



building towards

CIQC

canadian Immunohistochemistry Quality control

November 2016

Summary Report for Run 65 HER2 ISH

CIQC Run 65 HER2 ISH consisted of two FFPE engineered cell line samples (one non-amplified and one amplified) for HER2 ISH (FISH, CISH, SISH) on a single slide, provided by *Horizon Discovery* (Cambridge UK). Participating laboratories stained their slide as per their usual protocol for HER2 ISH and reported the average number of signals per nucleus (HER2 and CEP17), as well as final ISH results as Non-amplified, Equivocal or Amplified according to the following HER2/CEP17 ratio categories:

- < 1.8 = Non-amplified (N)
- 1.8 – 2.2 = Equivocal (E)
- > 2.2 = Amplified (A)

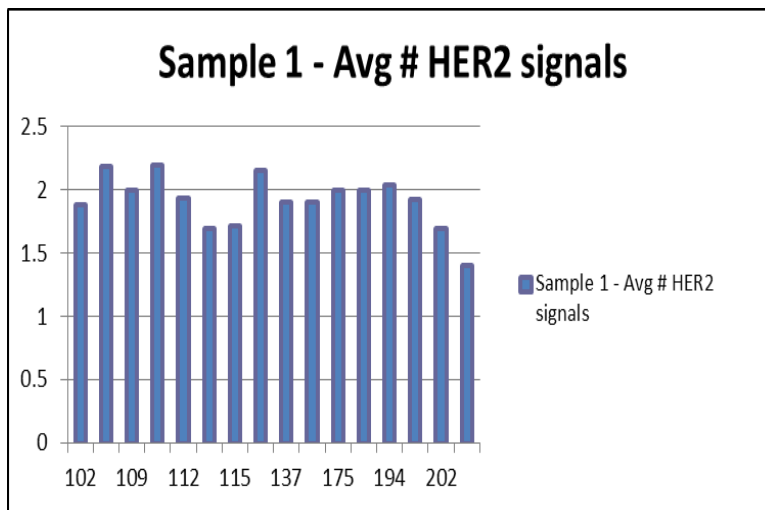
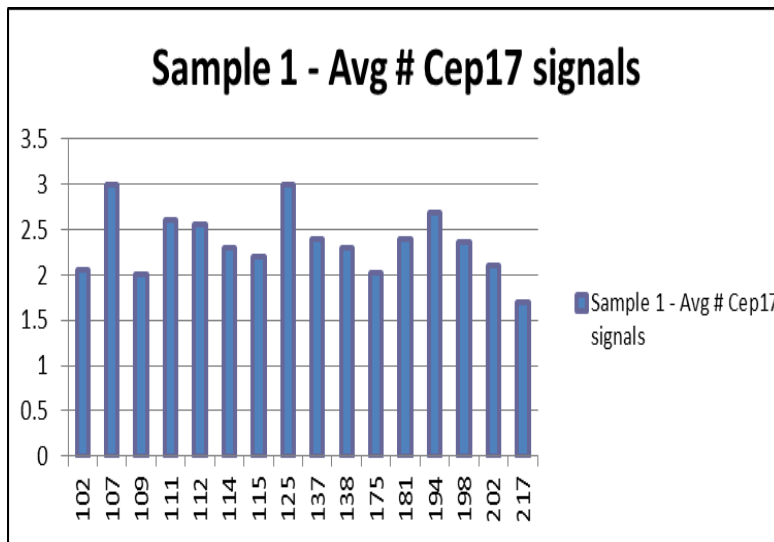
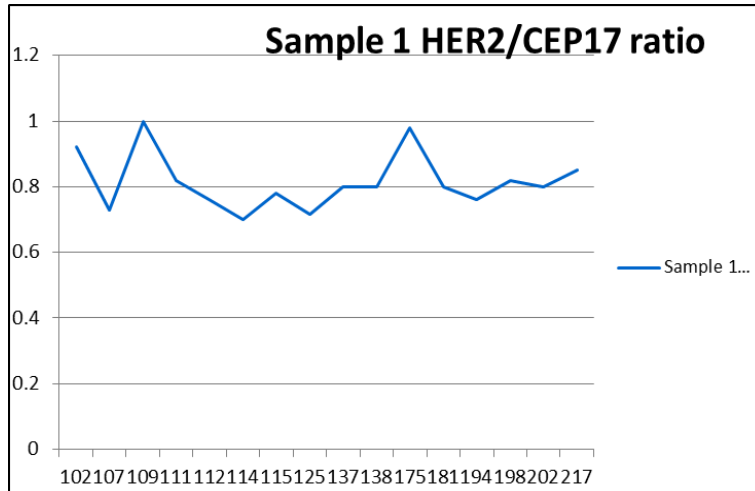
Results were received from 16 participants as summarized below. Counts were mostly consistent in both samples. Due to the high number of HER2 signals in Sample 2 it should be noted that some labs estimated the counts, so the Cep17 signal count is a better indication of accuracy.

Table 1. Interpretation of results by Run 65 participants

Lab/ Sample	102	107	109	111	112	114	115	125	137	138	175	181	194	198	202	217	Status
1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Non-amplified
2	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Amplified

Table 2. Signal counts by Run 65 participants

	102	107	109	111	112	114	115	125	137	138	175	181	194	198	202	217
Sample 1 - Avg # HER2 signals	1.88	2.19	2	2.2	1.94	1.7	1.72	2.15	1.9	1.9	2	2	2.04	1.93	1.7	1.4
Sample 1 - Avg # Cep17 signals	2.05	3	2	2.6	2.56	2.3	2.2	3	2.4	2.3	2.02	2.4	2.69	2.36	2.1	1.7
Sample 1 HER2/CEP17 ratio	0.92	0.73	1	0.82	0.76	0.7	0.78	0.717	0.8	0.8	0.98	0.8	0.76	0.82	0.8	0.85
Sample 2 - Avg # HER2 signals	31.1	24.05	12	29.5	30	18.7	27.31	29.15	25	22.4	27.6	14.4	37.03	>20 signals	25.4	20.8
Sample 2 - Avg # Cep17 signals	3.55	3.2	3	3.5	3.32	2.7	2.6	3.2	3.7	3.6	2.9	2.6	3.57	3.35	4.4	2.4
Sample 2 HER2/CEP17 ratio	8.76	7.52	4	8.34	9.26	6.9	10.51	9.109	6.8	6.2	9.5	5.6	10.39	>5.97	5.8	8.49



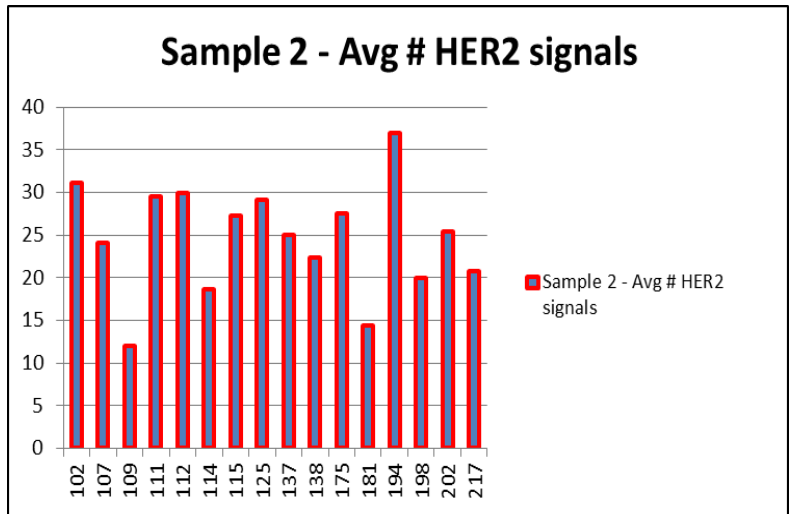
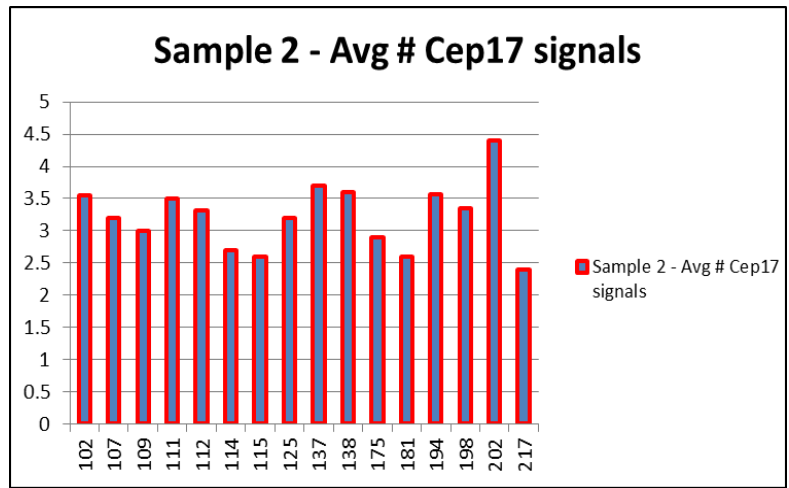
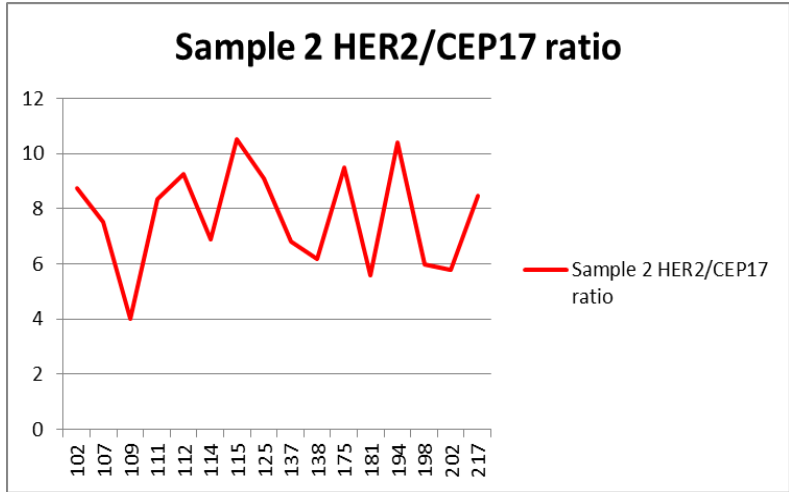


Table 3. Reported protocols

Lab/Field	Supplier/Vendor	Probe	Instrument	If manual protocol, specify method (use N/A if not manual)	Denaturation time/temp	Hybridization time/temp	Pre-treatment reagent/time/temp	Proteolytic digestion reagent/time/temp	Post-hybridization wash time/temp
102	Roche	HER2 DUAL ISH PROBE	BENCHMARK XT	N/A	20" @ 80	360" @ 44	CC2 32" @ 90	ISH PROTEASE 3 8" @ 37	8" @ 72
107	Abbott/Vysis	PathVysion HER2 dual colour	Thermobrite Elite Automated FISH processor	n/a	5 min/75C	overnight/37C	HCl/22min/RT; NaSCN40min/80C	Pepsin/13min/37C	2xSSC/0.3%Igepal/2min/73C; 1xPBS rinse (30sec-1min)
109	roche	INFORM HER2 DUAL ISH	BENCHMARK ULTRA	N/A	8 MIN@80DEG C	6 hrs	CC2 12 MIN @ 82 DEG C	PROTEASE 3 20 MIN AT 36 DEG C	SSC @ 72 DEG C x3 for 8 min
111	Intermedico	PathVysion HER2 DNA Probe kit	VP2000	NA	5 min/ 73C	16 hrs/ 37C	10mM sodium citrate/ 100 mins/ 80C	pepsin/ 20 mins/ 37C	2 mins/ 72C
112	Leica HER2 FISH System	LSI Her2/Cep17 Probe	BOND III	NA	10 minutes @95C	15 hours @ 37C	ER1 (Leica) pH6.0 40 minutes @ 97C	Bond enzyme concentrate 2 1:300 25 minutes @ 37C	Post Hybridization Wash 2 (Leica) 4 minutes @ 48C
114	PathVysion	LSI HER-2/neu (ERBB2)	Vysis HYbrite	Manual	5min/73C	16hrs/37C	40min/95C, then 20min cooled to room temp	250ug/mL Pepsin in 0.2N HCL/25min/RT	2min/73C
115	PathVysion	Her2-DNA Probe Vysis LSI Her2/neu Cep17	ThermoBrite hybridizer	Manual	2 min at 73°C	15 hours at 37°C	(1M NaSCN) 40 minutes at 80Å°C	(0.2N HCL + pepsin)20 minutes at 37Å°C	(2x ssc/0.3%NP40)8 minutes room temp and 8 minutes at 72Å°C
125	Roche/Ventana	Inform Her2 probe	Ventana Benchmark Ultra	N/A	N/a	50 degreeec C, 6 hours	CC1, 4min	ISH-Protease 3	SSC, 69 degrees Celsius, 8minx3
137	Abbott	Pathvysion	Thermobrite	FISH	5min/74c	overnight/37c	sodium citrate/2hr/80c	pepsin/15min/37c	72c 2min, RT 1min
138	Dako	HER2/CEN-17 IQISH Probe Mix	Dako Hybridizer	HER2 IQFISH pharmDx Kit Recommended Protocol	10 min/66C	120 min/45C	(HER2 IQFISH pharmDx,φ)Pre-Treatment Solution/10min/98C	Pepsin/3min/37C	(HER2 IQFISH pharmDx,φ)Stringent Wash/10min/63C
175	Roche diagnostics	HER2 DNP CHR17 D19	BenchMark Ultra	N/A	8 minutes /80 C	6 hrs/72 C	cc2/ 12 minutes /82 C	ISH protease 3/20 minutes/36 C	SSC/30 minutes/72 C
181	Ventana/Roche	Inform Her2 Dual ISH DNA probe CKTL.	Ventana Benchmark XT	N/A	20 minutes at 80 Deg. C	6 hours at 44 Deg. C	CC2 for 48 minutes at 90 Deg. C	Protease 3 for 4 minutes at 37 Deg. C	SSC for 16 minutes at 72 Deg. C.
194	Abbott	Pathvysion her2 DNA Probe kit	Thermobrite	NA	5min/74C	24 hrs/37C	Sodium citrate/2hrs/80C	pepsin/20min/37C	2 min/72C
198	InterMedico/Abbott	PathVysion HER2 DNA Probe Kit	done manually	manual pretreatment then codenaturization/hybridization done on ThermoBrite	73 deg 5 min	18 hrs 37 deg	0.2N HCL 30 min then 30 min in 1 M Sodium thiocyanate at 80deg C.	pepsin 37 deg 18 min	74 deg 2 min
202	Abbott	her2 dna probe	DAKO autostainer	na	5 min/73C	18 hrs/37C	30min/80C	5 min/125C citrateph 6.0	2 min/73C
217	Roche Ventana	HER2/CH17 dual BRISH	Ventana Benchmark Ultra	N/A	16 min	360 min	UltraCC2/120min/100C	ISH protease 3/16min/36C	24min/74C

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