

EXPERT ASSESSMENT FOR RUN 28

Assessment performed on Jan 29, 2013, at Vancouver General Hospital:
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This run was a smaller CIQC run on breast biomarkers ER and PR, for laboratories that were not participating in the larger run of breast biomarkers, that included ER, PR, HER2, Ki67 and basal markers.

The first task for the reviewers was to identify those cores where there was no tumour or insufficient tumour to allow assessment of staining. These cores were then removed from further consideration. We next did correlation of staining for each lab, compared to the reference results, based on their reported results (as recorded on the CIQC website). This allowed us to identify those labs with lesser levels of agreement with reference laboratory results (i.e. kappa statistic < 0.80) so that particular attention could be paid to those laboratories results.

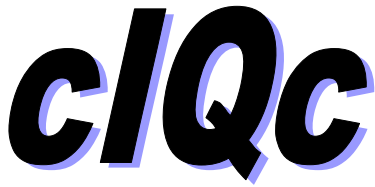
ER

Based on review of the Garratograms, after removal of data for "bad" cores, i.e. those cores with insufficient tumor to allow assessment, ER staining results were highly concordant between laboratories. Only laboratories 122 and 184 had kappa statistics less than 0.8/agreement rates with reference < 95%, and these labs were only slightly below these cut-offs. (cores considered inadequate for ER assessment were 6, 16, 17, 19, 21, 24, 26, 30, 35, 56)

Comments on individual laboratories

122: Overall very strong staining with increased background. Three cores were reported as positive that were negative in the reference lab (and most other labs participating in this run): cores 29, 54 and 60. On review core 29 was unsatisfactory for assessment and core 54 showed staining only in benign stromal cells and not in tumor cells (i.e. false positive results). Core 60 did show a few positive cells (and also showed focal staining in a few other labs - the "true" result for this core is uncertain). Thus, although the kappa statistic was 0.79, based on the self analysis, there was only one discrepant core based on review and results were therefore "optimal". Nonetheless, the overall intensity of staining was very strong with increased background staining.

184: Overall weaker staining than average. Four cores were reported as being different from the reference result (1 positive and 3 negative cores, as self reported). Core 3 was called negative but was



unsatisfactory on review. Cores 27 and 28 were also self reported as negative; on review core 27 was unsatisfactory and core 28 was weak positive. Core 20 was reported as positive but was negative on review. Thus, although there were four cores where there was disagreement with the reference lab, based on self reported results, none of these were discordant based on expert review. Overall staining intensity was weak, however, making interpretation more difficult.

117: core 41 was reported as positive but was considered negative on review. Core 60 did show a few positive cells. Optimal staining

128: Optimal staining

132: Core 13 was reported as negative, but on review showed an unusual edge artifact and was considered uninterpretable. Core 42 was called positive but was negative on review. Optimal staining

133: Optimal staining

134: Optimal staining

141: Core 54 was reported as positive but on review only stromal cells, not tumour cells were stained. Optimal staining

143: Optimal staining

144: Core 23 was reported as negative but was positive on review. Optimal staining

146: Optimal staining

148: Optimal staining

149: Optimal staining

159: Core 15 was reported as negative but shows strong positive staining on review. Optimal staining.

163: no slides available for review

165: A few positive cells were present on core 60. Optimal staining

168: no slides available for review

172: Optimal staining



173: Weak positive staining on core 60. Optimal staining

175: Core 20 was reported as positive but was negative on review. Weak positive staining on core 60. Optimal staining

177: Optimal staining

178: Core 3 called negative but unsatisfactory on review. Core 54 called positive but on review only stromal cells, not tumour cells staining. Optimal staining

183: Optimal staining

192: Cores 41 and 60 show rare positive cells. Optimal staining

196: Optimal staining

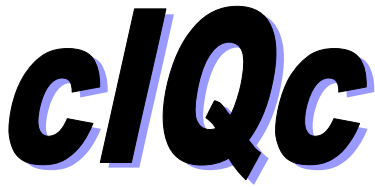
PR

As usual, PR stain shows more variability than ER staining. This is a very consistent observation from run to run, and no improvement is in sight, as we continue to use the same reagents. Unlike ER testing, where false positive staining is rare, with PR immunostaining it is possible to have false positive results.

There were several cores that showed significant variability in staining (cores 28, 31, 50 and 57); these cores were all ER positive and we have no way of knowing what the "true" result for these cores is. Although we have a reference value for these cores, it is not possible to be certain that these are the correct results. Because of the variability in staining (with only one lab, 149, having 100% agreement with the reference lab results) there was less stringency in considering PR staining to be optimal, compared to ER. Five laboratories (117, 128, 172, 173, 184) had kappa statistics less than 0.80. (cores considered inadequate for PR assessment were 3, 6, 16, 17, 19, 21, 24, 26, 30, 35, 56)

Comments on individual laboratories

117: Cores 7, 11, 50 and 60 were reported as positive; on review 7 and 11 showed equivocal staining, while cores 50 and 60 were positive. For cores 50 and 60, although negative in the reference laboratory, a significant minority of participating labs showed weak staining of these cores and it is not possible to know what the "true" result is for these cases (i.e. negative or weak positive). Therefore we considered this to be optimal staining.



128: Very intense staining with multiple cores that stained positively, but were negative for the reference laboratory (i.e. 7, 11, 37, 47, 50, 60). This was the only laboratory to see staining for core 47 and one of only two labs seeing positivity for core 37. Both of these cores were ER negative in all labs and thus we would interpret these as false positive results, and the overall staining was suboptimal.

172: The general impression was that there was increased background and uneven counterstaining, making interpretation difficult. Cores 11 and 33 showed false positive staining while core 15 showed was negative (but was uniformly positive in other labs). This is a puzzling pattern, that is rarely encountered (i.e. both false positive and false negative staining) and is most probably related to the uneven staining.
Suboptimal staining

173: Overall, the staining was weak. Core 12 was reported as negative but was equivocal on review. Core 31 was negative (also negative in a minority of participating laboratories, so not a definite false negative result). Core 60 was reported as positive but was negative on review. In summary, results were adequate but there was weaker than average staining.

184: The weak counterstain made interpretation difficult. Core 2 was called positive but it was a "floater" that was positive and the core was negative on review. Cores 5, 12, and 18 were unsatisfactory on review. Therefore, based on review, the agreement with reference results was satisfactory.

122: Optimal staining

132: Core 20 is negative on review. Optimal staining

133: Optimal staining

134: Optimal staining

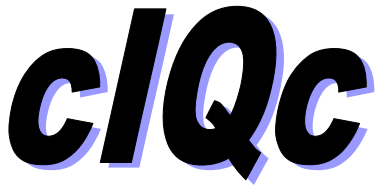
141: Core 2 was uninterpretable on review. Optimal staining

143: Optimal staining

146: Core 20 was uninterpretable on review. Optimal staining

149: Optimal staining

159: Optimal staining.



163: no slides available for review

165: Core 31 is positive on review. Optimal staining

168: no slides available for review

175: Optimal staining

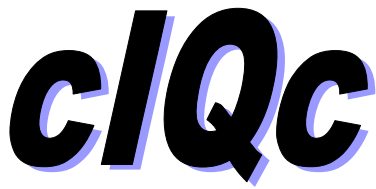
177: Cores 12 and 52 were both negative on review. The latter, in particular, was positive in all other laboratories. Although the staining looks good overall, with respect to intensity and background, there was a slight lack of sensitivity (core 52). Optimal staining

178: Core 5 called negative but positive on review. Core 31 called negative but positive on review. Optimal staining

183: Optimal staining

192: Optimal staining

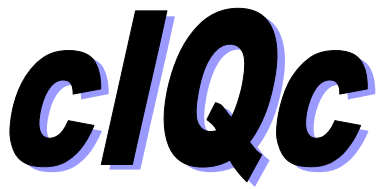
196: Optimal staining



Date	28-Jan-13
Excel worksheet name	ER
Reference lab name	R1
Number of labs	25

Descriptive Statistics

Test lab name	total n	% scorable	pairwise complete observations	concordance with reference (%)	sensitivity	specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
117	50	94	47	45/47 (96%)	1	0.78	0.95	1	0.85
122	50	98	49	46/49 (94%)	1	0.7	0.93	1	0.79
128	50	92	46	46/46 (100%)	1	1	1	1	1
132	50	90	45	43/45 (96%)	0.97	0.89	0.97	0.89	0.86
133	50	94	47	47/47 (100%)	1	1	1	1	1
134	50	86	43	43/43 (100%)	1	1	1	1	1
141	50	90	45	44/45 (98%)	1	0.88	0.97	1	0.92
143	50	96	48	48/48 (100%)	1	1	1	1	1
144	50	100	50	49/50 (98%)	0.97	1	1	0.92	0.94
146	50	92	46	46/46 (100%)	1	1	1	1	1
148	50	90	45	45/45 (100%)	1	1	1	1	1
149	50	90	45	44/45 (98%)	1	0.89	0.97	1	0.93
159	50	98	49	47/49 (96%)	0.97	0.9	0.97	0.9	0.87
163	50	92	46	46/46 (100%)	1	1	1	1	1
165	50	94	47	47/47 (100%)	1	1	1	1	1
168	50	98	49	49/49 (100%)	1	1	1	1	1
172	50	96	48	48/48 (100%)	1	1	1	1	1
173	50	96	48	47/48 (98%)	1	0.89	0.98	1	0.93
175	50	96	48	46/48 (96%)	1	0.78	0.95	1	0.85
177	50	96	48	48/48 (100%)	1	1	1	1	1
178	50	98	49	47/49 (96%)	0.97	0.9	0.97	0.9	0.87
183	50	92	46	46/46 (100%)	1	1	1	1	1
184	50	88	44	40/44 (91%)	0.92	0.86	0.97	0.67	0.7
192	50	86	43	42/43 (98%)	1	0.89	0.97	1	0.93
196	50	92	46	46/46 (100%)	1	1	1	1	1



Date	1/28/2013
Excel worksheet name	PR
Reference lab name	R1
Number of labs	23

Descriptive Statistics

Test lab name	total n	% scorable	pairwise complete observations	concordance with reference (%)	sensitivity	specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
117	50	92	46	41/46 (89%)	1	0.64	0.86	1	0.71
122	50	92	46	44/46 (96%)	0.97	0.93	0.97	0.93	0.9
128	50	94	47	39/47 (83%)	0.97	0.5	0.82	0.88	0.54
132	50	88	44	42/44 (95%)	1	0.87	0.94	1	0.9
133	50	92	46	45/46 (98%)	0.97	1	1	0.94	0.95
134	50	80	40	38/40 (95%)	1	0.83	0.93	1	0.87
141	50	84	42	39/42 (93%)	0.97	0.85	0.93	0.92	0.83
143	50	94	47	46/47 (98%)	1	0.93	0.97	1	0.95
146	50	80	40	38/40 (95%)	1	0.82	0.94	1	0.87
149	50	84	42	42/42 (100%)	1	1	1	1	1
159	50	100	50	49/50 (98%)	1	0.94	0.97	1	0.95
163	50	92	46	44/46 (96%)	1	0.87	0.94	1	0.9
165	50	94	47	45/47 (96%)	0.97	0.94	0.97	0.94	0.91
168	50	100	50	47/50 (94%)	1	0.82	0.92	1	0.86
172	50	94	47	42/47 (89%)	0.94	0.8	0.91	0.86	0.75
173	50	90	45	41/45 (91%)	0.91	0.92	0.97	0.8	0.79
175	50	100	50	46/50 (92%)	1	0.76	0.89	1	0.81
177	50	92	46	42/46 (91%)	0.87	1	1	0.79	0.81
178	50	94	47	43/47 (91%)	0.88	1	1	0.79	0.82
183	50	90	45	42/45 (93%)	1	0.8	0.91	1	0.84
184	50	86	43	36/43 (84%)	0.83	0.85	0.93	0.69	0.64
192	50	84	42	41/42 (98%)	1	0.93	0.97	1	0.95
196	50	86	43	42/43 (98%)	0.97	1	1	0.93	0.95



ER Protocols

Labs/Fields	117	118	122	128	132	133	134	136	141	143	144	146	148	149	159	163	165	168	172	173	175	177	178	180	183	184	192	196	R1
Clone	SP1		6F11	ER (SP1)	6F11	EP1	SP1		SP1	SP1	SP1	SP1	SP1	EP1	SP1	sp1	SP1	EP1	SP1	SP1	6F11	SP1		SP1	ER: ID5 & ER-2-123	SP1	sp1	SP1	
Dilution	NONE (COMMERCIAL)		RTU	Prediluted	1:80	1/100	Predilute		Predilute	no	pre-dilute	RTU	RTU	RTU	RTU	pre-diluted	nil	1/200	prediluted by Dako	Pre-diluted	Predilute	1:50	pre-dilute		predilute	RTU	Ready to use	No	1:50
Vendor	Ventana		Leica-Biosystems	Ventana	VECTOR	Dako	Ventana		Ventana	Confirm Ventana	Ventana	Dako	Ventana	Dako IR151	DAKO	Benchmrk Ventria	ventana	Cell Marque	Dako	Ventana	Ventana	Novocastra	Ventana		Ventana	Dako K4071	Ventana	ventana	Thermo
Antigen Retrieval	CC1		ER2(PH 9)-20 MIN.	CC1 mild 30 minutes	ENVISION FLEX HIGH PH	high ph	CC1		CC1	CC1 60 min	CC1 for 8 minutes	Flex TRS HIGH	CC1 36min	PT Link High pH 98 C 20 min	High pH	HIER	cc1	High pH	97C 20min pH 9	CC1 30 minutes	Heat	y	CC1 Mild		CC!	citrate buffer, low pH	CC1 mild (30 min.)	no	CC1 32mins
Detection System	Ultra View		POLYMER REFINE	Ultraview	HRP ENVISION FLEX	polymer-envision flex Dako	ultraview DAB Detection		ultraview Universal DAB	Uview Dab detection kit	Optiview	EnVision Flex Peroxydase/HRP	Ultraview	Envision Flex 20	FLEX	Peroxydase	ultraview	Envision Flex +	Envision Flex +	HRP-Multimer	I-View						Ultraview DAB (Ventana)	DAB ultraview	OptiView
Amplification	none		DAB ENHANCER	Copper	NONE	rabbit linker	no		No	no	none	None	N/A	None	none	Copper	-	None	no	Polymer complex	Copper	y	None		none	none	Copper	NO	Cu
Chromogen	Copper		DAB	Universal DAB	DAB	dab+	DAB		DAB	DAB	DAB	Substrat Working Solution Mix	DAB	DAB	DAB	DAB	dab	DAB	DAB	DAB	DAB	dab	DAB		DAB	DAB +	DAB	no	DAB

PR Protocols

Labs/Fields	117	118	122	128	132	133	134	136	141	143	146	149	159	163	165	168	172	173	175	177	178	180	183	184	192	196	R1	
Clone	100		16 PR (1E3)		16	1294	100		100	100	PgR 636	PgR636	PgR 636	100	100	PgR636	PGR 636	Y85	100	PGR636	100		100	PgR 1294	100	100	100	16
Dilution	None (commercial)		RTU	Prediluted	1:200	1/50	Predilute		Predilute	no	RTU	RTU	RTU	prediluted	nil	RTU	prediluted by Dako	1/50	Predilute	1:100	pre-dilute		predilute	Prediluted (K4071)	Ready to use	NO	1:25	
Vendor	Ventana		Leica-Biosystems	Ventana	VECTOR	Dako	Ventana		Ventana	confirm Ventana	Dako	Dako	DAKO	Benchmark Ventria	ventana	Dako	Dako	CellMarque	Ventana	Dako	Ventana		Ventana	Dako	Ventana	VENTANA	Leica	
Antigen Retrieval	CC1		ER2(PH9)-20 MIN.	CC1 Mild 30 minutes	ENVISION FLEX HIGH PH	low ph	CC1		ultraview Universal DAB	CC1 60 min	FLEX TRS HIGH	PT Link High pH 98 C 20 min	High	HIER	cc1	High pH	97C 20min pH 9	CC1-60 minutes	Heat	y	CC1 Mild		CC!	Citrate buffer 10X	CC1 mild (30 min.)	NO	CC1 32mins	
Detection System	Ultra View		POLYMER REFINE	Ultraview	HRP ENVISION FLEX	polymer-envision flex Dako	ultraView Detection		CC1	Uview DAB detection kit	EnVision Flex Peroxydase/HRP	Envision Flex + 20	FLEX	Peroxydase	ultraview	Envision Flex +	envision Flex +	HRP-Multimer	I-View						Dextran Polymer (K4071)	Ultraview DAB (Ventana)	DAB ultraview	OptiView
Amplification	none		DAB ENHANCER	Copper	NONE	none	no		No	endogenous biotin kit	none	None	none	Copper	-	Mouse Linker	no	Polymer complex	Copper	y	None		none	None	Copper	no	Cu	
Chromogen	Copper		DAB	Universal DAB	DAB	Dab+	DAB		DAB	DAB	Substrat Working Solution Mix	DAB	DAB	DB	dab	DAB	DAB	DAB	DAB	DAB	DAB	DAB		DAB	DAB+ (K4071)	DAB	no	DAB