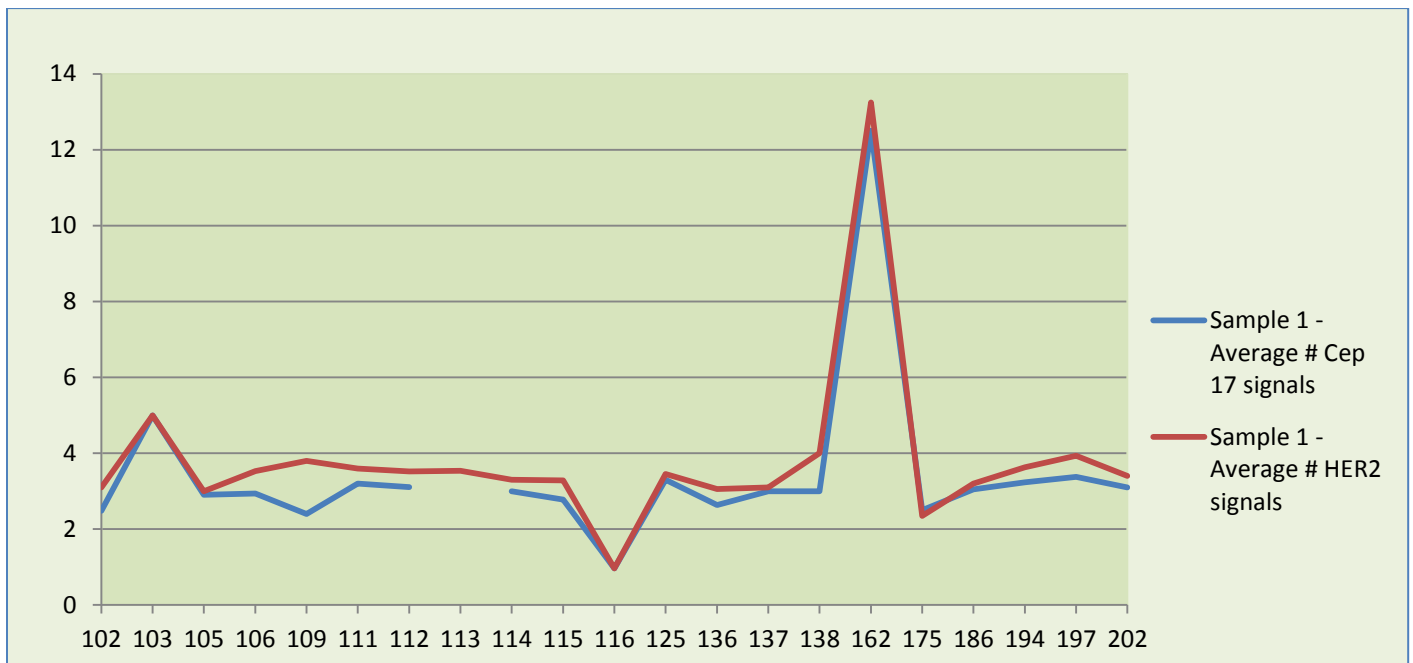
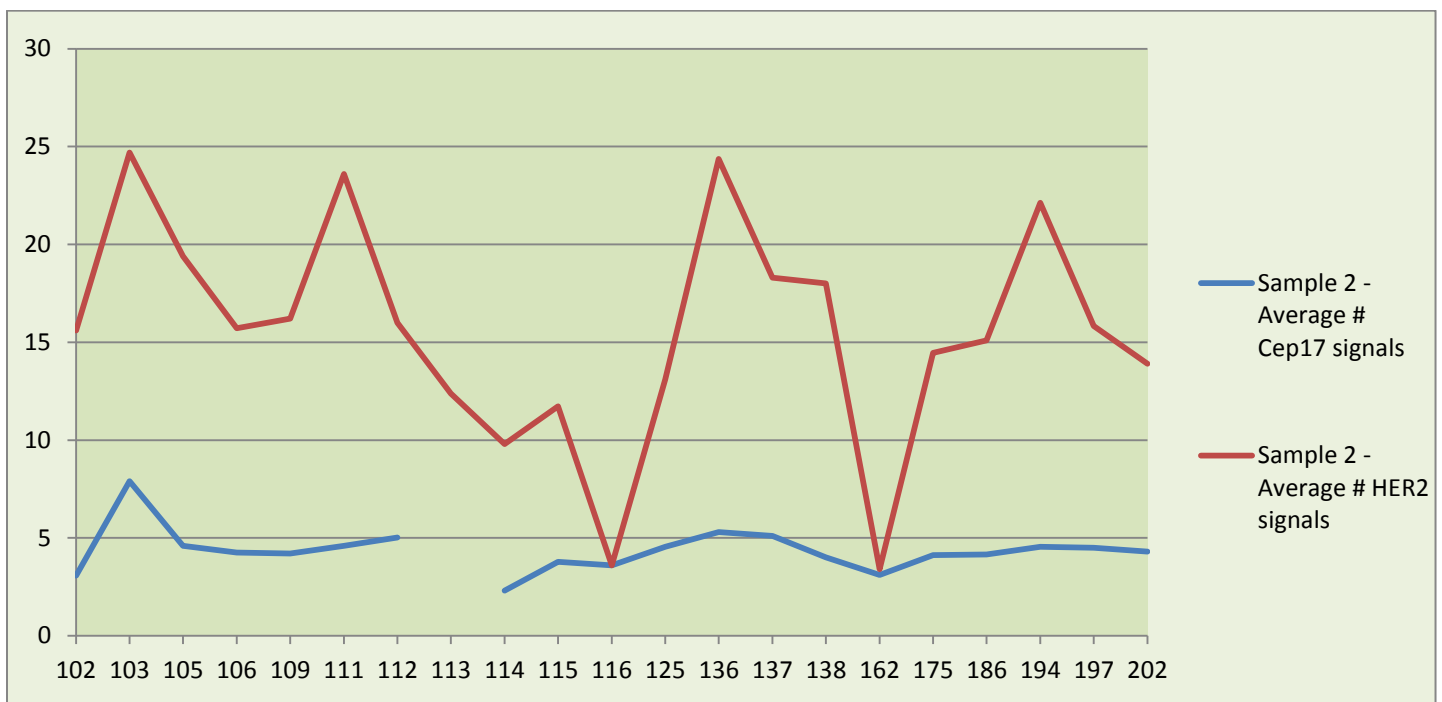


31 sets of slides were sent to registered laboratories. cIQc received results from 21 laboratories. This was the first time we have run a FFPE Cell Line for a HER2 ISH. The cell line was profiled using RT-qPCR and IHC for HER2.

Sample 1. Considered Non Amplified 20/20 laboratories. . Sample 1 had low levels of ERBB2 in the RT-qPCR analysis and was scored 1+ / 2+ with IHC. There were low numbers of signals which were easily counted which is reflected in the graph where Cep17 and HER2 signals parallel each other. Lab 162 appears to be an outlier with a high count of both signals.



Sample 2 Considered Amplified 20/21 laboratories Sample 2 had high levels of ERBB2 with RT-qPCR analysis and scored 3+ on IHC. There were a large number of HER2 signals which were difficult to count accurately which is reflected in the erratic nature of the graph.



## RAW DATA

Laboratories/Fields	Sample 1 - Average # Cep 17 signals	Sample 1 - Average # HER2 signals	Sample 2 - Average # Cep17 signals	Sample 2 - Average # HER2 signals
102	2.48	3.1	3.08	10
103	5	5	7.9	24.7
105	2.9	3	4.6	19.4
106	2.94	3.53	4.25	15.72
109	2.4	3.8	4.2	16.2
111	3.2	3.6	4.6	23.6
112	3.11	3.52	5.02	16
113		3.54		12.38
114	3	3.3	2.3	9.8
115	2.78	3.28	3.77	11.73
116	0.96	0.96	3.59	3.59
125	3.3	3.45	4.55	13.1
136	2.63	3.06	5.3	24.37
137	3	3.1	5.1	18.3
138	3	4	4	18
162	12.5	13.25	3.1	3.4
175	2.5	2.35	4.12	14.45
186	3.05	3.2	4.15	15.1
194	3.23	3.63	4.55	22.13
197	3.38	3.93	15.83	4.45
202	3.1	3.4	4.3	13.9

# Observations and Stain Protocols

Laboratories/Cores	Sample 1	Sample 2	Probe	Vendor	Instrument	Hybrization Time	Pretreatment Time	Post Hybridization Wash: Reagent, Temp, Time
102	Non Amplified	Amplified	HER2 DNA & Chr. 17	Ventana	BenchmarkXT	6 hours HER2/4 hours Chr.17	32" RB	SSC 24" 72C
103	Non Amplified	Amplified	as per kit	Ventana	Ultra bench mark	6 hours	16 mins	RB 72 deg c 8 min
105	Non Amplified	Amplified	Pathvysion vysis HER2	Abbott Molecular	na	18 hours	50 min	2Xssc/0.3% NP-40, 72C for 2 min.
106	Non Amplified	Amplified	Inform HER2 Dual ISH DNA Probe Cocktail	Ventana/Roche	Bench Mark Ultra	6 hours @44C	CC2+Protease for 40 minutes	2XSSC @72C for 8 minutes
108	No Result	provided						
109	Non Amplified	Amplified	INFORM HER2 DNA+ CHROMOSOME 17	ventana	benchmark XT	6 hours	cc2 extended(32min@90c)	ultraview silver wash 2,72 degrees C,24min
111	Non Amplified	Amplified	HER-2/neu - orange, CEP17 - green	Vysis, Abbott	Vysis VP2000 Processor, Vysis HYBrite	18 hours	100 min	2x SSC + 0.3% NP-40, pH 7.2, 75C, 2 min; 2x SSC, pH 7.3, RT, 1 min
112	Non Amplified	Amplified	Chrom17/Her2 DNA	Ventana	ultra BenchMark	3hrs C17 / 6hrs Her2	Reaction Buffer	SilverI 59deg C & 70 deg C for 24 minutes
113	Non Amplified	Amplified	HER2 DNA probe	Ventana/Roche	Benchmark XT	6 hours	36 minutes	Silver wash, 72 Deg C for 8 min each rinse - 3 rinses
114	Non Amplified	Amplified	LSI HER-2/neu Spectrum Orange/CEP17 Spectrum Green DNA Probe	Vysis PathVysion	Vysis HYBrite	16 hours at 37C	40min@95C + 20min@RT	SSC+NP40 Buffer (pH7.0-7.5), 73C, 2.0min
115	Non Amplified	Amplified	Har-2 new	PathVision	thermobrite	15 hours	30 min	8 min 72C wash buffer
116	Non Amplified	Amplified	LSI Her-2/neu SO/CEP 17 SG probe	PathVysion ( Vysis)	Hybridizer , Dako	24 hre	16-20 min	NP-40 0.3%, 72A°C, 4 min
118	No Result	provided						
123	No Result	provided						
125	Non Amplified	Amplified	Her2 DNA probe, Chr17 probe	Roche	Ventana Benchmark ultra	6 hrs	8 min	Roche SSC, 69 deg C, 8 min X3
136	Non Amplified	Amplified	vysis dual probe	Vysis	manual (hybrite)	overnight	vysis instructions	vysis instructions
137	Non Amplified	Amplified	Her2-neu (ERBB2)	Abbott	Thermobrite	17.5 hours	10 min + 18 min	Abbott paraffin pretreatment kit II (32-801210), 73 C, 2 min.
138	Non Amplified	Amplified	HER2/CEN-17 Probe Mix	Dako	Bench/Dako Hybridizer	20 hours	10 min @ 98 C	Stringent Wash; 10 min @ 65 C
152	No Result	provided						
160	No Result	provided						
162	Non Amplified	Non Amplified	ZytoLight spec HER2/CEN17 dual color probe	Zytovision			10 min	65C 5 min
166	No Result	provided						
171	No Result	provided						
175	Non Amplified	Amplified	Ish	Ventana	Benchmark	4 min	16 mins	Rinse Buffer, 44, 4 mins
181	No Result	provided						
186	Non Amplified	Amplified	Pathvysion Her 2 FISH probe	Abbott/ Vysis	Manual	16 hour	20 min	73+/- 2 degree C, 2.5 min
193	No Result	provided						
194	Non Amplified	Amplified	LSI HER-2/neu (SO) / CEP17 (SG)	Path Vysion	Thermobrite	18-20 hrs	n/a	40 (for both washes); Wash#1 @ Room Temp until coverslip falls off; Wash#2 at 72C
195	No Result	provided						
197	Non Amplified	Amplified	PathVysion Her-2 Kit	Abbott	Zeiss	18 hours	22 min HCl, 35min NaSCN	2xSSC/0.3%Igepal, 73,2 min
202	Non Amplified	Amplified	Path Vysion Her2 DNA probe kit (06N46-030 order #)	Vysis division Abbot Molecular	Thermobrite	5 minutes @ 73 degrees	80 degrees for 30 minutes	2x SSC @73 degrees x2 minutes
RT-qPCR ERBB2	2.89	7.29						