EGFR ring trial May 2012 - results

	Case1	Case2	Case3	Comments		Comments by distributor
definition	Adenocarcinoma, solid predominant	Adenocarcinoma predominant acinar and lepidic	Adenocarcinoma predominant lepidic and acinar			digital slides can be viewed www.iapaustria.com user: agpulmo PW: EGFR (case sensitive)
dates						
ID01				na	na	
ID02				28.05.2012	04.06.2012	
ID04				14.05.2012	16.05.2012	
ID05				na	na	
ID06				22.05.2012	30.05.2012	
ID07				na	na	
ID08				14.05.21012	25.05.2012	
ID09				10.05.12012	12.05.2012	x
ID10				10.05.2012	16.05.2012	
ID11				11.05.2012	14.05.2012	
ID12				11.05.2012 (8:00)	15.05.2012	
ID13				10.05.2012	15.05.2012	
ID14				10.05.2012	16.05.2012	
ID15				14.05.2012	28.05.2012	
ID16				10.05.2012	15.05.2012	
ID17				09.05.2012	18.05.2012	
ID 18				11.05.2012	21.05.2012	
ID19				21.05.2012	29.05.21012	
percentage of tumor cells within section (as in regular report)						
ID01	60-70%	10-15%	30%			
ID02	60%	30%	40%			
ID04	90%	65%	65%			
ID05	95%	60%	70%			
ID06	70%	50%	60%			
ID07						
ID08	80%	30%	30%			
ID09	50%	30%	30%			
ID10	80% tumor cells in md	10% tumor cells in md	30% tumor cells in md			
ID11	in marked area 80-90%	60 – 70%	70%			

ID12	70%	25%	60%		
ID13	60% tumor cells in analyzed tissue	30% tumor cells in analyzed tissue	40% tumor cells in analyzed tissue		
ID14	80%	60%	40%		
ID15	90%	45%	80%		
ID16	80%	40%	80%		
	markierte Region = 60%	markierte Region = 30%	markierte Region = 65%		
ID18	>80%	40 – 50%	approx 50%		
ID19	75%	25%	50%		
how was percentage calculated ?					
ID01	calculation of field				
ID02	histomorpology				
ID04	tumor cells minus stroma and inflammatory cells				
ID05	evaluated by molecular pathologist				
ID06					
ID07					
ID08	estimated by pathologist				
ID09	tumor cells vs. non-tumor cells by pathologist				
ID10	estimated				
ID11					
ID 12	ration of estimated number of tumor cells vs. normal cells				
ID13	amount of tumor cells in marked area				
ID14	estimated				
ID15	percentage of atypical eouthelial cells in tumor area				
ID16					
ID1´7	Kalulation durch den Pathologen				
ID18					
ID19	histological evaluation				
Tumor cell enrichment – yes/no : method of enrichment (e.g. macrodissection by needle)					
ID01	scratch diss – nearly 100% tu-cells	30 – 40% after dissection	dissection		
ID02	ys, macrodissection				
ID04	macrodissection by scrathcing				
ID05	macrodissection (by scraping)				
ID06	yes, case 2 and 3 macrodissection by needle				
ID07	macrodissection				
ID08	macrodissection by scratching				

ID09	macrodissection		
ID10	yes, macrodissection by scratching		good documentation of slide jpg
ID11	tumor area marked, macrodissection		
ID 12	no		
ID13	yes, tissue scratching of marked area		
ID14	macrodissection by scratching		
ID15	yes, scracthing		
ID16	no		
ID17	Makrodissektion		
ID18	no		
ID19	no		
method of DNA extraction			
ID01	Maxwell Promega		
ID02	QuiAmp DNA FFPE tissue kit Cat No 56404		
ID04	COBAS DNA Sample preparation kit (Roche)		
ID05	High Pure PCR Template Preparation Kit (Roche)		
ID06	Roche High Pure PCR Template Kit		
ID07	fibre glass extraction		
ID08	High Pure PCR Template Preparation kit Roche		
ID09	QIAmp DNA FFPE MiniKit (Quiagen)		
ID10	MAXWELL 16 Instrument , Fa.Promega		
ID11	DNA extraction from paraffin material		
ID12	EZ 1 Automat Fa. Qiagen		
ID13	QIAmp DNA FFPE tissue kit Fa Quiagen		
ID14	full automatic bo robot EZ1 Quiagen investigator kit		
ID15	QIAamp DNA Mini Kit (Qiagen)		
ID16	Quiagen FFPE		
ID17	QIAamp DNA FFPE Tissue Kit von Qiagen		
ID18	cobas DNA Sample Preparation Kit		
ID19	DNA Sample preparation kit Roche		
method of measurement of DNA			
ID01	Qubit fluorimetry		
ID02	Bio spec nano Shimatsu biotech		
ID04	Bio spec nano		
ID05	NanoDrop ND-1000 spectrophotometer		
ID06	Nanodrop		
ID07	UV photometry		
ID08	Nanodrop 1000		

ID09	Spectrophotometer NanoDrop 1000 (Peqlab)				
ID10	spectralphotometer Nanodrop				
ID 11	Biospec Nano				
ID11	Nanodrop ND-1000 Fa. Peqlab				
ID13	Quant-iT ds DNA BR Assay Kit, Fa Invitrogen(Qubit)				
ID14	photometry Glue Quant 1300				
ID15	NanoDrop ND-100 Spectrophotometer				
ID16	Bio Spec Nano – Simple Nucleic Acid Quant., Analyte dsDNA				
ID17	NanoDrop Peqlab				
ID18	QUBIT 2.0 Fluorometer				
ID19	Quan iT ds DNA HS Assay Kit (Invitrogen)				
Amount of DNA extracted (as in regular report)					
ID01	37,40 µg/ml (100µl)	7,22 µg/ml (100µl)	63,50 µg/ml (100µl)		
ID02	47,33	18,23	32,17		
ID04	3,79 ng/µl	15,74 ng/µl (diluted 1:2)	33,16 ng/µl (diluted 1:3)		
ID05	83,9	21,1	68,3		
ID06	57,92 ng/µl	45,81 ng/µl	107,29 ng/µl		
ID07					
ID08	60,60 ng/µl	14,54 ng/µl	71,43 ng/µl		
ID09	290,1 ng/µl	87, 7 ng/µl	224,4 ng/µl		
ID10	149 ng/µl (60 µl)	18 ng/µl (60 µl)	179 ng/µl (60 µl)		
ID11	268,27	88,05	130,82		
ID 12	43,6 ng/µl	29,9 ng/µl	53,0 ng/µl		
ID13	95,7 µg/ml	2,97 µg/ml	30,7 µg/ml	The amount is documented in a lab-report, not in the patients report	
ID14	48 ng/µl	14 ng/µl	31 ng/µl	Case 2. only 1 slide for DNA extr, because others broken	
ID15	33,6 ng/µl (V_{total} = 50 µl)	4,2 ng/µl (V_{total} = 50 µl)	36,6 ng/µl (V_{total} = 50 µl)		
ID16	193,95 ng/µl	39,42 ng/µl	323,77 ng/µl		
ID17	140 ng/µL	13,5 ng/µL	186 ng/µL		
ID18	17,8 ng/µl	1,4 ng/µl	62,0 ng/µl		
ID19	65,2 ng/ml	41,2 ng/ml	202 ng/ml		
qualitiy of DNA					
ID01					
ID02	good	good	good		
ID04	-36,08	2,23	2,29		
ID05	good	acceptable	good		

ID06	1,50	1,50	1,54		
ID08	good	good	good		
ID07					
ID08					
ID09	1,97	1,95	1,95		
ID10	1,83	2,03	1,85		
ID11					
ID 12	260/280, 1,87	260/280, 1,99	260/280, 1,90		
ID13	good	good	good		
ID14	260/280 nm 2,1	3,5	2,2		
ID15	$A_{260/280}$ = 1,85; $A_{260/230}$ = 3,45	$A_{260/280}$ = 1,63; $A_{260/230}$ = 1,20	$A_{260/280}$ = 1,91; $A_{260/230}$ = 2,94		
ID16					
ID17	1,9	2,2	2,0	(260/280)	
ID18	optimal	low	high		
ID19	not done	not done	not done		
evaluation of DNA qualitiy					
ID01					
ID02	ratio				
ID04	OD 260/280				
ID05	spectrophotometric analysis				
ID06	suitable				
ID07	sufficient for mutation testing				
ID08	Nanodrop 1000 (260/280)				
ID09	included control PCR				
ID10	ratio 260/280 (pure DNA :				
ID11					
ID12	0,8 % Agarosegel and control				
ID13	Estimation in relation to peak height of the program and in relation to the control DNA				
ID14	(exon2) control mix (kit) assay + inhibitor control (kit)				
ID15	NanoDrop ND-100 Spectrophotometer measurement + β-globin control PCR				
ID16					
ID17	-				
ID18	convenient				
ID19	not done				
Method of sequencing (as in regular report)					
ID01	EGFR RGQ PCR kit (therascreen); cobas Z480			all	

ID02			
ID04	COBAS 4800 Roche		
ID05	Sanger sequencing (ABI 3130 Genetic Analyser)		
ID06	Pyromark Quiagen 24 pyrosequencing with Quiagen therascreen EGFR kit for IVD		
ID07	Dye terminator		
ID08	Next generation sequencing		
ID09	allele specific PCR (EGFR)	Thera Screen and/or PyroMark Quiagen	
ID10	Thera Screen EGFR Pyro kit Quiagen		
ID11	COBAS EGFR mutation test COBAS 4800 System		
ID 12	Sanger Sequencing ABI 3500xL Dx		
ID13	Pyrosequencing Therascreen EGFR PYro kit Fa Quiagen "Method used: DNA-extraction with Quiagen QIAmp FFPE Kit, sequencing with Therascreen EGFR Pyro Kit (Quiagen, Hilden, Germany)"		
ID14	(real time PCR) thera screen EGFR RGQ PCR kit Quiagen		
ID15	7500 Real Time PCR System + TaqMan Mutation Detection Assays, Applied Biosystems Dideoxy sequencing (Applied Biosystems, 3100-Avant)		
ID16			
ID17	Parosequenzierung		
ID18	cobas z 480 analyser for real time PCR		
ID19	Real Time PCR – Cobas EGFR Mutation Test		
What types of mutations can be detected by your method? Sensitivity of your method, if known?			
ID01	therascreen: T790M ,Del., L585R, L861Q, G719X, S786I, Ins.; COBAS: G719X, Del.; T790M, S768I, Ins., L858R		
ID02	s. COBAS EGFRmutation test		
ID04	41 different mutations in exons 18,19,20,21 (Exon 19 Del., Exon 20 Insertion, S768I, T790M, exon 18 G719X, exon 21 L858R		
ID05	all types by 10% sensitivity		
ID06	exon 18 codon 719, exon 20 codon 768, exon 20 codon 790 exon 21 codon 858 and 861 exon 19 10 most frequent deletions form codon 2255		
ID07	EGFR exon 19 and 21		
ID08	EGFR exon 19 and 21 sensitivity (1-) 5%		
ID09	29 mutations detected by the TheraScreen EGFR29 Mutation Kit. Sensitivity according to the manufacturer: 15%		
ID10	exon 18 mutations on codon 719: G719X (S,C,A,D) exon 19: all deletions within codons 746-750 exon 20:mutation codon 768 (S768I), insertion codon 770 774, mutation codon 790: T790M exon 21: mutation codon 858 and 861: L858R and L:861Q	the limit of detection is about 5% (LOD values represent the lowest signal (measured frequency) that can be regarded as positive for the	

				respective mutation.	
ID11	exon 18: pointmutation in codon 719 exon 19: all deletions listed in Version 1.0/Oct2011 of kit exon 20: pointmutations in codon 790 (T790M) and codon 768 (S768I) insertions exon 21: mutation in codon 858(L858R)				
Id 12	all types (del, ins, sub, dup)				
ID13	Point mutations codons 719, 768, 790, 858-861; Deletions Exon 19, Sensitivity 5-10% mt in wildtype				
ID14	19 deletions in exon 19, T790M,L858R. L861Q, G719X, S763I, 3 insertions in exon 20; sensitivity 1-10% mutated DNA				
ID15	Exon 18: G719A, G719S, G719C Exon 19: 19 deletions (L747_T751>S, L747_E749del, E746_S752>D, E746_A750del (2235_2249del15), E746_A750del (2236_2250del15), L747_T751del, L747_T752del, E746_S752>A, L747_T751del, L747_P753>S, L747_A750>P, L747_A751>P, E746_S752>V, L747_P753>Q, L747_T751>Q, L747_A750>P, E746_T751>A, E746_T751del and E746_T751>I) Exon 20: T790M, S768I, V769_D770insASV, H773_V774insH and D770_N771insG Exon 21: L858R and L861Q TaqMan Mutation Detection Assays can detect 0,1% mutated DNA in a background of wild type DNA.				
ID16	41 mutations in exons 18, 19, 20, 21 sensitiviy: >5 % mutation in Wildtype background				
ID17	Exon 18: Codon 709, 712, 719, 721 Exon 21: Codon 858, 863 Exon 19: Codon 754 Exon 20: Codon 769, 790				
ID18	detects 41 specific mutations (insertions and deletions) in EXONS 18, 19, 20, 21 of the EGFR gene sensitivity >5% mutation copies of FFPET DNA in a background of wild type DNA				
ID19	by our method 3 point mutations can be detected in exon 18 (G719A, G719C, G719S), 29 deletions and complex mutations in exon 19, 2 point mutations (S768I, T790M) and 5 insertions in exon 20 and L858R in exon 21				
result as in regular report					
ID01	negative	L858R Mut	G719X Mut		
ID02	WT	L858R	G719X		
ID04	mutation not detected	mutation detected exon 21 L858R	mutation detected exon 18 G719X		case 1+2 inverted only in form (s.diagnosis)
ID05	WT	c.2573T>G p.L858R	c.2126 A>C E709A*, c.2155 G>A G719S		as shown here reports should be written using HGVS nomencclature

ID06	exon 18, 19, 20(codon 768,790), 21 wild type	exon 18, 19, 20(codon 768,790), wild type exon 21 L858R mutation 21%	exon 19, 20 (codon 768, 790) wild type, exon 18 G719S mutation 17,1%		
ID07	wt	exon 21 p.L858R	wt		no method and sensitivity indicated
ID08	A895D 10%	L858R 7%	WT		no analysis of exon 18 in used method
ID09	WT	mutation –p.L858R (c.2573T>G), exon 21	mutation G719X		
ID10	wildtype	exon 21 cod 858 L858R 23%	exon 18 G719S 32%		
ID11	mutation not detected	mutation detected: exon 21 L858R	mutation detected; exon 18 G719X		
ID12	EGFR (exon 18, exon 19, exon 20, exon 21) : no mutation	EGFR exon 21: c.2573T>G p.L858R	EGFR exon 18: c.[2126A>C;2155>A] p.[E709A;G719S]	Auf die Angabe von stillen Mutationen (Exon 20) und Varianten (Intron) wurde verzichtet)	
D13I	moleculargenetic analysis for EGFR: no mutation /deletion in EGFR gene	mutation in codon 858 of EGFR gene p.L858R	mutation in codon 719 of EGFR gene p.G719S		
ID14	no mutation detected	L858R +	G719X +		
ID15	negative	positive L858R mutation	positive G719S and E709A mutation	Mutation Q787Q (silent mutation) was also detected in Case 1,2 and 3	
ID16	mutation not detected	exon 21 L858R	exon 18 G719X		
ID17	keine Mutation (wt)	p.L8585R	p.E709A; p.G719S		
ID18	N/A	Exon 21 L858R	Exon 18 G719X		
ID19	no mutations have been detected in sample	activating mutations have beendetected in sample L858R	activating mutations have been detected in sample G719X		
interpretation of result as in regular report					
ID01	s.a.	s.a	s.a.		
ID02	*	**	***	*no mutation in EGFR gene in specimen ** EGFR mutation (L858R) ifound in specimen – repsonse to therapy with TKi to be expected. *** response to therapy with TKI erlotinib to be expected	
ID04	no mutation of EGFR gene	activating mutation detected: exon 21	activating mutation detected: exon 18		

		L858R	G719X		
ID05	Wild type EGFR	EGFR exon 21 mutated adenocarcinoma	EGFR exon 18 mutated adenocarcinoma		
ID06	activating mutation of EGFR cannot be detected	activating mutation in exon 21 indicating response for TKI	activating mutation in exon 18 indicating response for TKI		
ID07	wild type	EGFR activating mutation	wild type		
ID08	resistance mutation in exon 21	activating mutation in exon 21	no mutation found in exon 19 and 21		
ID09				not done in our institute	
ID10	no mutation	activating mutation	activating mutation		
ID11					
ID 12					
ID13					
ID14	s.a.	s.a.	s.a.		
ID15	sample ist negative for EGFR gene mutation	sample is positive for activating L858R mutation	sample ist positive for activating G719S mutation and for E709A mutation		
ID16					
ID17		Die p.L858R Mutation ist assoziiert mit einer erhöhten Sensitivität auf EGFR TKIs.	Die p.G719S Mutation ist assoziiert mit einer erhöhten Sensitivität auf EGFR TKIs		
ID18	mutation not detected	mutation detected in exon 21	mutation detected in exon 18		
ID19	EGFR negative, no benefit with TKI treatment	EGFR positive result confers TKI sensitivity	EGFR positive result confers TKI sensitivity		
Additional comments to oncology department or recommendations					
ID01					
ID02	*	**	***		
ID04					
ID05	patient is not eligible for EGFR TK inhibitor therapy	patient is eligible for EGFR TK inhibitor therapy (TARCEVA and IRESSA)	patient is eligible for EGFR TK inhibitor therapy (TARCEVA and IRESSA)	Case 3:*Non-classical exon 18 activating mutation	
ID06					

ID07					
ID08	tumour sample shows resistance to anti EGFR therapy	tumour sample shows sensitiviy to anti EGFR therapy	tumour sample shows resistance to anti EGFR therapy		
ID09		mutation in EGFR- or KRAS gene is reported to exclude ALK fusions	mutation in EGFR- or KRAS gene is reported to exclude ALK fusions		
ID10					
ID11					
ID 12	no supportive data for use of IRESSA	data support usage of IRESSA	double-mutation; no supportive data for use of IRESSA		attenuated response to gefitinib in double mutation E709A+G719**C**
ID13		good response to anti EGFR-therapy propable	good response to anti EGFR-therapy propable		
ID14					
ID15		presence of the activating L858R mutation is associated with EGFR TKI sensitivity: EGFR TKI therapy is recommended Reference: Travis W. D. et al. Proc Am Thorac Soc. 2011 Sep;8(5):381-5.	presence of these mutations in combination with attenuated response to TKI therapy Reference: Tam I Y et al Mol. Cancer Ther 2009 Auf 8(8): 2142-51		attenuated response to gefitinib in double mutation E709A+G719**C**
ID16					
ID17		Rücksprache mit dem Onkologen	Rücksprache mit dem Onkologen		
ID18	continue with FISH analyses	mutation activating the EGFR tyrosine kinase and are sensitive to TKIs	mutation activating the EGFR tyrosine kinase and are sensitive to TKIs		
ID19			discuss the case multidisciplinary tema meeting (rare type of activating EGFR mutation)		

Diagnosis of case as done in regular reports					
ID01	solid, partially large cell adneocarcinoma G3	acinar adenocarcinoma with in situ component G2/3	acinar adenocarcinoma with in situ component G2		
ID02	NSCLC 60%	adeno G2 30%	adeno G2 40%		
ID04	solid adenocarcinoma	adenocarcinoma, 80 acinar, 20% papillary	adenocarcinoma, papillary, acinar		diagnosis congruent (see results)
ID05	EGFR WT adenocarcinoma	EGFR mutated adenocarcinoma	EGFR mutated adenocarcinoma		
ID06	pulmonary adenocarcinoma	pulmonary adenocarcinoma	pulmonary adenocarcinoma		
ID07					
ID08	adenocarcinoma with solid pattern estimated percentage of EGFR mutation in tumor cells 12,5%	adenocarcinoma with papillary pattern estimated percentage of EGFR mutation in tumor cells 23,3%	adenocarcinoma with papillary pattern		
ID09	adenocarcinoma, solid predominant	adenocarcinoma, lepidic predominant (60%), acinar (35%) and micropapillary (5%)	minimally invasive adenocarcinoma, lepidic predominant (80%), acinar (20%), AAH		
ID10	solid adenocarcinoma partial mucinous G3	acinar adenocarcinoma G2	aciinar (80%) and papillary(20%) adenocarcinoma G2		
ID11	solid adenocarcinoma G3	adenocarcinoma lepidic type G1	daenocarcinoma papillary and lepidic G2		
ID 12					
ID13	EGFR negative	EGFR positive	EGFR positive		
ID14	predominant solid lung carcinoma, susp. adenosqamous	invasive adenocarcinoma – lepidic and papillary type G2	invasive adenocarcinoma tubulopapillary G2		
ID15	adenocarcinoma with predominant solid pattern	adenocarcinoma with predominant lepidic pattern	adenocarcinoma with predominant papillary pattern		
ID16					
ID17	Gering differenziertes bronchogenes Karzinom, andeutungsweise mit	Bronchogenes Adenokarzinom (Grad2)	Bronchogenes Adenokarzinom (Grad2)		

	squamöser Differenzierung (Grad3)				
ID18	NSCLC epidermoid Ca (growing on periphery with predominance of alveolar space filling pattern) versus pleomorphic Ca, versus solid adenocarcinoma with mucin production versus large cell Ca G3 (G4 resp.) V1	adenocarcinoma bronchioloalveolar type, non mucinouse type	adenocarcinoma mixed, classic lepidic ca 40% NOS up to 60% (micorpapillary? tubuloacinar?) V1, susp pleura infiltration		
ID19	adenocarcinoma predominant solid pattern (100%) EGFR negative	adenocarcinoma predominant acinary pattern (60%) with lepidic pattern (40%) EGFR postivie	adenocarcinoma predominant papillary pattern (50%) with acinary (20%) and lepidic (30%) pattern, EGFR positive		