



December, 2013

RUN 32 consisted of a single FFPE tissue sample that was stained by IHC for HER2 and was scored 2+ by a cIQc affiliated pathologist.

Laboratories were requested to stain the slide as per their usual protocols for HER2 by ISH methodology and enter the results in the cIQc TMA Scorer website

Sites were requested to:

- Provide the average number of signals per nucleus (HER2 and CEP17)
- Report the sample to be either Amplified, Non Amplified, Equivocal or unsatisfactory for analysis
 - <1.8 = non-amplified
 - 1.8 – 2.2 = equivocal
 - 2.2 = amplified

Slides were sent to 27 laboratories and results were received from 22 sites. 21 sites reported the sample to be non-amplified, while one laboratory called the sample amplified (site 103). This site received a second slide and repeated the test and rescored the sample. It was reported non-amplified on the repeat. The discordance could potentially be attributed to background “noise” around the black SISH signals resulting in a high HER2 count.

Review your laboratories performance in Run 32 below.

Run 32 Results

Laboratories/Fields	102	103	103 rpt	105	106	109	111	112	114	115	116	123	125	137	138	162	175	181	186	194	197	202	207
Average # CEP17 signals	2.23	1.65	1.95	2.7	2.31	2.4	2.6	2.51	2.1	2.65		2.6	2.45	2.6	2.3	3.2	2.2	2.05	2.52	2.8	2.5	1.8	2.705
Average # HER2 signals	3.5	5.35	3.15	3.2	3.62	3.3	3.4	2.53	2.9	3.25		4.1	3.55	3.6	2.9	2.5	2.35	3.35	3.39	3.9	3.31	2.4	3.035
HER2/CEP17 Ratio	1.56	3.24	1.61	1.19	1.56	1.37	1.31	1.01	1.38	1.23	0.96	1.57	1.39	1.38	1.26	0.78	1.07	1.63	1.34	1.39	1.32	1.33	1.12



Run 32 Protocols

Laboratories/Fields	Average # Cep 17 signals	Average # HER2 signals	Amplification	Probe	Vendor	Instrument	Hybridization Time	Pretreatment Time	Post Hybridization Wash: Reagent, Temp, Time
102	2.23	3.5	Non-Amplified	HER2 DNA & CHR17	VENTANA	BENCHMARK XT	6' & 4'	8"	SSC 72DEG 24" & 59DEG 24"
103	1.65	5.35	Amplified	inform her2 dual ish probe cocktail	roche	Bench mark ultra	6hrs	protease 3 16 mins	buffer, 72 degrees .8 mins
103 repeat	1.95	3.15	Non-Amplified	inform her2 dual ish probe cocktail	roche	Bench mark ultra	6hrs	protease 3 16 mins	buffer, 72 degrees .8 mins
105	2.7	3.2	Non-Amplified	LSI HER-2/CEP 17	Path Vysion	manually	16-18 hours	50min	2xSSC0.3%NP40, 72C, 2min
106	2.31	3.62	Non-Amplified	Inform Dual ISH HER2	Roche Ventana	Bench Mark Ultra	6 hours	CC2-12 min, P3-8 min	SSC@72C for 24 min
109	2.4	3.3	Non-Amplified	inform her2/inform chr17	VENTANA	BENCHMARK XT	6 HOURS	CC2 EXTENDED	SILVER WASH 11, 72 DEGREES, 24 MIN
111	2.6	3.4	Non-Amplified	VYSIS	ABBOTT	Vysis 2002T	16h	120min	2xSSC 0.3%NP-40, 73C, 2 min
112	2.51	2.53	Non-Amplified	Chrom17/HER2 DNA	Ventana/Roche	ultraBenchMark	3hrs Chr17/ 6hrs Her2	reaction buffer @ 86c 24min	SilverII 59C & 70C 24min
114	2.1	2.9	Non-Amplified	Her-2 DNA Probe Kit	Vysis Path Vysion	Vysis Hybrite	16 hours	95C/40min then 20min at RT	20XSSC buffer/73C/2min
115	2.65	3.25	Non-Amplified	path vision Her 2 Dna	path vision	path vision	15 hours	2 min	...
116	ratio 0.96	ratio 0.96	Non-Amplified	HER2 DNA PROBE, Chr17 Probe	Ventana Roche	Benchmark Xt - Ultra	8 hres	ISH Prot@ase 3: 12 min	Her2 : 72Å °C: 24 min Chr17; 59Å °C 24min
123	2.6	4.1	Non-Amplified	Her2/neu-D17Z1	Abbot Labs	Thermotron	denature@72C for 5 min, hybridize overnight	2hr sodium citrate, 15 min pepsin	0.4xSSC/NP40, 72C, 2 min. 2XSSC0.1%NP40, RT, 1 Min
125	2.45	3.55	Non-Amplified	Inform Her2 DNA probe	Ventana	Ventana Benchmark Ultra	6 hours	8 min (ISH+protease 3)	SSC, 69 degrees, 8 min x 3
137	2.6	3.6	Non-Amplified	PathVysion HER-2/neu	Abbott Molecular	Thermobrite	18 hours	2.25 hours	0.4xSSC0.3% NP40, 72C, 2 min: 2xSSC0.1%NP40, RT, 1 min
138	2.3	2.9	Non-Amplified	HER2/CEN-17 Probe Mix	Dako	Dako Hybridizer	20 hours	10 minutes	Stringent Wash Buffer, 65C, 10 min
162	3.2	2.5	Non-Amplified	ZytoLight spec HER2/CEN17 dual color probe	Zytovision	non	10 min 75 dg	10 min 99 dg	5 min 65 dg
175	2.2	2.35	Non-Amplified	Inform Ventana	Ventana	Benchmark XT	4	16	Pinse Buffer, 44, 4 mins
181	2.05	3.35	Non-Amplified	Inform Her2 Dual ISH DNA probe CKTL	Ventana/Roch	Ventana Bench Mark XT	6 hours	56 minutes	SSC buffer @ 72 deg. for 16 minutes
186	2.52	3.39	Non-Amplified	Pathvysion Her2 neu	Vysis (Abbott)	manual	16 hour	20 min	73 +/- 2 C, 2.5 min
194	2.8	3.9	Non-Amplified	D17Z1/ERBB2	Abbott	Thermobrite	18-20 hours	NA	2xSSC0.3% NP40(for both w ashes); Wash#1 @ RoomTemp until coverslip falls
197	2.5	3.31	Non-Amplified	PathVysion HER2/cep17	Abbott/Inter Medico	Scored on Zeiss Axiomager Z2	5 min denaturation, 18 hours hyb time	22 min 1 N HCl, 35 min NaSCN	2 x SSC0.3% Igepal at 73C for 2 min
202	1.8	2.4	Non-Amplified	vysis 30-161060	abbott	hybrite	5 minutes	18hours	room temperature 2X SSC0.3%/NP40 for 2 minutes
207	2.705	3.035	Non-Amplified						

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Run 32 Results

