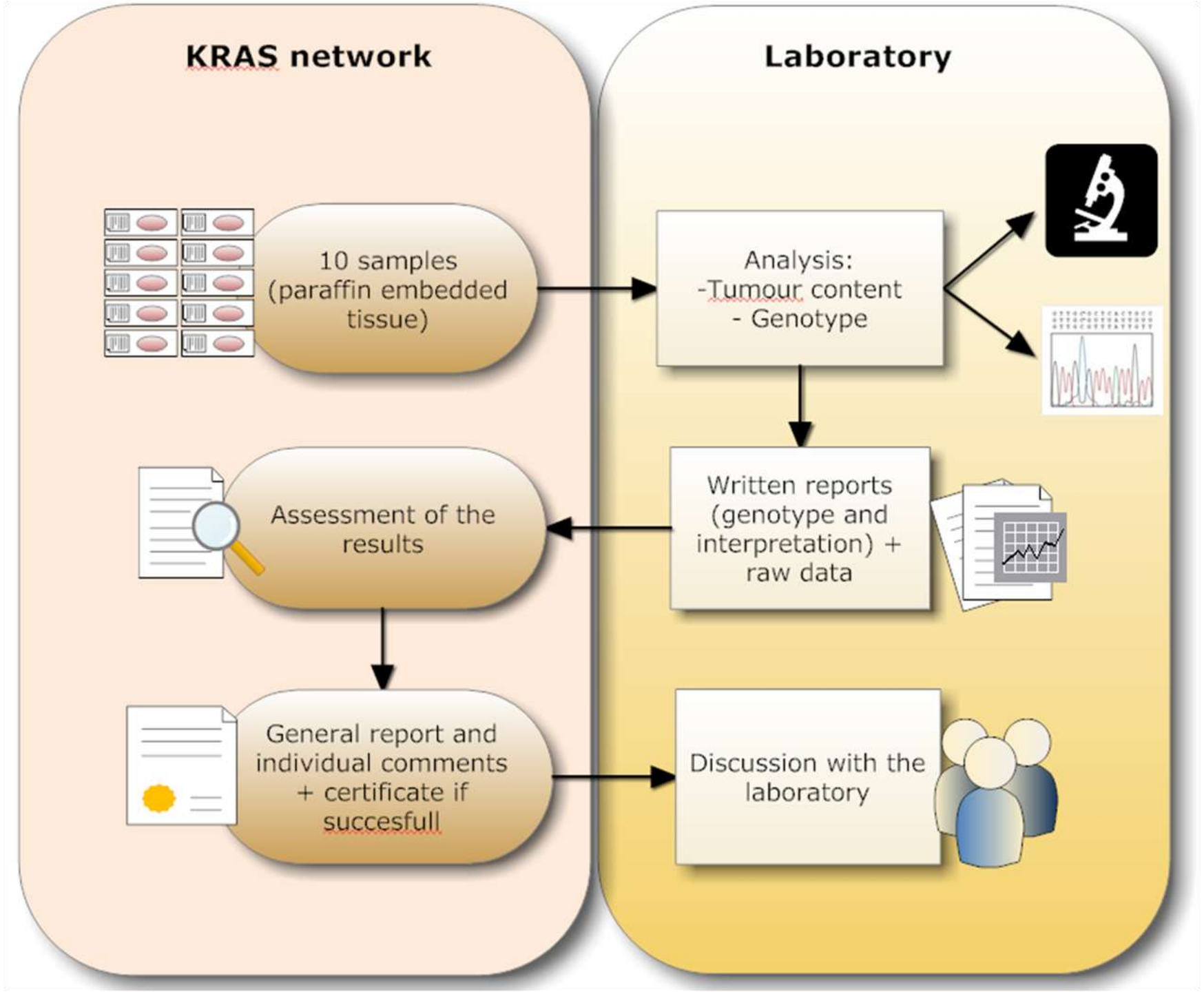
Received: 24 June 2008 / Accepted: 21 August 2008 / Published online: 18 September 2008

KRAS-ESP European Quality Assurance (EQA) program

The European Society of Pathology (ESP) established a European QA program for testing KRAS mutations in colorectal cancer. This program **aims to ensure optimal accuracy and proficiency in KRAS mutation testing across all countries or institutions in the European Union.**

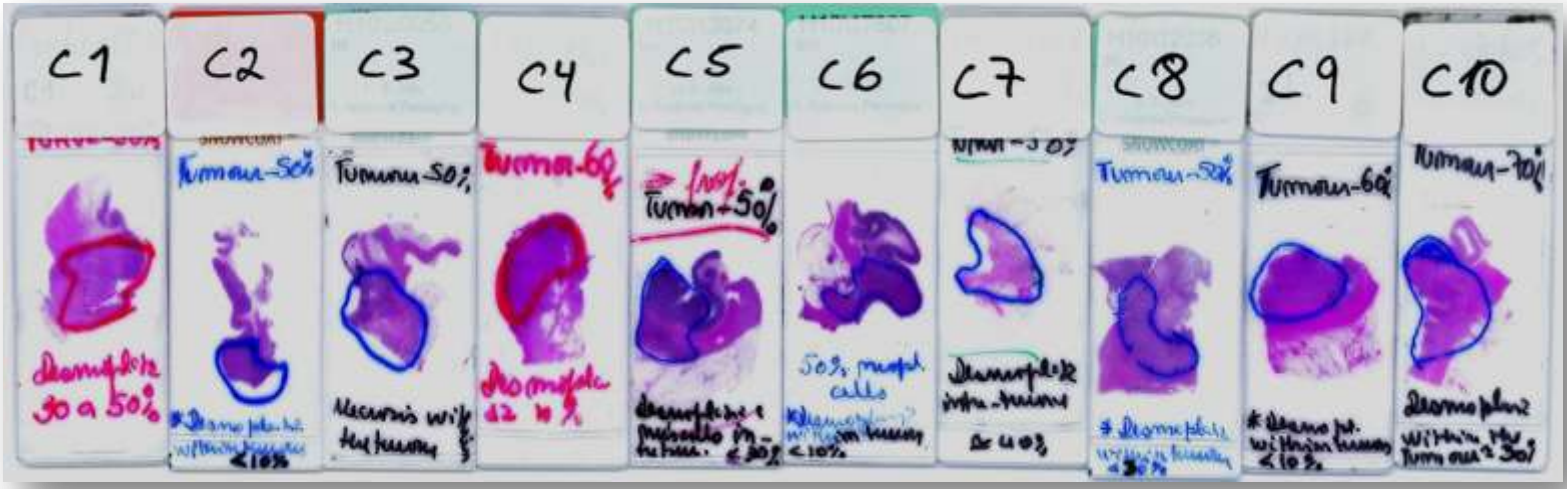
A first **European pilot** KRAS EQA scheme was running May – June 2009. Based on these experiences **Regional** and **European EQA schemes** were organized in different countries in 2009 and 2010.

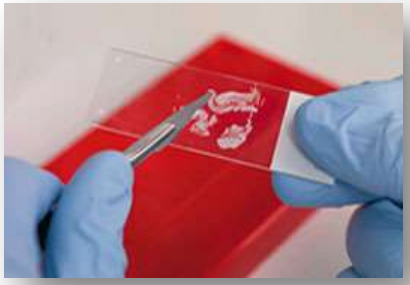


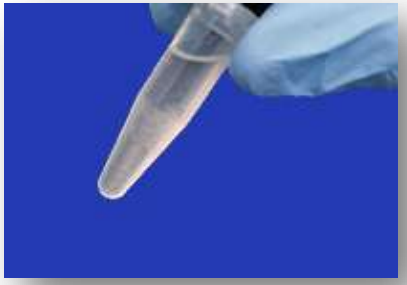

KRAS network
Laboratory
10 samples (paraffin embedded tissue)
Analysis: -Tumour content - Genotype
Written reports (genotype and interpretation) + raw data
Assessment of the results
General report and individual comments + certificate if succesfull
Discussion with the laboratory

Regional KRAS EQA scheme 2010

(IPATIMUP was one of the 6
Regional Laboratories responsible for the
preparation and validation of the samples)







LOGO
Institute/Laboratory

EQA ID
Institution – Department - Address
Contact person laboratory
Tel - Fax

Leuven, 10-10-2010

Referring clinician: Doctor X
Street
City, Country

Molecular genetic analysis for *KRAS*

Name:	John Doe (*)
Date of birth:	01/01/1980 (*)
Reason for analysis:	KRAS mutation present?
Sample received:	01-10-2010
Sample type:	Paraffin embedded section of adenocarcinoma
Your reference:	CF07-2
Our reference:	KRAS 10.199

RESULT:

- **MICROSCOPY**: Microscopic analysis of the sample showed 70% tumor cells. Sample is suitable for KRAS mutation analysis
- **MUTATION ANALYSIS: Mutation present in codon 12 of the KRAS gene: p.Gly12Ser (c.34G>A)**

INTERPRETATION:

In a DNA extract of the sample tissue, we detected an activating mutation in the KRAS gene: p.Gly12Ser (c.34G>A). This indicates likely resistance to anti-EGFR-therapy (cetuximab, panatimumab)

Analysis performed by

Molecular biologist X

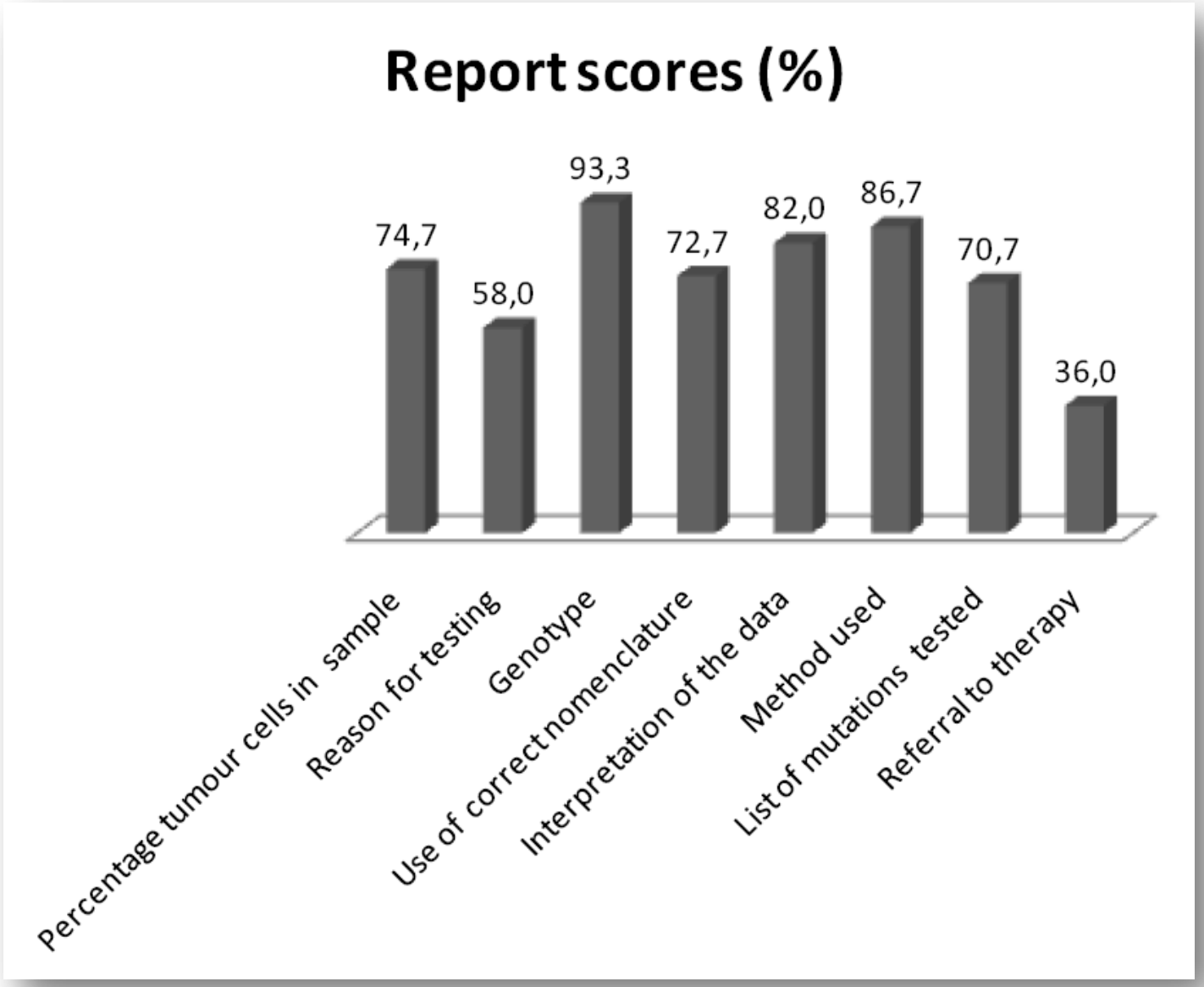
Microcopy performed by

Pathologist Y

The method used: DNA-extraction with the Qiagen QIAamp® DNA FFPE-kit, K-RAS mutation analysis by the TheraScreen® K-RAS Mutation Kit (CE-IVD) (DxS Ltd, Manchester, UK), which combines two technologies (Amplification Refractory Mutation System, Astrazeneca, and Scorpions, DxS) to detect the most commonly reported KRAS mutations by realtime quantitative PCR. Mutations screened for: p.Gly12Asp (c.35G>A), p.Gly12Ala (c.35G>C), p.Gly12Val (c.35C>T), p.Gly12Ser (c.34G>A), p.Gly12Arg (c.34G>C), p.Gly12Cys (c.34G>T), p.Gly13Asp (c.38G>A)

These mutations represent about 98% of the KRAS mutations in colorectal cancer. The Therascreen method is highly selective. If sufficient DNA is present, presence of 1% mutant DNA can be detected in a wild type background. A negative test does not exclude the presence of a mutation.

(*) Data might be not provided in EQA schemes: enter / or not applicable (NA)



Scores of important criteria in written reports sent by the participants. Percentages indicate in how many reports the item was present (n=75).

Participants of the Regional *KRAS* EQA scheme 2010

In total **76 laboratories** participated in the EQA scheme 2010. The number of laboratories that participated (and submitted results) in 2010 is listed per country in Table 1.

Country	**Number of laboratories that participated in the scheme and submitted results**
Austria	7
Belgium	13
Denmark	8
France	12
Germany	3
Greece	2
Italy	6
The Netherlands	16
Norway	2
Portugal	1
Spain	2
Sweden	2
Switzerland	1
Turkey	1
TOTAL	**76**

Table 1: Number of laboratories that participated in the Regional KRAS EQA scheme, per country, in 2010.

Table with the individual genotype results:

Subscheme	EQA ID/lab	KRASRx10.301	KRASRx10.302	KRASRx10.303	KRASRx10.304	KRASRx10.305	KRASRx10.306	KRASRx10.307	KRASRx10.308	KRASRx10.309	KRASRx10.310	Total score genotyping
SCHEME A	Result	c.35G>A, p.Gly12Asp	c.34G>T, p.Gly12Cys	No mut	c.38G>A, p.Gly13Asp	No mut	c.34G>A, p.Gly12Ser	No mut	c.35G>C, p.Gly12Ala	c.35G>T, p.Gly12Val	No mut	
		a	a	a	a	a	a	a	a	a	a	10,0
		a	a	a	a	a	a	a	a	a	a	10,0
		a	a	a	a	a	a	a	a	a	a	10,0
		a	a	a	a	a	w (no mut)	a	a	a	n	8,5
		a	a	a	a	a	a	a	n	a	a	9,5
		a	a	a	a	a	a	a	a	a	a	10,0
		a	a	a	n	a	a	a	n	a	a	9,0
		a	a	a	a	a	a	a	w (no mut)	a	a	9,0
		a	a	a	a	a	a	a	a	a	a	10,0
		a	a	a	a	a	a	a	a	a	n	9,5
		a	a	a	a	a	a	a	a	a	a	10,0
		a	a	a	a	a	a	a	a	a	a	10,0
		a	a	a	a	a	a	a	a	a	a	10,0
		w (no mut)	w (c.35G>A, p.Gly12Asp)	w (c.34G>T, p.Gly12Cys)	w (no mut)	a	w (no mut)	w (c.34G>A, p.Gly12Ser)	w (no mut)	w (c.35G>C, p.Gly12Ala)	w (c.35G>T, p.Gly12Val)	1,0
		a	a	a	n	a	a	a	n	a	a	9,0
		a	a	a	a	a	a	a	a	a	a	10,0
SCHEME B		c.35G>A, p.Gly12Asp	c.34G>T, p.Gly12Cys	No mut	c.38G>A, p.Gly13Asp	No mut	c.34G>A, p.Gly12Ser	No mut	c.35G>C, p.Gly12Ala	c.35G>T, p.Gly12Val	No mut	
		a	a	a	a	a	a	a	a	a	a	10,0
		a	a	a	a	n	a	a	a	a	a	9,5
		a	a	a	a	n	a	a	a	a	a	9,5
		a	a	a	a	n	a	a	a	a	a	9,5
		a	a	a	a	a	a	w (c.38G>A, p.Gly13Asp)	a	a	a	9,0
		a	a	a	a	a	a	a	a	a	a	10,0
		a	a	a	a	a	a	a	a	a	a	10,0
		a	a	a	a	a	a	a	a	a	a	10,0
		a	a	a	a	n	a	a	a	a	a	9,5
		a	a	a	a	a	a	a	w (c.35G>C, p.Gly12Ala + c.35G>A, p.Gly12Asp)	a	a	9,0
		a	a	a	a	a	a	a	a	a	a	10,0
		a	a	a	a	n	a	n	a	w (no mut)	a	8,0
		a	a	a	a	n	a	a	a	a	a	9,5

SCHEME E	Result	c.35G>A, p.Gly12Asp	c.34G>T, p.Gly12Cys	No mut	c.38G>A, p.Gly13Asp	No mut	c.34G>A, p.Gly12Ser	No mut	c.35G>C, p.Gly12Ala	c.35G>T, p.Gly12Val	No mut	
	PT0001	a	a	a	a	a	a	a	a	a	a	10,0
	ES0001	a	a	a	a	a	a	a	a	a	a	10,0
	ES0003	a	a	a	a	a	a	a	a	a	a	10,0
	IT0001	a	a	a	a	a	a	a	a	a	a	10,0
	IT0002	a	a	a	a	a	a	a	a	a	a	10,0
	IT0003	a	a	a	a	a	a	a	a	a	w (c.35G>A, p.Gly12Asp)	9,0
	IT0004	a	a	a	a	a	a	a	a	a	a	10,0
	IT0005	a	a	a	a	a	a	a	a	a	a	10,0
	IT0006	a	a	a	a	a	a	a	a	a	a	10,0
SCHEME F	Result	c.182A>G, p.Gln61Arg	No mut	c.34G>T, p.Gly12Cys	No mut	c.38G>A, p.Gly13Asp	c.35G>C, p.Gly12Ala	c.183A>T, p.Gln61His	c.35G>A, p.Gly12Asp	c.34G>A, p.Gly12Ser	c.35G>T, p.Gly12Val	
	FR0001	b	a	a	a	a	a	b	a	a	a	10,0
	FR0002	a	a	a	a	a	a	a	a	a	a	10,0
	FR0003	b	a	a	a	a	a	b	a	a	a	10,0
	FR0004	b	a	a	a	a	a	b	a	a	a	10,0
	FR0005	b	a	a	a	a	a	b	a	a	a	10,0
	FR0006	b	a	a	a	a	a	b	a	a	a	10,0
	FR0007	b	a	a	a	a	a	b	a	a	a	10,0
	FR0008	b	a	a	a	a	a	b	a	a	a	10,0
	FR0009	b	a	a	a	a	a	b	a	a	a	10,0
	FR0010	b	a	a	a	a	a	b	a	a	a	10,0
	FR0011	b	a	a	a	a	a	b	a	a	a	10,0
	FR0012	a	a	a	a	a	a	a	a	a	a	10,0

CERTIFICATE OF PARTICIPATION
IN THE 2010 REGIONAL QUALITY ASSESSMENT SCHEME FOR KRAS

IPATIMUP Diagnostics

Genetic Diagnostics
IPATIMUP
Porto, Portugal

European Society of Pathology

KRAS
European EQA program

The KRAS EQA scheme is coordinated from the Biomedical Quality Assurance Research Unit of the Katholieke Universiteit Leuven, Belgium

Score genotype: 10.0/10.0
Average score genotype: 9.5/10.0
Average score genotype (subscheme): 9.9/10.0

Prof. Dr. E Dequeker	1/3/2011
Prof. Dr. Han Van Krieken	1/3/2011

European external quality assessment for the improvement of KRAS testing

Bellon E.[1], Ligtenberg M.J.L.[2], de Hertogh G.[3], de Stricker K.[3], Edsjö A.[3]Laurent-Puig P.[3], Machado JC.[3], Rouleau E.[3], vandenberghe P.[3],vander Borght S.[3], van Krieken J.[2] and Dequeker E.[1]

[1] University of Leuven, Centre for Human Genetics, Biomedical Quality Assurance Research Unit Leuven, Belgium . [2] Department of Pathology, Radboud University Nijmegen Medical Centre, The Netherlands. [3] Regional scheme organizers of the 2010 ESP KRAS Regional EQA scheme (alphabetically).

- In total 76 laboratories from 14 different European countries participated.
- From the 76 participating laboratories, 50 reported all 10 genotypes correctly (66%).

KRAS European QA Program

http://kras.eqascheme.org

Agenda invitational conference on quality assurance in molecular pathology

June 9 2011, Office of the ESP, Rue Bara 6, Brussels

12.00	informal, sandwiches	
13.00	Han van Krieken	Welcome, what is the ESP, goal of the conference
13.10	Els Dequeker	The ESP KRAS EQA program: organization and results
13.20	Marjolein Ligtenberg	The ESP KRAS EQA program: practical aspects
13.30	Erik Thunnissen	EQA for EGFR testing in lung cancer
13.40	Simon Patton	The vision of the European Thoracic Oncology Platform
13.50	Frank Opdam	Development and potential of artificial samples
14.00	Hans Kreipe	The German approach to EQA for molecular pathology
14.10	Andreas Jung	Experiences from the German EQA
14.20	Etienne Rouleau	The French approach to EQA for molecular pathology
14.30	Sandi Deans	The NEQAS approach to EQA for molecular pathology
14.40	Nicola Normanno	The Italian approach to EQA for molecular pathology
14.50	Ed Schuuring	The Dutch approach to EQA for molecular pathology
15.00		Tea break
15.30	Peter Collins	Test regulatory approval and registration
15.40	Sabine Tejpar	The view of the clinician
15.40	Nicola Normanno	The approach in Italy and the view of ESMO
15.50	Scott Patterson	The view of industry: Amgen
16.00	Ivonne Marondel	The view of industry: Pfizer
16.10	discussion on statements	

IPATIMUP

(FOUR EXAMPLES OF BIOMARKERS ROUTINELY TESTED)

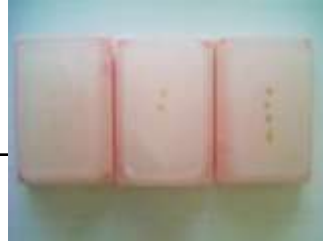
- EGFR mutations and EML4/ALK translocations in lung carcinoma
- KRAS mutations in colorectal carcinoma
- KIT/PDGFRA mutations in GIST tumours
- HER-2 amplification in breast carcinoma

DNA extraction from paraffin-embedded material
(Biopsy and surgical specimens)
Crucial role of the pathologist

KRAS mutations in colorectal carcinoma

KRAS mutations	Results - IPATIMUP			Results from literature	
	N	%	Rank	%	Rank
12Asp	169	37%	1	31-47	1
12Val	125	28%	2	22-29	2
13Asp	73	16%	3	14-21	3
12Cys	43	9%	4	6-9	4
12Ser	20	4%	5	4-6	5
12Ala	17	4%	6	3-6	6
12Arg	4	1%	7	1-2	7
13Cys	3	1%	8	0,5-1	8
1 mutation	454				
>1 mutation	9				
Total (mutations)	**463**	**38%**			30-50%
Wild-type	763	62%			
Total (cases)	1226				

EGFR mutations in lung carcinoma



EGFR mutations	Results - IPATIMUP		Results from literature
	N	%	%
Exon 18	3	7%	5-10
Exon 19	17	41%	45-50
Exon 20	7	17%	5-14
Exon 21	14	34%	20-45
Total (mutations)	**41**	**12%**	10-20
Wild-type	295	88%	
Total (cases)	336		

KIT/PDGFRA mutations in GIST tumours

GIST mutations	Results - IPATIMUP		Results from literature
	N	%	%
KIT			
Exon 9	4	14%	10%
Exon 11	12	43%	70%
Exon 13	3	11%	1%
Exon 17	1	4%	1%
PDGFRA			
Exon 12	1	4%	1%
Exon 14	1	4%	1%
Exon 18	6	21%	6%
Total (mutations)	**28**	**57%**	90%
Wild-type	21	43%	10%
Total (cases)	49		

TAKE HOME LESSONS

- Quality control is a must-have! The crucial words are **competence and harmonization.**
- Oncologists, Pathologists and Geneticists have to be perfectly sychronyzed for **patients to take full advantage from available technology!**
- The involvement of Pathologists in these ventures will increase the **quality and the public awareness** (clinicians and patients) **of our profession.**

Thanks for your attention

25th European Congress of Pathology

First week of September 2013

Lisbon, Portugal

European Society of Pathology

Sociedade Portuguesa de Anatomia Patológica

SPAP