ESP 2014

- EGFR
- ALK

Disclosures

- Lung cancer
  - Adv board: MSD, Pfizer, Clovis
  - Grants: IIR Pfizer, Astra Zeneca

External Quality Assessment

- Sample preparation,
- Validation
- Distribution
- Participants testing
- Assessor meeting
- Preliminary report to participants
- Final report

Laboratory	Responsible person
Preparation and validation of samples	
VU University Medical Centre, Amsterdam	E. Thunnissen (coordinator)
University Medical Center Groningen	E. Schuuring
Validation of samples	
Center of Excellence on Aging, University-Foundation, Chiety	A. Marchetti
University Hospital Basel, Basel, Switzerland	L. Bubendorf
Universitätsklinik Köln Institut für Pathologie, Koln	R. Büttner

The assessors:

Dequeker E. – KU Leuven, Biomedical Quality Assurance Research Unit, Belgium
Marchetti A. – University-Foundation Chiety, Italy
Schuuring E. – University Medical Center Groningen, The Netherlands
Tack V. – KU Leuven, Biomedical Quality Assurance Research Unit , Belgium
Tembuyser L. – KU Leuven, Biomedical Quality Assurance Research Unit , Belgium
Thunnissen E. – VU University Medical Centre Amsterdam, The Netherlands
Tornillo L. – University Hospital Basel, Switzerland

EGFR	Number of participants
The Netherlands	23
Turkey	18
Spain	11
Italy	10
France	9
Belgium	8
Denmark	7
Portugal	6
Austria	6
Germany	5
Poland	4
Sweden	4
Czech republic	3
Hungary	3
Romania	3
Greece	3

Romania	3
Greece	3
Switzerland	3
Norway	3
Brazil	2
Slovenia	2
United kingdom	2
Israel	2
Slovakia	1
Bulgaria	1
Finland	1
Russia	1
Croatia	1
Malta	1
Lebanon	1
Total	144

Sample number	Sample nature	EGFR Genotype	allelic frequency
L14.EGFR1	Cell line 90/10%	exon 20: c.2369C>T ; p.Thr790Met	79%
		exon 21: c.2573T>G; p.Leu858Arg	74%
L14.EGFR2	Cell line 20/80%	exon 19: c.2235_2249del; p.Glu746_Ala750del	13%
L14.EGFR3	Cell line 50/50%	exon 20: c.2369C>T; p.Thr790Met	65%
		exon 21: c.2573T>G; p.Leu858Arg	54%
L14.EGFR4	Cell line 50/50%	exon 18: c.2155G>A; p.Gly719Ser	20%
L14.EGFR5	Cell line 10/90%	exon 19: c.2235_2249del; p.Glu746_Ala750del	10%

Cell lines: Schuuring, UMCG, Groningen, NL

Sample number	Sample nature	EGFR Genotype	Allelic frequency
L14.EGFR6	Resection specimen	c.2155G>A; p.Gly719Ser	69%
		c.2327G>A; p.Arg776His	77%
		c.2369C>T; p.Thr790Met	11%
L14.EGFR7	Resection specimen	c.2236_2250del15; p.Glu746_Ala750del	34%
L14.EGFR8	Resection specimen	wild-type EGFR	0%
L14.EGFR9	Resection specimen	wild-type EGFR*)	0%

*) reference.

	Marking criteria	Marks.
Analytical failure	Test failure giving no result on the sample (analytical failure) and mention no test result due to analytical failure, ask for a new sample	0.5
Accurate result	Correct genotype (true positive or true negative)	2
Major genotyping error	Incorrect genotype (false-positive or false negative)	0
Other genotyping error 1 point	Genotype mis-positioned or mis-called (e.g. incorrect base/amino acid detected)	1
Other genotyping error minor 1.5 point	Not correctly using HGVS nomenclature for either the nucleotide or amino acid changes	1.5

Scoring Code	Marking criteria	Marks
a	The sample was assigned correctly	2,00
0	The sample was incorrectly assigned (false positive, false negative, or wrong mutation)	0,00
b	The sample mutation was not screened for p.R776H; c.2327G>A (assumed is that screening for p.719S and p.T790M is routine)	2,00
c	Samples were likely switched	0,00
d	The sample mutation was incorrectly filled in in the datasheet, but correctly found on raw data and/or reports	1,00
e	Aberration miss-positioned or miss-called (e.g. incorrect base/amino acid detected for genotypes)	1,00
f	Technical error - no or not enough amplified product for a reliable result / test failure giving no result for the sample	0,50

L	missed c.2155G>A; p.Gly719Ser and c.2327G>A; p.Arg776His (only for case 6)	1,00
m	missed c.2327G>A; p.Arg776His and c.2369C>T; p.Thr790Met (only for case 6)	1,00
n	missed c/2369C>T; p.Thr790Met (only for case 6)	1,00
q	missed c.2327G>A; p.Arg776His (only for case 6)	2,00
r	If SNPs were reported in wild-type samples as a mutation (not calling it a SNP)	0,00
s	In case the right mutation was found and an additional SNP was reported as a mutation (not calling it a SNP)	1,00
u	if the laboratory reported that the sample's neoplastic cell content was below the method's detection limit	2,00

Sample ID	nr of errors	nr of technical failures
L14.EGFR1	20	0
L14.EGFR2	22	2
L14.EGFR3	17	0
L14.EGFR4	14	1
L14.EGFR5	17	0
L14.EGFR6	73	0
L14.EGFR7	8	3
L14.EGFR8	8	12
L14.EGFR9	3	1

DNA extraction method	Number of labs	% of labs (n=144)
QIAamp DNA FFPE Tissue Kit (Qiagen)	54	37,50%
Cobas® DNA Sample Preparation Kit (Roche)	25	17,36%
QIAamp DNA Mini Kit (Qiagen)	20	13,89%
Maxwell 16 FFPE Tissue LEV DNA Purification kit (Promega)	12	8,33%
Raw proteinase K lysate	9	6,25%
NucliSENS easyMAG (bioMérieux)	3	2,08%
NucleoSpin FFPE DNA(Macherey-Nagel)	2	1,39%
QIAsymphony DSP DNA Mini Kit	2	1,39%
NucleoSpin Tissue (Macherey-Nagel)	2	1,39%
Other	16	11,11%

Primary mutation detection method	Number of labs	Percentage of labs (n=144)
Prescreening		
HRM	10	6,94%
Commercial test kit		
Therascreen EGFR RGQ PCR Kit (Qiagen DxS)	24	16,67%
EGFR Mutation Test (Cobas)	24	16,67%
Therascreen EGFR Pyro Kit (Qiagen DxS)	14	9,72%
EGFR Mutation Analysis Kit (EntroGen)	12	8,33%
EGFR StripAssay (ViennaLab)	4	2,78%
EGFR TKI response (Diatech Pharmacogenetics)	4	2,78%
Therascreen EGFR PCR Kit (Qiagen DxS)	2	1,39%
Myriapod Cancer Status (Diatech Pharmacogenetics)	2	1,39%
Other	6	4,17%

	6	4,17%
Next generation sequencing		
Ion Ampliseq (Ion Torrent, Life Technologies)	3	2,08%
MiSeq (Illumina)	1	0,69%
GS Junior System (454 Life Sciences, Roche)	1	0,69%
Amplicon based home made panel	1	0,69%
PCR and sequencing		
Dideoxy sequencing	24	16,67%
Pyrosequencing	2	1,39%
Other		
Taqman	3	2,08%
Sequenom Massarray Assay panel, in-house developed	1	0,69%
Length analysis of PCR amplified products	1	0,69%
Denaturing Capillary electrophoresis	1	0,69%
Fragment analysis	1	0,69%
ARMS-PCR	1	0,69%
Hydrolysis probe assay	1	0,69%

Overall EGFR mutation analysis

Sample

- Average genotyping score 15,8 /18

Laboratory

- 88/ 144(61%) successful
- 52/144 (36%) no genotyping errors
- 56/144 (39%) 1 genotyping error
- 36/144 (25%) 2 genotyping errors
- 13/144 (9%) combi genotyping + technical error

EGFR assays

- Not test dependent for most mutations

Cases > 60% allelic frequency T790M

case1	T790M +		T790M negative		p-value
	n	%	n	%	
Cobas	24	100%	0	0%	0,18
other	60	90%	7	10%	
case 3	T790M +		T790M negative		p-value
	n	%	n	%	
Cobas	24	100%	0	0%	0,32
other	62	93%	5	7%	

Cases 11% allelic frequency T790M

case 6	T790M +		T790M negative		p-value
	n	%	n	%	
Cobas	2	8%	22	92%	<0.0001
other	46	69%	21	31%	

Conclusion EGFR mutation analysis

- EQA is important
- This round more challenging samples
- Feed back to laboratories is essential
- Feed back to commercial company provided.

ALK

- FISH
- IHC
- (RT-PCR)

Samples for ALK analysis		
Sample number	Sample nature	ALK status
L14.ALK1	cell line (90/10%)	negative
L14.ALK2	cell line (90/10%)	negative
L14.ALK3	cell line (90/10%)	negative
L14.ALK4	cell line (90/10%)	positive
L14.ALK5	resection specimen	negative
L14.ALK6	resection specimen	negative
L14.ALK7	resection specimen	negative
L14.ALK8	resection specimen	negative (educational for ALK FISH)
L14.ALK9	resection specimen	positive
L14.ALK10	resection specimen	positive (educational for ALK FISH and ALK IHC)

Laboratory	Responsible person
Preparation of samples	
Department of Pathology, VU University Medical Centre, Amsterdam, The Netherlands	E. Thunnissen (coordinator)
Department of Pathology, University Medical Center Groningen, Groningen, The Netherlands	E. Schuuring
UKNEQAS, London, UK	K. Miller
Institute for Pathology, University Hospital Basel, Basel, Switzerland	L. Bubendorf
Validation of samples	
Service de Cytogénétique et Biologie Cellulaire, University of Rennes, Rennes, France	M.A. Belaud-Rotureau
Institute for Patholog, University Hospital Heidelberg, Heidelberg, Germany	A. Warth
Department of Pathology, University Medical Center Groningen, Groningen, The	E. Schuuring

EQA NSCLC ALK 2014

- **Labs registered / submitted results**

- ***ALK* FISH 136 / 116**
- ***ALK* FISH Digital 93 / 81**
- ***ALK* IHC 106 / 96**
- ***ALK* RT-PCR 16 / 6**

The assessors:

Cabillic F. – University Hospital Rennes, Rennes, France
Dequeker E. – KU Leuven, Biomedical Quality Assurance RU, Leuven, Belgium
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Tembuyser L. – KU Leuven, Biomedical Quality Assurance RU, Belgium
Thunnissen E. – VU University Medical Centre Amsterdam, The Netherlands
Tornillo L. - University Hospital Basel, Basel, Switzerland
Weichert W. – University Hospital Heidelberg, Heidelberg, Germany
Warth A. – University Hospital Heidelberg, Heidelberg, Germany

Scoring code	Marking criteria	Marks
a	Correct result (≥ 50 evaluable nuclei counted)	2.00
a*	Correct outcome, but misleading technical information	1.00
b	False positive / false negative	0.00
b*	Sample indicated as borderline ALK FISH positivity	0.40
c	Not contributive	0.00 points for FISH digital 0.50 points for the other subschemes
d	No cores available	0.50
e (ALK FISH digital)	<50 nuclei evaluated	0.00
f	<50 nuclei evaluated in a positive case, with correctly assigned outcome	1.90
g	<50 nuclei evaluated in a negative case: if total nr of nuclei with FISH signal - (total split signal or single red) = 43-49. The reason behind the range 43-49 is that counting more would not change the outcome. Below 43 counting more might in theory change outcome. The score of 1.9 in stead of 2 is because beforehand evaluation of 50 nuclei was mentioned to be required	1.90
h	<50 nuclei evaluated in a negative case: if total nr of nuclei with FISH signal - (total split signal or single red) <43. For an explanation see “g”	0.00
i	Sample from block that did not pass QC check (too few cells available)	not assessed (sample left out of scoring)

ALK FISH methods

Method	# labs	% of total (n=116)
Vysis ALK break apart FISH probe kit (Abbott Molecular)	79	68%
ZytoLight SPEC ALK/EML4 TriCheck™ Probe (ZytoVision)	15	13%
ALK FISH DNA Probe, Split Signal (Dako)	11	9%
ZytoLight SPEC ALK Dual Color Break Apart Probe (ZytoVision)	6	5%
REPEAT-FREE™ POSEIDON™ ALK (2p23) Break Probe (Kreatech Diagnostics)	4	3%
Cytocell Aquarius ALK LPS 019 Breakapart probe	1	1%

ALK FISH

ALK FISH	Outcome	Nr of labs without cores	Nr of labs that counted <50 nuclei (with correct outcome)	Nr of FP/FN	Nr of labs with non contributive result	Mean % positive nuclei	SD of % positive nuclei
1	-	/	6	4	8	4,7	6,7
2	-	/	5	4	11	5,2	8,3
3	-	/	3	1	7	2,4	6,3
4	+	/	5	1	6	63,6	21,4
5	-	2	5	1	9	5,7	14,2
6	-	3	6	1	2	3,5	4,8
7	-	3	5	4	3	3,3	9,0
8	-	3	1	3	36	6,0	13,4
9	+	2	3	14	5	51,7	26,7
10*	+	3	6	11	20	46,1	23,7

DIGITAL ALK

Digital ALK FISH	Outcome	Nr of labs that counted <50 nuclei (with correct outcome)	Nr of FP/FN	Nr of labs with non contributive result	Mean % FISH positive nuclei	SD of % FISH positive nuclei
1	-	10	1	2	5,6	9,7
2	+	7	1	3	76,6	15,6
3	-	5	0	1	3,4	3,1
4	+	6	0	0	78,8	11,7

ALK IHC Antibody	Number of labs	% total (n=96)
1A4 SDIX (Origene)	1	1%
5A4 (Abcam)	3	3%
5A4 (Biocare Medical)	1	1%
5A4 (Clinisciences)	2	2%
5A4 (Leica)	1	1%
5A4 (Monosan Xtra)	2	2%
5A4 (Novocastra)	20	21%
5A4 (Zytomed Systems)	1	1%
ALK01 (Ventana)	3	3%
ALK1 (Dako)	4	4%
D5F3 (Cell Signalling Technology)	27	28%
D5F3 (Ventana)	30	31%
NCL-ALK-1 (Leica)	1	1%

ALK IHC	Outcome	Nr of labs with no cores	Nr of FP/ FN	Nr of labs with non contributive result
1	-	/	5	0
2	-	/	5	0
3	-	/	5	1
4	+	/	2	0
5	-	1	3	0
6	-	1	3	0
7	-	2	12	3
8	-	1	6	1
9	+	0	13	1
10*	+	1	30	7

EQA NSCLC ALK

- **Average score**

- ***ALK* FISH** **90%**
- ***ALK* FISH Digital** **90%**
- ***ALK* IHC** **93%**
- ***ALK* RT-PCR** **83%**

Overall NSCLC ALK analysis

Sample

- Average genotyping score 15,8 /18

Laboratories

- 82/ 116 (71%) successful ALK FISH
- 67 / 96 (70%) successful ALK IHC

Conclusion

- EQA useful feedback for participants/ assays
- Level never 100%
- Continous monitoring shows improvement