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Report for cIQc Run 94: HER2 ISH (February 2019)

cIQc Run 94 HER2 ISH consisted of three FFPE engineered cell line controls on a single slide. These are histoid-type controls (3D Reference Standard Controls) contain cancer cells grown in a matrix of stromal cells. More information on the cell lines is available at statlab.com. IHC staining of the cores shows amplified, equivocal and negative staining. Samples used in this block have been formalin-fixed and processed through alcohol and xylene into paraffin wax in the usual manner. If you do not use the same tissue processing in your laboratory, the results obtained with this test may or may not be representative of your laboratory performance.

HER2 IHC

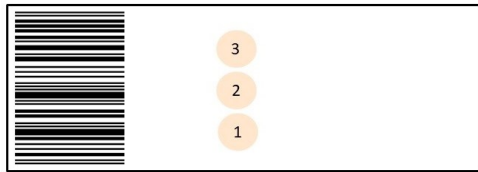
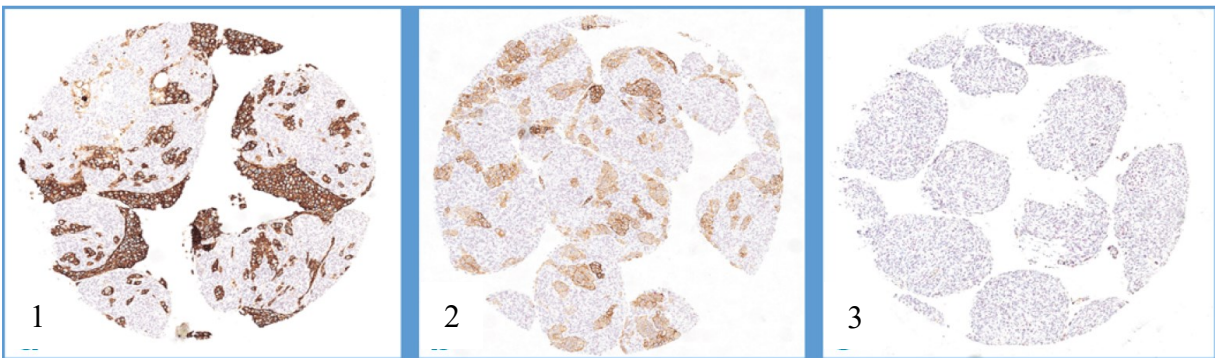


Image not to scale

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)

Lab/ Core	102	107	109	111	114	115	120	123	137	138	181	189	194	198	202	211	R1
1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Amplified
2	E	A	A	E	N	E	N	N	E	E	A	N	N	E	N	E	Truly Equivocal
3	N	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Negative

A Amplified **E** Equivocal **N** Negative



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As mentioned above, the 3D Reference Standard Control samples used in this challenge comprised of a mix of cell types, which caused some problems for counting.

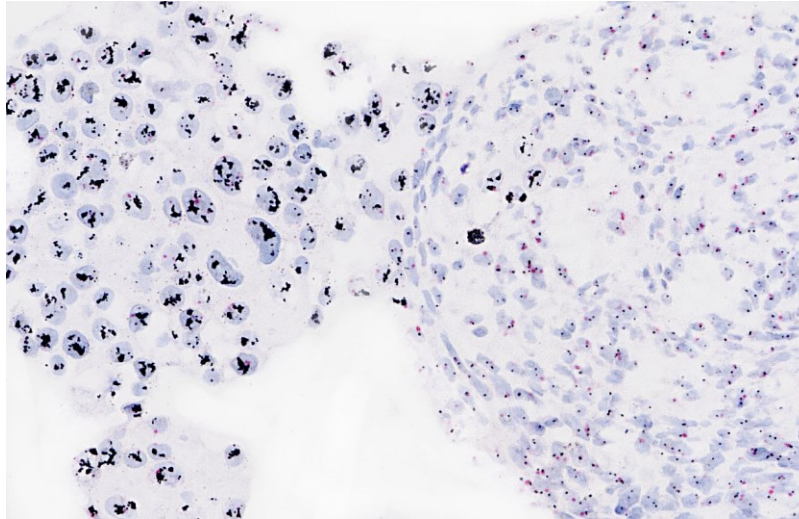
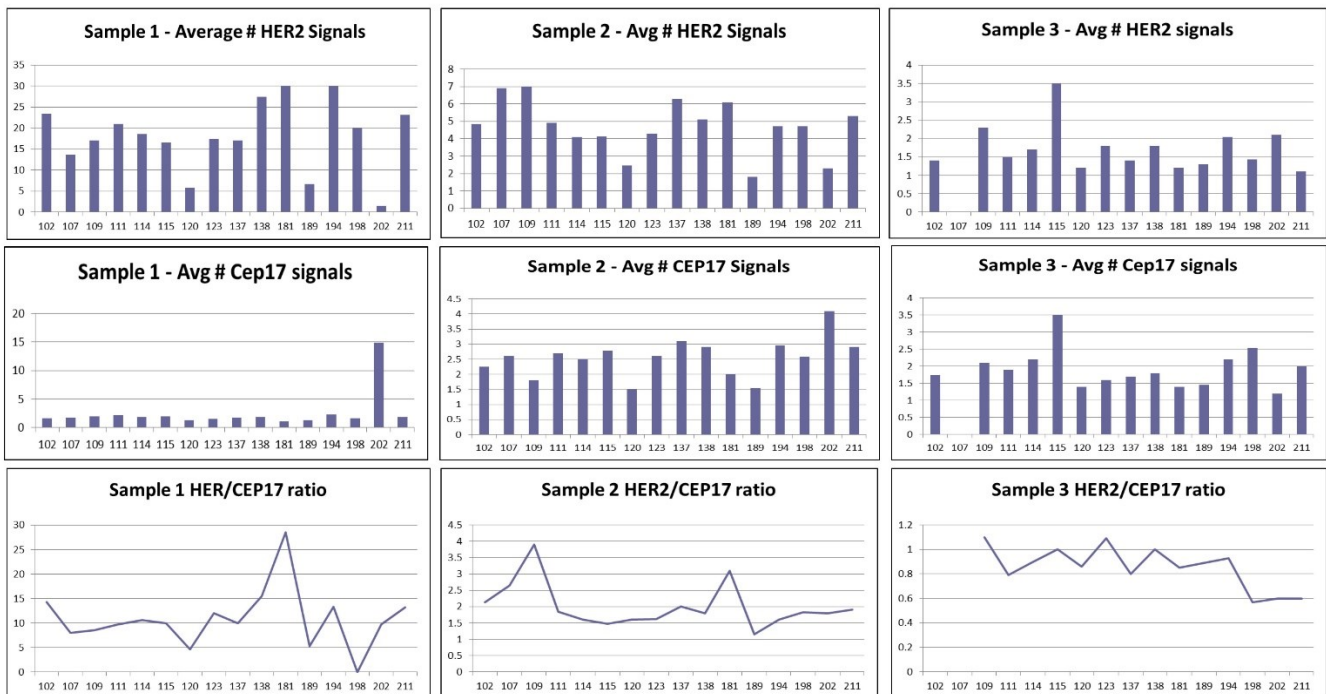


Figure 1. Core 1 stained with Ventana Dual ISH, showing the distinct cell populations.

NOTE: 2018 CAP/ASCO HER2 guidelines require the combined interpretation of the IHC and ISH results in certain circumstances (Wolff AC, Hammond ME, Allison KH, Harvey BE, Mangu PB, Bartlett JM, Bilous M, Ellis IO, Fitzgibbons P, Hanna W, Jenkins RB. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. Archives of pathology & laboratory medicine. 2018 May 30;142(11):1364-82.).

Reported HER2 counts, CEP17 counts and HER2/CEP17 ratios are summarized below:





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Reported Protocols:

Lab ID	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	Ventana (Roche)	Inform Her2 Dual ISH DNA Probe	Ventana Benchmark XT	NA	20"/80	360"/44	CC2 32"/90	ISH Protease 3 8"/37	8"/72
107	PathVysion	HER-2 DNA Probe Kit II	N/A	Manual	75oC/5min	37oC 16-18hrs (overnight)	HCL for 22 min, RT; NaSCN 40 min, 80oC	Pepsin/13min/37oC	2min/73oC
109	ROCHE	HER2/CHR 17	BENCHMARK ULTRA	N/A	8 MIN AT 80 DEGREES	6 HOURS	CC2 12 MIN AT 86	PROTEASE 3 20 MIN AT 36 DEGREES	24 MIN 72 DEGREES
111	Intermedico	PathVysion HER2	VP 2000	NA	5 min/ 73C	16 hrs/ 37C	10 mM sodium citrate/ 100 min/ 80C	pepsin/ 20 min/ 37C	2 min/ 72C + 1 min/ 4C
114	Dako	Her2 IQ FISH pharm Dx	Dako Omnis	N/A	10/66	75/45	ISH Pretreatment Soln(Dako Omnis)/15/97	ISH Pepsin(Dako Omnis)/17/32	10/61
115	PathVysion / Intermedico	Her2/Cep17	Hybridizer	FISH	2 mins @ 73 Degrees	15 Hours @ 37 Degrees	1M NaScN / 40 Minutes / 80 Degrees	pepsin/ 20 mins @ 37 Degrees	8 Mins @ rt & 8 Mins @ 72 Degrees
120	Roche - Ventana	INFORM HER2 Dual ISH DNA Probe cocktail	Benchmark ULTRA	N/A	8 min / 80°C	6 hrs / 80°C	CC2 / 12 min / 82°C	ISH-PROTEASE 3 / 20min / 80°C	?? / 72°C
123	Abbott Molecular	Her-2	ThermoBrite	FISH	5min/74Â°C	12hrs/37°C	Sodium citrate/80Â°C/2hrs	Pepsin/20min/37Â°C	72Â°C
137	Abbott Molecular	Pathvysion kit	Thermobrite	ours	5min/74c	overnight/37c	sodium citrate/2hrs/80c	HCl pepsin/15min/37c	2min@72c, 1min@room temp.
138	Agilent Dako	HER2/CEN-17 IQISH Probe Mix pharmDX	Omnis	N/A	10 min / 66C	75 min / 45C	ISH Pretreatment Solution / 15 min / 97C	ISH Pepsin / 20 min / 32C	10 min / 61C
181	Ventana/Roche	InformHer2 Dual ISH DNA probe CKTL	Venana Benchmark XT	N/A	20 minutes @ 80 Deg c	6 hours @ 33 Deg c	CC2 for 48 minutes @ 90 Deg c	Protease 3 for 4 minutes @ 37 Deg c	24 minutes @ 72 Deg c
189	Roche/Ventana	HER2 Dual ISH Cocktail	BenchMark ULTRA	N/A	8 min. @ 94Â°C	6 hrs.	CC2 Standard	P3 for 20 min.	Stringency Wash @ 72°C
194	Abbott Molecular	Pathvysion Her-2 DNA probe kit	Thermobrite	NA	5min/74C	overnight 37C	10mM Sodium Citrate 2.5 hours/80C	Pepsin in HCl Sol'n 20 min/37C	1. 2XSSC/0.3%NP40 until coverslip falls offat room T 2. 2XSSC/0.3%NP40 2min/72C
198	PathVysion/Abbot/Inter Medico	Dual LSI HER2/neu spectrum orange/CEP 17 spectrum green	Use ThermoBrite for denature and hyb- rest of method done manually	Developed in house- dewax, pretreat with 1M Sodium thiocyanate, digest with Pepsin, dehydrate, add probe coverslip and add to ThermoBrite.	73 deg / 5 min	37 deg / 18 hrs	1M Sodium thiocyanate/ 80 deg/ 30 min	Pepsin/ 37 deg/ 18 min	2 min/ 74 deg
202	Abbot	VYsis Her2 DNA probe	Hybrite	n/a	5 min 73C	18 hrs/ 37C	30 min /80C	5 min 124C	2 min/74C
211	Abbott Molecular	Pathvysion HER-2	Thermobrite	N/A	5min/75C	20hrs/37C	1N NaSCN/30min/80.6C	Pepsin/10min/37.3C	2min/72.3C