EGFR ring trial December 2012 - results

	Case1	Case2	Case3	Comments		Comments by distributor
definition	Adenocarcinoma, lepidic predominant	Adenocarcinoma solid predominant	Adenocarcinoma solid predominant			digital slides can be viewed www.iapaustria.com user: agpulmo PW: EGFR (case sensitive)
dates						
ID01				02.01.2013	14.01.2013	
ID02				28.12.2012	03.01.2013	
ID04				31.12.2012	09.01.2013	
ID05				na	na	
ID06				na	na	
ID07				21.12.2012	15.01.2013	
ID08				03.01.2013	23.01.2013	
ID09				07.01.2013	17.01.2013	
ID10				20.12.2012	04.01.2012 only 6 working days with reduced staff	
ID11				31.12.2012	11.01.2013	
ID12				27.12.2012 (12:00)	02.01.2013 (16:00)	
ID13				27.12.2012	09.01.2013	
ID14				26.12.2012	28.12.2012	
ID15				03.01.2013	15.01.2013	
ID16						
ID 18				02.01.2013	09.01.2013	
ID19				02.01.2013	31.01.2013	
percentage of tumor cells within section (as in regular report)						
ID01	20%	20%	85%			
ID02	20%	20%	80%			
ID04	20	10	60			
ID05	50%	30%	80%			
ID06	50% of marked area	30% of marked area	40%			
ID07	20% - 60%	30% - 50%	60% - 80%	before - after enrichment		
ID08	50%	5%	65%			
ID09	50%	40%	60%			

ID10	80% tumor cells in marked area	40% tumor cells in marked area	70% tumor cells in marked area		
ID11	60%	30%	70%		
ID12	40%	35%	80%		
ID13	Das analysierte Gewebe zeigte 90% Tumorzellen.	Das analysierte Gewebe zeigte 40% Tumorzellen.	Das analysierte Gewebe zeigte 60 % Tumorzellen.		
ID14	in markierten Tumorarealen 30%	in 2 markierten Tumorarealen 30%	70%		
ID15	65%	40%	75%		
ID16	75%	75%	90%		
ID18	30 – 40%	30%	70%		
ID19	30%	15%	50%		
how was percentage calculated ?					
ID01	see +				
ID02	histomorpology				
ID04	tumor cells minus stroma and inflammatory cells				
ID05	evaluated by molecular pathologist				
ID06					
ID07	relative tumor cell quantity within selected region (microscopy)				
ID08	estimated by pathologist				
ID09	tumor cells vs. non-tumor cells by pathologist				
ID10					
ID11	estimated				
ID 12	ratio of estimated number of tumor cells vs. normal cells				
ID13	amount of tumor cells in marked area				
ID14	percentage of atypical epithelial cells in tumor area				
ID15	percentage of atypical eouthelial cells in tumor area				
ID16	estimated				
ID18	semiquantitatively ba agreement of both evaluationg pathologists				
ID19	histological evaluation				
Tumor cell enrichment – yes/no : method of enrichment (e.g. macrodissection by needle)					
ID01	macordissectin by scratching 20%	macrodissection by scratsching 20%	whole tissue section sby scratching 85%		
ID02	yes, macrodissection				
ID04	Only in Case B - macrodissection by scrathcing				
ID05	macrodissection (by scraping)				
ID06	yes macrodissection	no	yes macrodissection		

ID07	macrodissection of selected region		
ID08	macrodissection by scratching		
ID09	yes: macrodissection		
ID10	macrodissection by scratching (in Case A and B only the marked area, in Case C whole section)		good documentation of slide jpg
ID11	macrodissection		
ID 12	Case A: macrodissection by scratching, Cases B and C: no		
ID13	yes, tissue scratching of marked tumor area		
ID14	macrodissection by scratching		
ID15	yes, scracthing		
ID16	yes, yes, no (scratching)		
ID18	no		
ID19	No enrichment; material was scratched into microcentrifuge tube by sterile scalpel.		
method of DNA extraction			
ID01	Maxwell Promega		
ID02	QuiAmp DNA FFPE tissue kit		
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	DNA FFPE MiniKit (Quiagen)		
ID10	MAXWELL 16 Instrument , Fa.Promega		
ID11	DNA sample preparation kit		
ID12	EZ 1 AdvancedLS Automat Fa. Qiagen		
ID13	QIAmp DNA FFPE tissue kit Fa Quiagen		
ID14	full automatic bio robot EZ1 Quiagen/investigator kit		
ID15	QIAamp DNA Mini Kit (Qiagen)		
ID16	Roche, DNA Sample Prearation		
ID18	cobas DNA Sample Preparation Kit		
ID19	DNA Sample preparation kit Roche		
method of measurement of DNA			
ID01	Qubit fluorimetry		
ID02	Bio spec nano		
ID04	Bio spec nano		
ID05	NanoDrop ND-1000 spectrophotometer		
ID06	Nanodrop		
ID07	UV photometry		

ID08	Nanodrop 1000				
ID09	NanoDrop Peqlab				
ID10	spectralphotometer Nanodrop				
ID 11	Biospec Nano				
ID 12	Nanodrop ND 1000 Fa Peqlab				
ID13	Quant-iT ds DNA BR Assay Kit, Fa Invitrogen(Qubit)				
ID14	photometry Gene Quant 1300				
ID15	NanoDrop ND-100 Spectrophotometer				
ID16	Bio Spec Nano				
ID18	QUBIT 2.0 Fluorometer				
ID19	Quan iT ds DNA HS Assay Kit (Invitrogen)				
Amount of DNA extracted (as in regular report)					
ID01	57,70 µg/mL (100µl)	38,40 µg/ml (100µl)	48,60 µg/ml (100µl)		
ID02	2,63 ng/µL	3,61 ng/µL	5,53 ng/µL		
ID04	22,04 ng/µl	24,52 ng/µl (diluted 1:2)	37,28 ng/µl		
ID05	17,2	20,5	47,5		
ID06	13,97 ng/µl	739,14 ng/µl	77,29 ng/µl		
ID07	26ng/ul	52ng/ul	17ng/ul		
ID08	63,9 ng/µl	45 ng/µl	114,3 ng/µl		
ID09	17,9 ng/µl	11,9 ng/µl	23,4 ng/µl		
ID10	103 ng/µl	12 ng/µl	144 ng/µl	in 60 µl eluat	
ID11	42,89 ng/µl	30,78 ng/µl	92,65 ng/µl		
ID 12	12,9 ng/µl	17,5 ng/µl	37,8 ng/µl		
ID13	14,9 µg/ml	4,77 µg/ml	14,9 µg/ml	The amount is documented in a lab-report, not in the patients report	
ID14	20 ng/µl	20ng/µl	67 ng/µl		
ID15	47,27 ng/µl (V_{total} = 50 µl)	15,17 ng/µl (V_{total} = 50 µl)	73,77 ng/µl (V_{total} = 50 µl)		
ID16	35,20 ng/µl	26,56 ng/µl	55,65 ng/µl		
ID18	16,6 ng/µl	7,64 ng/µl	16,0 ng/µl		
ID19	62,6 ng/ml	14,9 ng/ml	37,2 ng/ml		
qualitiy of DNA					
ID01					
ID02	good	good	good		
ID04	1,86	1,181	1,75		
ID05	good	good	good		
ID06	good	good	good		
ID08	good	good	good		

ID07	1,7	1,7	1,75		
ID08					
ID09	1,88 260 nm/280nm	1,61 260 nm/280nm	1,80 260 nm/280nm		
ID10	ratio 1,84	ratio 1,8	ratio 1,85		
ID11	ok	ok	ok		
ID 12	260/280, 2,52	260/280, 2,14	260/280, 1,95		
ID13	good	good	good		
ID14	ratio 2,5	2,5	1,9		
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					
ID18	optimal	low	high		
ID19	not done	not done	not done		
evaluation of DNA qualitiy					
ID01					
ID02	internal control of DOBAS EGFR mutation test				
ID04	OD 260/280				
ID05	spectrophotometric analysis				
ID06	OD 260/280, 260/230				
ID07	sufficient for mutation testing				
ID08	Nanodrop 1000 (260/280)				
ID09	control PCR				
ID10	ratio 260/280 (pure DNA 1,7 – 1,8):				
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				
ID15	NanoDrop ND-100 Spectrophotometer measurement + β-globin control PCR				
ID16					
ID18	convenient				
ID19	not done				
Method of sequencing (as in regular report)					
ID01	cobas Z480 EGFR RGQ PCR kit (therascreen)			all	
ID02					
ID04	COBAS 4800 Roche				
ID05	1.Sanger sequencing, 2.controlled by ROCHE COBAS Z480				
ID06	pyrosequencing Pyromark Q24				
ID07	Dye terminator				

ID08	Sanger sequencing, next generation sequencing		
ID09	allele specific PCR	Thera Screen and/or PyroMark Quiagen	
ID10	Thera Screen EGFR Pyro kit Quiagen		
ID11	COBAS EGFR Mutationsanalyse		
ID 12	Sanger Sequencing ABI 3500xL Dx		
ID13	Pyrosequencing; Therascreen EGFR Pyro Kit, Fa. Qiagen „Verwendete Methode: DNA-Extraktion mit Qiagen QIAmp® DNamp FFPE Kit, Sequenzierung mit Therascreen® EGFR Pyro Kit (Quiagen, Hilden, Deutschland).“		
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	Cobas 4800 EGFR Mutation Test, Roche, Realtime-PCR		
ID18	cobas z 480 analyser for real time PCR		
ID19	Real Time PCR – Cobas EGFR Mutation Test (Roche)		
What types of mutations can be detected by your method? Sensitivity of your method, if known?			
ID01	cobas Z480: T790M ,Del., L585R, L861Q, G719X, S786I, Ins.; RG: G719X, Del.; T790M, S768I, Ins., L858R		
ID02	s. COBAS EGFRmutation test		
ID04	41 different mutations in exons 18,19,20,21 …… Sensitivity is about 5% mutated cells in wild type background…….		
ID05	5%		
ID06	exon 18 , 19, 20, 21		
ID07	EGFR exon 19 and 21		
ID08	18 – 21 exon any		
ID09	29 mutations detected by the TheraScreen EGFR29 Mutation Kit. Sensitivity according to the manufacturer: 1%		
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		
ID11	Exon 18: Punktmutationen im Codon 719 Exon 19: sämtliche derzeit in Version 1.0/ Oktober 2011 beschriebene Deletionen Exon 20: Punktmutationen Codon 790 (T790M) und 768 (S768I) Insertionen Exon 21: Mutationen 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	T790M, Deletionen Exon 19, S768i, L858R, G719X, Insertionen Exon 20				
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. overall 41 mutations				
result as in regular report					
ID01	negative	negative	negativ		
ID02	Wildtype	Wildtype	Wildtype		
ID04	Im vorliegenden Untersuchungsmaterial ist eine Mutation des EGFR Gens nicht nachweisbar	Im vorliegenden Untersuchungsmaterial ist eine Mutation des EGFR Gens nicht nachweisbar	Im vorliegenden Untersuchungsmaterial ist eine Mutation des EGFR Gens nicht nachweisbar		
ID05	WT	WT	WT		
ID06	wild type	wild type	wild type		
ID07	exon 19: wt exon 21: wt wt	exon 19: wt exon 21: wt	exon 19: wt exon 21: wt		
ID08	ELREA deletion in 1% (rs1050171 SNP found)	Wild type (rs1050171 SNP found)	Wild type		
ID09	no mutation detected	no mutation detected	no mutation detected		
ID10	using pyrosequencing technology, a normal peak pattern ist detected. No other peaks are detectable. This	using pyrosequencing technology, a normal peak pattern ist detected. No other peaks are detectable. This	using pyrosequencing technology, a normal peak pattern ist detected. No other peaks are detectable. This		

	covers the above mentioned DNA segments	covers the above mentioned DNA segments	covers the above mentioned DNA segments		
ID11	Mutation not detected	Mutation not detected	Mutation not detected		
ID12	EGFR Ex 18, 19, 20, 21: Keine Veränderung der Gensequenz	EGFR Ex 18, 19, 20, 21: Keine Veränderung der Gensequenz	EGFR Ex 18, 19, 20, 21: Keine Veränderung der Gensequenz	Auf die Angabe von stillen Mutationen und Varianten wurde verzichtet!!!	
D13I	Molekulargenetische Analyse für EGFR: Keine Mutation bzw. Deletion im EGFR-Gen	Molekulargenetische Analyse für EGFR: Keine Mutation bzw. Deletion im EGFR-Gen	Molekulargenetische Analyse für EGFR: Keine Mutation bzw. Deletion im EGFR-Gen		
ID14	no mutation detected	no mutation detected	no mutation detected		
ID15	no mutation	no mutation	no mutation	Mutation Q787Q (silent mutation) was also detected in Case 1,2 and 3	from last round?
ID16	negative	negative	negative		
ID18	EGFR mutation not detected	EGFR mutation not detected	EGFR mutation not detected		
ID19	no mutation detected	no mutation detected	no mutation detected		
interpretation of result as in regular report					
ID01					
ID02	Im vorliegenden Untersuchungsmaterial eine aktivierende Mutation des EGFR-Gens NICHT nachweisbar				
ID04	no mutation of EGFR gene	activating mutation detected: exon 21 L858R	activating mutation detected: exon 18 G719X		
ID05	Wild type EGFR	Wild type EGFR	Wild type EGFR		
ID06	no activating mutation that would increase response for EGFR inhibitor	no activating mutation that would increase response for EGFR inhibitor	no activating mutation that would increase response for EGFR inhibitor		
ID07	EGFR exon 19 and 21 mutation not detected	EGFR exon 19 and 21 mutation not detected	EGFR exon 19 and 21 mutation not detected		
ID08	EGFR activating mutation in exon 19 (1%)*	No mutation found in EGFR gene (exon18-21)	No mutation found in EGFR gene (exon18-21)	*discuss the case on the onco team needed	
ID09	sensitivity to EGFR TKI unlikely	sensitivity to EGFR TKI unlikely	sensitivity to EGFR TKI unlikely		
ID10					
ID11	Im vorliegenden	Im vorliegenden	Im vorliegenden		

	Untersuchungsmaterial die häufigsten Mutationen im EGFR Gen nicht nachweisbar	Untersuchungsmaterial die häufigsten Mutationen im EGFR Gen nicht nachweisbar	Untersuchungsmaterial die häufigsten Mutationen im EGFR Gen nicht nachweisbar		
ID 12					
ID13					
ID14	s.a.	s.a.	s.a.		
ID15	Sample is negative for EGFR gene mutation.	Sample is negative for EGFR gene mutation.	Sample is negative for EGFR gene mutation.		
ID16					
ID18	Neither activating nor resistance-associated EGFR mutation detected	Neither activating nor resistance-associated EGFR mutation detected	Neither activating nor resistance-associated EGFR mutation detected		
ID19	EGFR negative, no benefit with TKI treatment	EGFR negative, no benefit with TKI treatment	EGFR negative, no benefit with TKI treatment		
Additional comments to oncology department or recommendations					
ID01					
ID02					
ID04					
ID05	Patient is not eligible for EGFR TK inhibitor therapy	Patient is not eligible for EGFR TK inhibitor therapy	Patient is not eligible for EGFR TK inhibitor therapy	Case 3:*Non-classical exon 18 activating mutation	
ID06					
ID07					
ID08	response to therapy with TKI to be expected	response to therapy with TKI not expected	response to therapy with TKI not expected		
ID09	ALK-rearrangement possible. Respective testing advised	ALK-rearrangement possible. Respective testing advised	ALK-rearrangement possible. Respective testing advised		
ID10					
ID11					
ID 12	Datenlage unterstützt **NICHT** den Einsatz von IRESSA	Datenlage unterstützt **NICHT** den Einsatz von IRESSA	Datenlage unterstützt **NICHT** den Einsatz von IRESSA		
ID13					
ID14					
ID15	Good response to EGFR TKI	Good response to EGFR TKI	Good response to EGFR TKI		

	therapy is not expected. Reference: Travis W. D. et al. Proc Am Thorac Soc. 2011 Sep;8(5):381-5.	therapy is not expected. Reference: Travis W. D. et al. Proc Am Thorac Soc. 2011 Sep;8(5):381-5.	therapy is not expected. Reference: Travis W. D. et al. Proc Am Thorac Soc. 2011 Sep;8(5):381-5.		
ID16					
ID18	To be continued with IHC/FISH analysis of ALK	To be continued with IHC/FISH analysis of ALK	To be continued with IHC/FISH analysis of ALK		
ID19	FISH ALK testing recommended (IHK positivity?)		need fpr IHC (combined tumor?) KRAS gene mutation detected (codon 12/13)		
Diagnosis of case as done in regular reports					
ID01	azinäres focal micropapilläres Adenoca mit in situ Anteil G2	gerin diff NSCLC ,G§ NOS? Adnonca, adenosquamös?	Adenoca G2/3, neuroendokrine Anteile?, Acinuszellanteil?		
ID02	Minimal invasives Adenocarcinom, nichtmuzinös	Invasives Plattenepithelcarcinom, basaloid	Gemischt SCCL und NSCLC, DD: LCNEC, NSCLC-NOS		
ID04	Adenocarcinom,predominant lepidic growth pattern	Adenocarcinom predominant solid growth pattern	Adenocarcinom predominant solid growth pattern		
ID05	EGFR WT adenocarcinoma	EGFR WT adenocarcinoma	EGFR WT adenocarcinoma		
ID06	adenocarcinoma micropapillare of the lung grade II	Adenocarcinoma solidum of the lung grade III.	Adenocarcinoma partim clear cell, solidum of the lung grade III.		
ID08	pulmonary adenocarcinoma predominant papillary with mucinous acinar pattern	pulmonary adenocarcinoma predominant squamous	pulmonary adenocarcinoma predominant squamous		
ID09	Moderately differentiated Adenocarcinoma of the lung (70% lepidic, 25% acinary, 5% papillary)	Poorly differentiated, solid adenocarcinoma of the lung	Poorly differentiated, solid adenocarcinoma of the lung		
ID10	Acinar Adenocarcinoma with lepidic pattern. The most common mutations of the EGFR gene are not present	Solid Adenocarcinoma G3 The most common mutations of the EGFR gene are not present	Solid Aenocarcinoma with mucin The most common mutations of the EGFR gene are not present		

ID11	Adenokarzinom überwiegend lepidisch, partiell tubulär und kribriform (G2)	Adenokarzinom überwiegend solide, fokal mikropapillär (G3)	Kombiniertes großzelliges neuroendokrines Karzinom mit Adenokarzinom überwiegend solid (G3)		
ID 12	Adenocarcinom mixed type (acinär, lepidic) G2	Solides großzelliges Carcinom G3	Adenocarcinom mixed type (acinär, solide) G3		
ID 13					
ID14	invasives AdenoCa GII, papillär und cribriform 20%, 80 % Adenocarcinoma in situ	gering diff, solid u. foll, cribirf.. AdenoCa GIII	invasives gerind diff AdenoCa GIII, teils solid, azinär, teils enteral muzinbildend, teils komedoartig		
ID15	Adenocarcinoma with predominant lepidic pattern	Predominant solid pattern, no clear adeno or plano differentiation In this section, need more samples or immunohistochemistry for diagnosis.	Large cell neuroendocrine carcinoma		
ID16	Adenocarcinom präd. lepid mit acinären und papillären Anteilen, hoch bis mäßig differenziert	Adenocarcinom wenig differenziert, solid	Adenocarcinom mäßig diff. präd. solid, partiell kleinzellig		
ID18	predominantly lepidic invasive adenocarcinoma (75%), with acinary (approx. 15 %) and macro-/micropapillary growth (cca 10%)	necrotising NSCLC NOS, preferably adenocarcinoma, with predominance of solid growth, with clear and few spindle and giant cells (in the background of fibroelastosis and regressive changes).	NSCLC NOS (hepatoid variant of a solid adenocarcinoma ?)	In case Nr. 2 we would like to see IHC slides (at least p63, CK5/6, CK7, TTF1, etc) and mucin staining (acc. to HE morphology dg of adenocarcinoma would be preferred). In case Nr. 3 we also would like to see IHC slides (incl. CK5/6, CK7, CK20, TTF1, CDX2, CD30, AFP) and mucin staining for the diff. dg. Serum level of AFP ?	
ID19	Adenocarcinoma, predominant lepidic pattern (75% lepidic, 10% acinar, 10% solid/cribriform, 5% papillary)	Adenocarcinoma, predominant solid pattern (80% solid, 20% micropapillary)	Adenocarcinoma, predominant solid pattern (70% solid, 30% tubuloacinar)		