

EQA rondzending *KRAS*

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Some slides obtained from  
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Role of Scheme organizers\*

- Inventarisation of adequat FFPE material:
  - Type of mutation (similar among schemes)
  - Sufficient material
  - ≥ 30% tumor cells after microdissection
  - Quality control of samples in reference lab
- Preparation and distribution of slides:
  - 3 slides consecutive unstained slides/lab
  - Highest and lowest slide should be comparable
  - One or two spare sets
  - Last set of three slides > reference lab



Role of Coordination centre Leuven

- Coordination role between all scheme organizers and participants
- Responsible for the harmonization of the samples
- Responsible for all communications
- Responsible for the website and electronic submission form
- Data collection of the results, draft first report and overview of results
- Logitudinal research on performance

Information submitted by the laboratory to the European QA coordinator

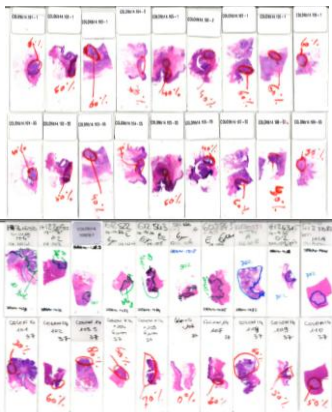
- Tabular reporting form** (electronic data submission)
- which mutations were tested
  - which method was used
  - % tumor cells and genotype results
  - general information of the lab

Raw data of the lab results and the reports sent to treating physion of the first 3 samples

Sample identifier	Harmonized cell content?	Which mutation was tested?	Which method was used?	Preprocessing performed	Primary method used	Secondary method used	Are method (compare with not multiple for samples)
20090104-101	90%	NRAS G12C (R101)	qPCR (100%)	qPCR (100%)	qPCR (100%)	qPCR (100%)	qPCR (100%)
20090104-102	90%	No mutation	qPCR (100%)	qPCR (100%)	qPCR (100%)	qPCR (100%)	qPCR (100%)
20090104-103	90%	NRAS G12C (R101)	qPCR (100%)	qPCR (100%)	qPCR (100%)	qPCR (100%)	qPCR (100%)

Data-analysis

- **Results have to be submitted within 10 workdays**
  - Mutation analysis of the samples
  - Analysis of tumor percentage
  - Written reports of the first 3 samples
- Raw data
- List with general questions
- Minimal requirement 2014:  
*KRAS & NRAS* codons 12,13, 59, 61, 117, 146



B

D

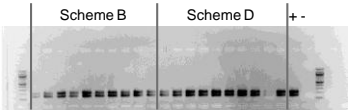
Genotypes of Scheme 2014-2015

Sample number	Genotype
COLONx14.101	KRAS c.38G>A; p.Gly13Asp
COLONx14.102	wild-type KRAS/NRAS/BRAF
COLONx14.103	subscheme A,B,D,E: KRAS c.182A>G; p.Gln61Arg subscheme G: KRAS c.181C>A; p.Gln61Lys
COLONx14.104	wild-type KRAS/NRAS/BRAF
COLONx14.105	subscheme A,B,C,E: KRAS c.183A>C; p.Gln61His subscheme G,L,I: KRAS c.183A>T; p.Gln61His subscheme D,E: KRAS c.182A>T; p.Gln61Leu wild-type KRAS/NRAS/BRAF
COLONx14.106	insufficient neoplastic cells (expected result: sample not contributive)
COLONx14.107	KRAS c.35G>A; p.Gly12Asp
COLONx14.108	BRAF c.1798T>A; p.Val600Glu
COLONx14.109	KRAS c.436G>A; p.Ala146Thr
COLONx14.110	wild-type KRAS/NRAS/BRAF

Dutch labs in schemes  
B n= 15  
D n= 7

Some labs just tested normal tissue

DNA quality of schemes B and D



Size ladder with amplicons of 119 bp and 216 bp

All samples performed well in AmpliSeq panel analyzed on PGM

Techniques in Dutch labs

- Sanger seq 9
- NGS 7x (PGM & Roche Junior)
- Therascreen 2
- Sequenom 1
- HRM 1
- Pyrosequencing 1
- Taqman 1

2014-2015	Scheme	Sample 14.101	Sample 14.102	Sample 14.103	Sample 14.104	Sample 14.105	Sample 14.106	Sample 14.107	Sample 14.108	Sample 14.109	Sample 14.110
		KRAS c.38G>A; p.Gly13Asp	wild-type KRAS/NRAS/BRAF	subscheme A,B,D,E: KRAS c.182A>G; p.Gln61Arg subscheme G: KRAS c.181C>A; p.Gln61Lys	subscheme A,B,C,E: KRAS c.183A>C; p.Gln61His subscheme G,L,I: KRAS c.183A>T; p.Gln61His subscheme D,E: KRAS c.182A>T; p.Gln61Leu wild-type KRAS/NRAS/BRAF						
		2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
		Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found
		0	0	0	0	0	0	0	0	0	0
		7	0	0	0	0	0	0	0	0	0
		8	0	0	0	0	0	0	0	0	0
		12	0	0	0	0	0	0	0	0	0
		14	0	0	0	0	0	0	0	0	0
		16	0	0	0	0	0	0	0	0	0
		18	0	0	0	0	0	0	0	0	0
		21	0	0	0	0	0	0	0	0	0
		24	0	0	0	0	0	0	0	0	0
		41	0	0	0	0	0	0	0	0	0
		42	0	0	0	0	0	0	0	0	0
		44	0	0	0	0	0	0	0	0	0
		47	0	0	0	0	0	0	0	0	0
		48	0	0	0	0	0	0	0	0	0
		52	0	0	0	0	0	0	0	0	0
		53	0	0	0	0	0	0	0	0	0
		75	0	0	0	0	0	0	0	0	0
		77	0	0	0	0	0	0	0	0	0
		81	0	0	0	0	0	0	0	0	0
		96	0	0	0	0	0	0	0	0	0
		108	0	0	0	0	0	0	0	0	0
		122	0	0	0	0	0	0	0	0	0

2014-2015	Scheme	Sample 14.101	Sample 14.102	Sample 14.103	Sample 14.104	Sample 14.105	Sample 14.106	Sample 14.107	Sample 14.108	Sample 14.109	Sample 14.110
		KRAS c.38G>A; p.Gly13Asp	wild-type KRAS/NRAS/BRAF	subscheme A,B,D,E: KRAS c.182A>G; p.Gln61Arg subscheme G: KRAS c.181C>A; p.Gln61Lys	subscheme A,B,C,E: KRAS c.183A>C; p.Gln61His subscheme G,L,I: KRAS c.183A>T; p.Gln61His subscheme D,E: KRAS c.182A>T; p.Gln61Leu wild-type KRAS/NRAS/BRAF						
		2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
		Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found
		0	0	0	0	0	0	0	0	0	0
		7	0	0	0	0	0	0	0	0	0
		8	0	0	0	0	0	0	0	0	0
		12	0	0	0	0	0	0	0	0	0
		14	0	0	0	0	0	0	0	0	0
		16	0	0	0	0	0	0	0	0	0
		18	0	0	0	0	0	0	0	0	0
		21	0	0	0	0	0	0	0	0	0
		24	0	0	0	0	0	0	0	0	0
		41	0	0	0	0	0	0	0	0	0
		42	0	0	0	0	0	0	0	0	0
		44	0	0	0	0	0	0	0	0	0
		47	0	0	0	0	0	0	0	0	0
		48	0	0	0	0	0	0	0	0	0
		52	0	0	0	0	0	0	0	0	0
		53	0	0	0	0	0	0	0	0	0
		75	0	0	0	0	0	0	0	0	0
		77	0	0	0	0	0	0	0	0	0
		81	0	0	0	0	0	0	0	0	0
		96	0	0	0	0	0	0	0	0	0
		108	0	0	0	0	0	0	0	0	0
		122	0	0	0	0	0	0	0	0	0

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		2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
		Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found	Wrong mutation found
		0	0	0	0	0	0	0	0	0	0
		7	0	0	0	0	0	0	0	0	0
		8	0	0	0	0	0	0	0	0	0
		12	0	0	0	0	0	0	0	0	0
		14	0	0	0	0	0	0	0	0	0
		16	0	0	0	0	0	0	0	0	0
		18	0	0	0	0	0	0	0	0	0
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		48	0	0	0	0	0	0	0	0	0
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		53	0	0	0	0	0	0	0	0	0
		75	0	0	0	0	0	0	0	0	0
		77	0	0	0	0	0	0	0	0	0
		81	0	0	0	0	0	0	0	0	0
		96	0	0	0	0	0	0	0	0	0
		108	0	0	0	0	0	0	0	0	0
		122	0	0	0	0	0	0	0	0	0

	2014	
Total scores Dutch labs	20,00	B
	19,00	B
	20,00	B
	20,00	B
	19,50	B
	20,00	B
	20,00	B
	20,00	B
	18,00	B
	20,00	B
labs without major phenotype error and >18 will be mentioned on the website	20,00	B
	20,00	B
	14,00	B
	20,00	B
	20,00	B
	20,00	B
	19,50	D
	14,00	D
	18,00	D
	9,50	D
	18,00	D
	10,00	D
	18,00	D

Conclusion

- The ESP RAS EQA schemes **highlight the need for continuing EQA** in this field
- Still some labs do not test all required RAS codons
- EQA scheme assesses not only the laboratory's **ability to obtain accurate, reliable results**, but also **the ability to safely interpret the results** and ensure that the referring clinician has the correct information.
- The **quality of the reports improved**

Acknowledgement

**Scheme co-ordinator and assistants co-ordinator**  
E Dequeker,L Tembuyser  
Biomedical Quality research Unit KU Leuven, Belgium

**Medical and technical expert**  
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**Scheme organisers**

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