



building towards

cIQc

canadian Immunohistochemistry Quality control

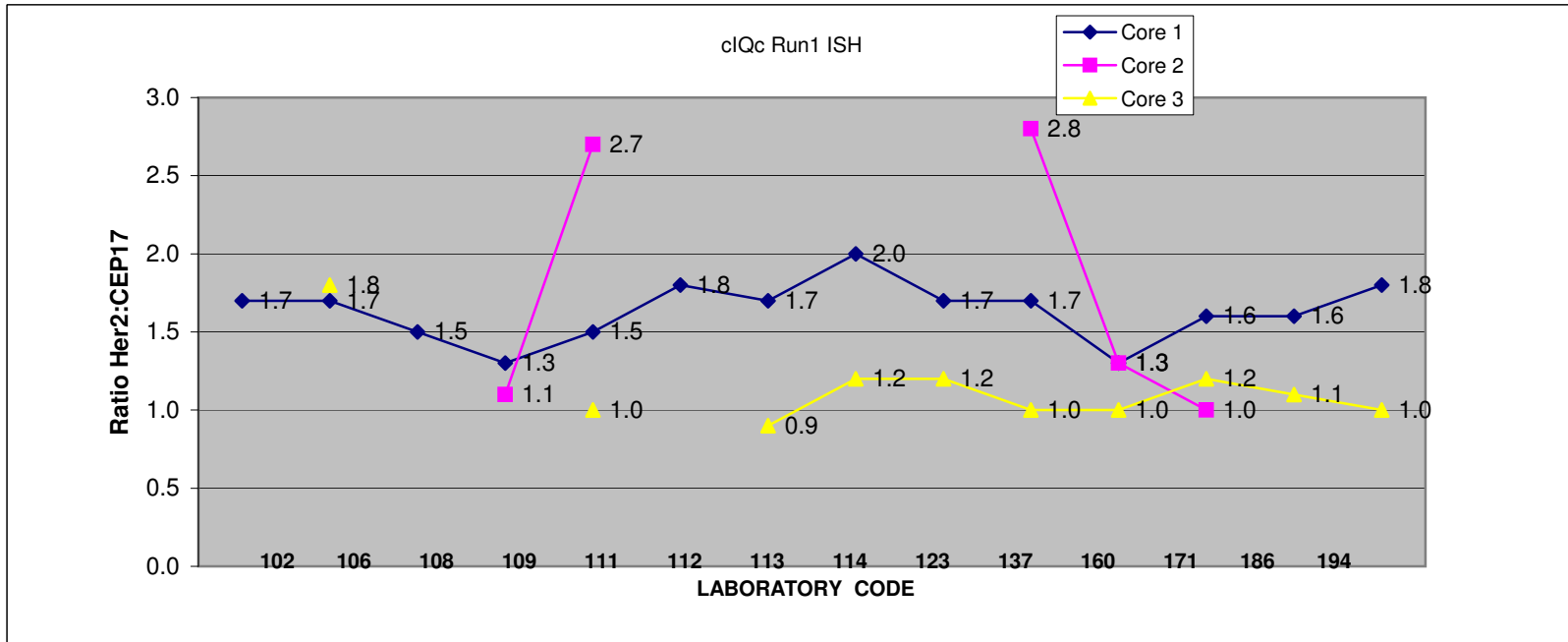
Assessors' report for cIQc Run 21: HER2 ISH

The three tissues used in Run 21 were selected because they were challenging. Core 2 appeared to be particularly challenging technically and 50% of laboratories did not submit results. This appears to be a problem with the nature of the tissue rather than in the protocols of the laboratories. As such do not consider Core 2 when evaluating your laboratories performance. In addition to the ISH results, the ISH protocols are also posted. These are interesting in themselves since they show the variety of protocols that are being used in Canadian laboratories.

HER2:Cep17 RATIO

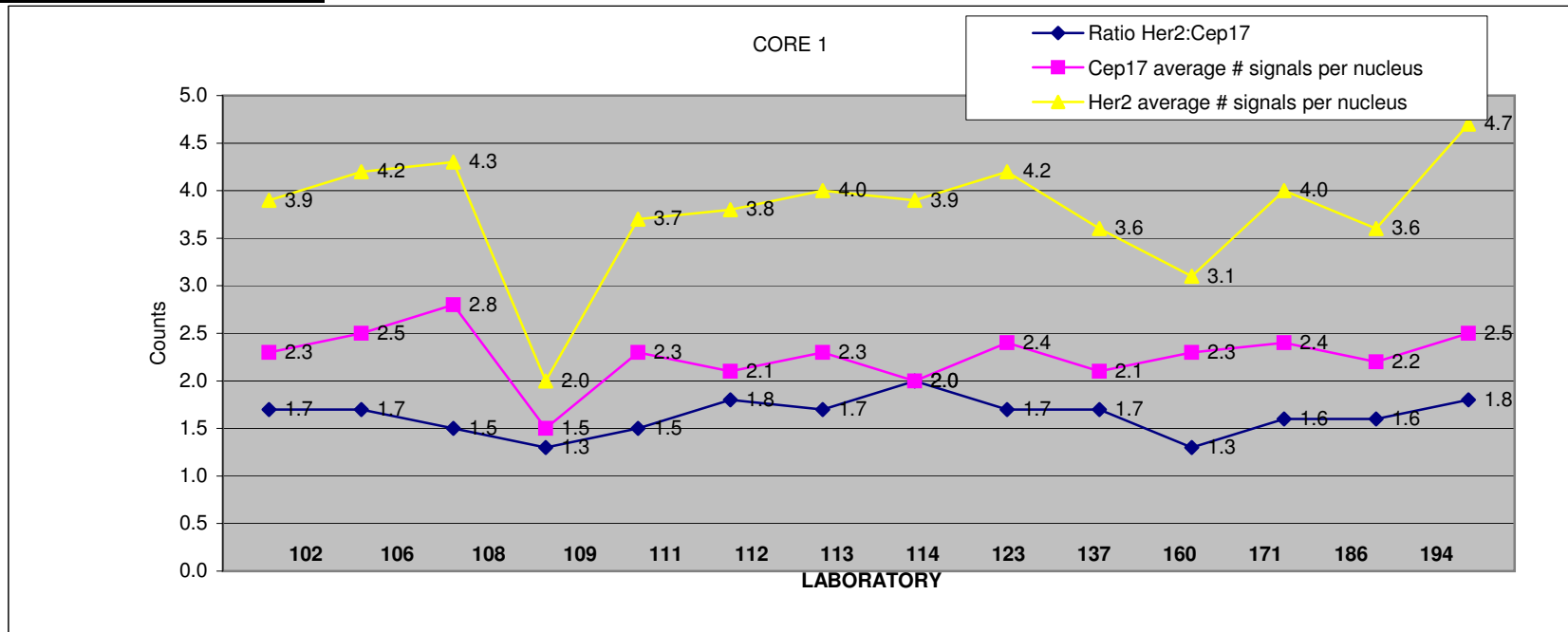
clQc Run 21 ISH

LAB	Core 1	Core 2	Core 3
102	1.7		
106	1.7		1.8
108	1.5		
109	1.3	1.1	
111	1.5	2.7	1.0
112	1.8		
113	1.7		0.9
114	2.0		1.2
123	1.7		1.2
137	1.7	2.8	1.0
160	1.3	1.3	1.0
171	1.6	1.0	1.2
186	1.6		1.1
194	1.8		1.0
Average	1.6	1.8	1.1



Core1

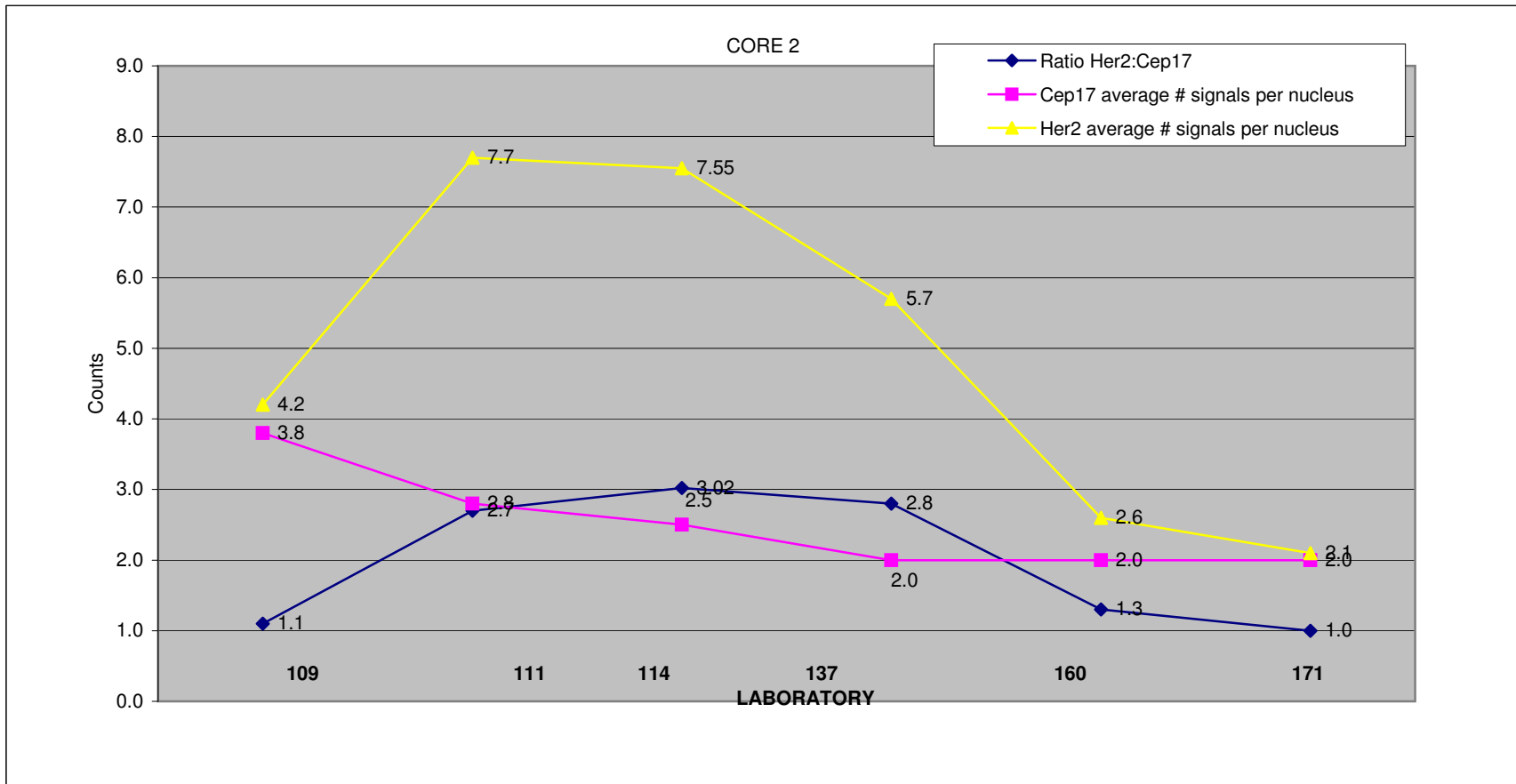
LAB	ratio	CEP17	Her2
102	1.7	2.3	3.9
106	1.7	2.5	4.2
108	1.5	2.8	4.3
109	1.3	1.5	2.0
111	1.5	2.3	3.7
112	1.8	2.1	3.8
113	1.7	2.3	4.0
114	2.0	2.0	3.9
123	1.7	2.4	4.2
137	1.7	2.1	3.6
160	1.3	2.3	3.1
171	1.6	2.4	4.0
186	1.6	2.2	3.6
194	1.8	2.5	4.7
Average	1.6	2.3	3.8



Core 2

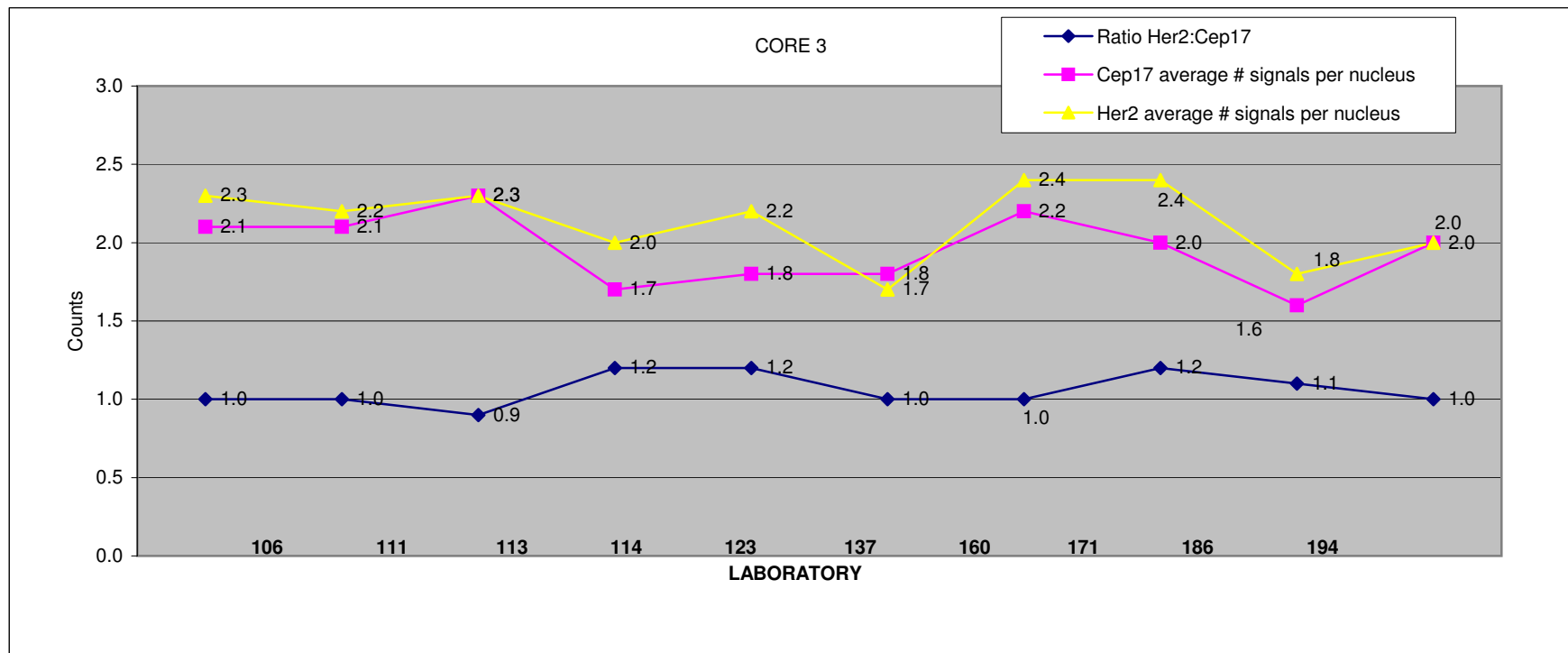
Core 2

LAB	ratio	CEP17	Her2
109	1.1	3.8	4.2
111	2.7	2.8	7.7
114	3.02	2.5	7.55
137	2.8	2.0	5.7
160	1.3	2.0	2.6
171	1.0	2.1	2.0
Average	2.0	2.5	5.0



Core 3

LAB	ratio	CEP17	Her2
106	1.0	2.1	2.3
111	1.0	2.1	2.2
113	0.9	2.3	2.3
114	1.2	1.7	2.0
123	1.2	1.8	2.2
137	1.0	1.8	1.7
160	1.0	2.2	2.4
171	1.2	2.0	2.4
186	1.1	1.6	1.8
194	1.0	2.0	2.0
Average	1.1	2.0	2.1



cIQC Run 21 Protocols

Lab/Fields	102	105	106	108	109	111	112	113	114	115
Vendor	Ventana	PathVysion	Vysis Abbott	Ventana	Not provided	Abbott Molecular	Ventana/ Roche	Invitrogen	Abbott (Vysis)	Abbott Molecular - Vysis
Probe	Inform HER2 DNA Probe	Her2/CEP17 dual color probe	PathVysion	SISH	Not provided	Vysis	HER2 DNA/ Chromosome 17 Probe	Her2/CEP17	Her2/CEP17 dual color probe	HER2
Instrument	Benchmark XT	Manual with ThermoBrite	ThermoBrite	Benchmark LT	Not provided	VP-2000	ULTRA BENCHMARK	DAKO hybridizer	ThermoBrite	manual
Pretreatment	Reaction Buffer	Buffer at 80C for 50min	Citrate Buffer, Pepsin	N/A	Not provided	2XSSC	Reaction Buffer	Spotlight pretreatment	Dako target Retrieval	sodium thiocyanate
Denaturation Temp	90	73 degrees	73C	N/A	Not provided	37C	86 deg C	75C	95	73
Denaturation Time	32"	5 minutes	5 minutes	N/A	Not provided	20 min	28 minutes	5min	40	2 min
Hybridization Time	6 hours	16-18 hours	18 hours	N/A	Not provided	16 hr	6 HOURS/ 3 HOURS	16hr	16hrs	15 hours
Post Hybridization Wash Reagent	SSC	2X SSC/ 0.3% NP40	0.3% NP-40 in 2XSSC	N/A	Not provided	2XSSC/ 3%NPP-40	ultraView Silver Wash II	SSC	SSC with NP40	vysis
Post Hybridization Wash Temp	72	72 degrees	75C	N/A	Not provided	72 C	70 deg C / 59 deg C	79	73 C	72
Post Hybridization Wash Time	24"	2 minutes	6 minutes	N/A	Not provided	3 min	24 minutes	5min	2 mins	5 min

cIQC Run 21 Protocols

Lab/Fields	116	123	137	160	171	175	186	194	197
Vendor	Ventana	Abbott (Vysis)	Abbott (Vysis)	Abbott	ABBOTT	Ventana	Vysis/ Abbott Molecular	VYSIS	Abbott
Probe	sonde HER2 - sonde chromo 17	HER2/CEP17	LSI Her2/neu	Path-Vysion	PATHVISION	Her2 DNP	Pathvysion Her2/ CeP17	Pathvysion HER-2NEU	PathVysion Her2 DNA
Instrument	Benchmark XT	Little Dipper/ manual	Thermobrite	Thermo Brite	Thermobrite	Benchmark	Manual	Abbott Molecular Thermobrite	Hybrite
Pretreatment		at 60degrees) , Na citrate, pepsin	Vysis paraffin pretreatment reagent kit	proteinase K sodium thiocyanate	Thiocyanate Proteinase K	Antigen Retrieval and Protease 3	1M Thiocyanate	DAKO A/R PH 6.0	Pepsin
Denaturation Temp		74 degrees celsius	68 C	80C	80Â°c	80	73 degree C	73 C	75 C
Denaturation Time		5 minutes	15 min	8 min.	8 MIN	20 min	5 min	5 MINS	4 minutes
Hybridization Time	6 heures	20 hours	17 hours at 37 C.	18 hrs	16 HRS	6 hours	16 hr	18 HRS 37 C OVERNIGHT	18 hours
Post Hybridization Wash Reagent	SSC	0.4X SSC/ 0.3%NP-40	2xSSC with .3% NP40	2xSSC + NP40	2X SSC, NP40	SSC buffer	SSC, NP40	2X SSC/0.3 % NP-40X2	2Xscc /0.3%igepal
Post Hybridization Wash Temp	72 degrÃ©	68 degrees celsius	73 C	65 C	65Â°c	72	73 degree C	ROOM TEMP/72 C	73.5 C
Post Hybridization Wash Time	24 minutes	2 minutes	2 min	4 min.	4 MIN	8 minutes	2 and 1/2 minutes	UNTIL COVERSIP FALLS OFF	2 minutes