



Summary report for cIQc Run 51:

HER2 ISH Run 51 consisted of two FFPE cell line samples (one non-amplified and one amplified) for HER2 ISH (FISH, CISH, SISH) on a single slide. Laboratories were requested to stain the slide as per their usual protocol. Each participant was asked to provide the average number of signals per nucleus (HER2 and CEP17) and report final ISH results online as Non-amplified, Equivocal or Amplified according to the following HER2/CEP17 ratio categories:

< 1.8 = Negative (N)

1.8 – 2.2 = Equivocal (E)

> 2.2 = Amplified (A)

Results were received from 16 participants as summarized below. Data from Lab 116 has not been included in the results section of this report as quantitative results were not reported.

These were two challenging cores selected because they were both laying on the edge of equivocal. Labs performed well with no false positives and a single false negative (Lab 115) which was actually border-line.

Run 51 Signal Count

Labs/Fields	106	109	111	112	113	115	116	123	125	137	138	149	170	189	197	207	211
Sample 1 - Avg # Cep17 signals	2.67	2.2	2.6	1.65	we do CEP17 separately	2.28	na	7.1	2.4	2.82	2.6	2.4	2.55	2.8	2.91	2.1	2.7
Sample 1 - Avg # HER2 signals	5.18	5.9	5.7	3.81	2.57	4.07	na	2.9	7.85	7.11	5.7	5.4	5.1	4.9	5.51	4.8	6.3
Sample 2 - Avg # Cep17 signals	2.68	0	3	1.81	We do Cep17 separately	2.88	na	2.5	3.25	3.2	2	3.2	2.45	NA	2.45	2.35	3.8
Sample 2 - Avg # HER2 signals	3.22	0	3.2	2.62	4.23	3.28	na	2.2	4.35	4	2.1	6.5	2.8	NA	3.35	2.23	6.1

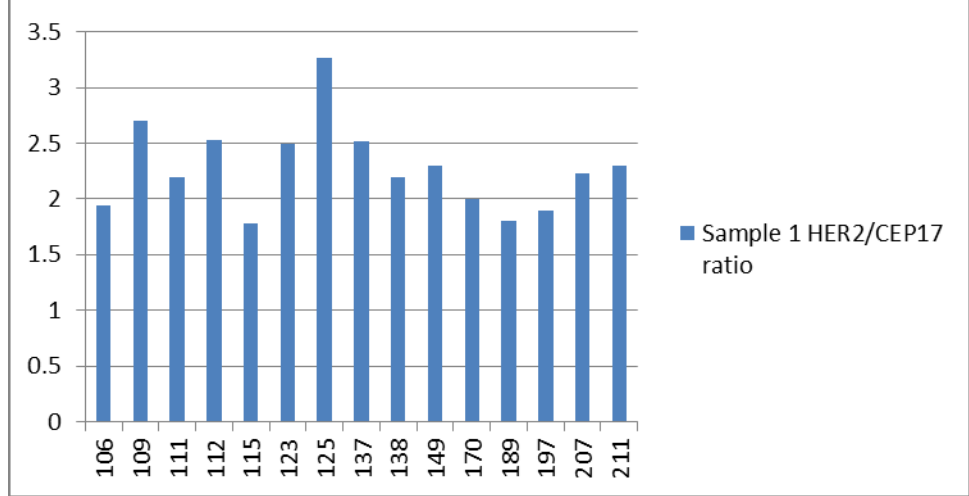


building towards

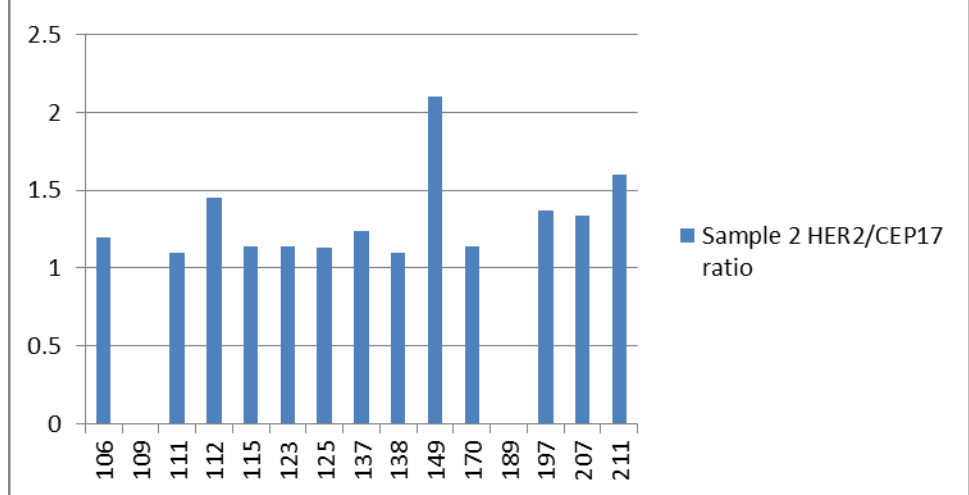
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Sample 1 HER2/CEP17 ratio



Sample 2 HER2/CEP17 ratio





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ER2 ISH Protocols

Labs/ Fields	Supplier/Vendor	Probe	Instrument	If manual protocol, specify method (input NA if not manual)	Denaturation time/temp	Hybridization time/temp	Pre-treatment reagent/time/temp	Proteolytic digestion reagent/time/temp	Post-hybridization wash time/temp	
106	Intermedico/Vysis	Pathvysion	ThermoBrite	NA	5 minutes @73C	18 hours@37C	citrate buffer,2 hrs@81C	pepsin,2.5 min@37C	0.3%NP40in2XSSC,6m in@75C	
109	ventana	Her2/CHR17 DUAL ISH	BENCHMARK ULTRAVIEW	NA	8 MIN AT 80 DEGREES	6 HOURS	CC2 12 MIN AT 36DEG	ISH PROTEASE 3 - 20MIN	3 WASHES AT 72 DEG	
111	Intermedico	Pathvysion Her2 DNA Kit	VP2000 and Hybrite	na	5 min/ 73C	16 hrs/ 37C	10mM sodium citrate/ 100 min/ 80C	pepsin/ 20 min/ 37C	2 min/ 72C	
112	Leica HER2 FISH SYSTEM	LSI HER2/CEP17 PROBE	BOND III	NA	10 minutes @ 95C	15 hours @ 37C	ER1 (Leica) pH 6.0 40 minutes @ 97C	BOND ENZYME CONCENTRATE 2 1:300 25minutes @ 37C	POST HYBRIDIZATION WASH 2 (LEICA) 4 minutes @ 48C	
113	Roche	Her2 DNA Probe	Benchmark XT	NA	12/95 Celcius	368/52Celcius	CC2/28/C	ISH Protease 3/8/37C	Silver wash 24/72C	
115	PathVysion	Her DNA probe kit (Her2/cep17)	Thermobrite	NA	2min 73C	15 hours 37C	45min 80C	20min 37C	8min RT, 8min 37C	
116	Vysis	HER2	DAKO	na	na	na	na	na	na	
123	Abbott Labs/Vysis	Pathvysion	Hybrite	n/a	74 C / 5 min	37 C / overnight	NaCitrate/80C/2hr	pepsin/15min/37 C	0.4xSSC/0.3%NPO40/ 2 min/72 C	
125	Roche	Roche HER2 DNA probe	Ventana Ultra	NA	NA	6 hours, 50 degrees	NA	roche ISH Prot 3, 8 min	3x SSC, 8 min	
137	Abbott	Pathvysion	Thermobrite	FISH	5min/74c	overnight/37c	sodium citrate buffer/2hrs/80c	pepsin/15min/37c	72c 2min, RT 1min.	
138	Dako	Dako IQFISH Probe Mix (HER2 DNA probe and CEN-17 PNA probe)	Dako Hybridizer	Semi-manual		66	45	97	37	63
149	Abbott	PathVysion	Thermobrite	Only denaturation performed on thermobrite. Remainder of protocol is performed manually.	73C for 6 min	37C overnight	8% sodium thiocyanate at 80C for 8 minutes initially and changed if tissue is either over or under digested.	0.2N HCl + 0.2g pepsin ta 37C for 6 minutes and then adjusted is tissue is either over or under digested.	2XSSC+0.3% NP-40 at 72C for 2 minutes	
170	DAKO	Her2 IQFISH pharmDX	none	DAKO protocol	10min/66	120min/45	DAKO reagent/10min+15min/ 98+cooling	Pepsin/25min/37	10min/63	
189	Ventana	HER2 Dual Probe	BM XT	NA	20/95Å°	6hr/72Å°	CC2/8/12/8/86Å°	P3/16 min/36Å°	8 min./72Å°	
197	InterMedico (Abbott)	HER 2/cep 17 dual colour	Thermobrite Elite	n/a	5 min at 75Å°C	18 hours at 37Å°C	0.1N HCl: 22 min at RT; 1M NaSCN for 40 min at 80Å°C	Pepsin for 13 min at 37Å°C	2xSSC/0.3% Igepal: 2 min at 72Å°C; 1XPBS rinse for 30 sec at RT	
207	Abbot	PathVysion Her2 DNA kit	manual	NA	115 degrees -2 min	18 hours/37 degrees	NA	Pepsin- 2 min	2min-73 degrees	
211	Abbott Molecular	PathVysion HER-2 DNA Probe Kit II	ThermoBrite	Vysis Paraffin Pretreatment Kit I	5 min / 75C	20 hours / 37C	Vysis Paraffin Pretreatment Kit I Pretreatment Solution / 30 min / 80C	Vysis Paraffin Pretreatment Kit I Protease in Buffer / 20- 40 min / 37C	2 min / 72C	