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Assessors' report for cIQc Run 77: ALK IHC (September 2017)

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Assessment performed on November 16, 2017 at Vancouver General Hospital, Vancouver, BC.

Health Canada Summary

Canadian laboratories are required by Health Canada to demonstrate proficiency in IHC and/or FISH testing of NSCLC of ALK. cIQc is providing regular EQA for ALK (NSCLC) IHC and FISH to enable laboratories to comply with Health Canada regulations. Canadian laboratories performing ALK testing of NSCLC must show compliance with the regulations. Provided is the link to the Health Canada Summary basis of decision for XALKORI (crizotinib) http://www.hc-sc.gc.ca/dhp-mps/prodpharma/sbd-smd/drug-med/sbd_smd_2012_xalkori_145155-eng.php#a3.3.3.

The above-mentioned document states the following:

"The labelling also highlights the importance of the requirement to utilize laboratories with demonstrated proficiency in using a validated diagnostic assay to assess ALK fusion, to avoid inappropriate treatment in ALK-negative patients for whom the benefit of Xalkori is not established.

The approval of Xalkori for ALK+ patients is linked to the use of a validated diagnostic assay with high sensitivity and specificity and by a laboratory with demonstrated proficiency in using this validated assay.

Using a validated ALK assay, assessment for ALK-positive locally advanced or metastatic NSCLC should be performed by laboratories with demonstrated proficiency in the specific technology being utilized. Improper assay performance can lead to unreliable test results."

OVERVIEW

Run 77 consisted of a single-core tissue microarray containing 30 NSCLC cases with accompanying ALK FISH data. Core 13 was a weak positive case and served as a good on-slide weak positive control for IHC. In total, 21 laboratories participated in Run 77 and all participants returned slides to the cIQc office in time for the assessment meeting.

RESULTS

Overall ALK IHC results were excellent, with all labs having either adequate or optimal staining. Participant-specific feedback for is summarized below:

Table with 3 columns: Lab ID, IHC Status*, Comments. Rows include Lab IDs 101, 102, 107, 109, 110, 111, 112, 114, 115, 116, 120 with various staining status and comments.

Table with 3 columns: Lab ID, IHC Status*, Comments. Rows include Lab IDs 125, 146, 149, 191, 194/194a, 202, 217, 220, 222, 234 with various staining status and comments.

*based on cIQc assessment



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Lab/ Core	101	102	109	110	111	112	114	115	116	120	125	146	149	191	194	194a	202	217	220	222	234	FISH	
1	U	C	C	C	C	C	C	C	N	N	N	C	C	C	N	N	C	N	C	C	N	N	
2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
5	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	
6	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
7	P	P	U	P	P	P	P	P	P	E	P	P	P	P	P	P	P	P	P	P	P	P	
8	N	N	N	N	N	N	N	E	E	N	N	N	N	E	E	E	N	N	N	E	N	N	
9	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
11	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
12	N	N	U	N	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
13	P	P	E	P	P	P	P	P	P	E	P	P	P	P	P	P	P	E	P	P	P	P	
14	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
16	U	U	U	U	N	U	U	U	N	N	N	U	N	U	U	N	U	N	U	U	N	N	
17	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
18	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
19	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
20	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
21	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
22	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
23	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
24	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
25	N	U	U	N	N	N	N	U	N	N	N	U	N	U	N	N	N	N	U	U	N	N	
26	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	
27	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
28	P	P	E	P	E	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
29	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
30	N	N	N	N	N	N	N	U	N	N	N	U	N	N	N	N	U	N	N	U	N	N	

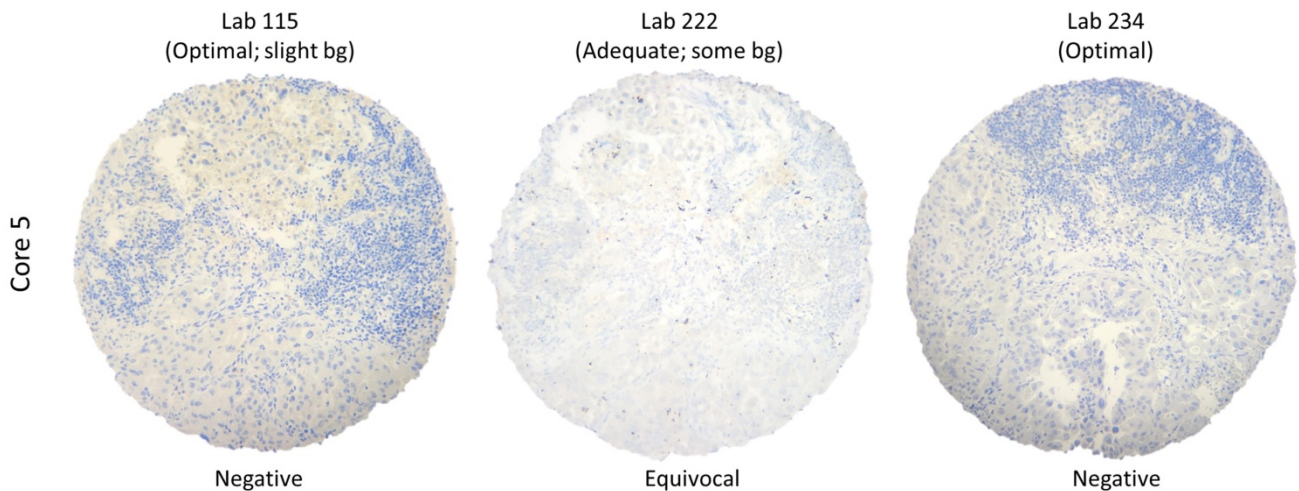


Figure 1. Representative images of staining variability seen in Core 5, an ALK IHC-negative core. bg = background



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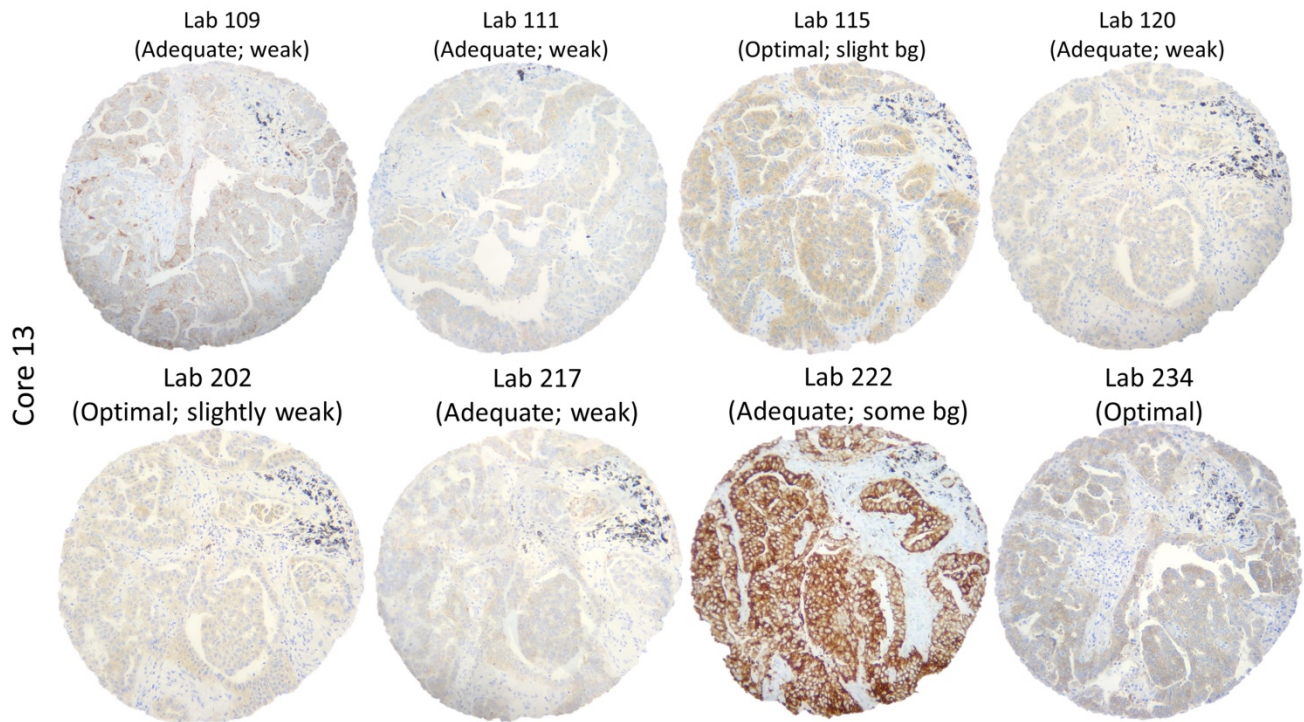


Figure 2. Representative images of staining variability seen in Core 13, a weak positive core. bg = background

Supplementary Table 1 summarizing staining protocols and Supplementary Table 2 summarizing descriptive statistics can also be found at the end of this document. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with this reservation. Your regular participation in cIQc is greatly appreciated and we look forward to continuing to work with you and the Canadian Association of Pathologists – Association Canadienne des Pathologistes.

Table S1. Reported ALK IHC staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/ Vendor	Ab Lot No.	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	64 min	5A4	1:25	Novocastra	6046565	16 min	OptiView	y	y	DAB
102	DAKO PT - HIGH PH	20	5A4	1:40	NOVOCASTRA	6039189	60"	DAKO ENVISION FLEX+	YES	YES CUSO4	DAB+
107	cc1	48	5A4	1:20	Novocastra	6049748	48	Optiview DAB	Y	Y	DAB
109	HIER high pH(cc1)	40 min	D5F3	RTU	ROCHE	F09750	16 MIN	OPTIVIEW	Y	Y	DAB
110	DAKO PT High ph 9.0	20 min @ 97 C	5A4	1:50	Biocare Medical	81016	30 min	Dako Envision Flex-FLEX +30 Ms	Y	N	DAB
111	HIER	72	5A4	1/25	Leica	6050551	60	optiview	y	y	DAB
112	BOND Epitope Retrieval 2	30 minutes	5A4	1:50	Leica (Nococastra)	6032589	30 minutes	BOND Polymer Refine Detection	none	none	DAB
114	CC1	64	5A4	1/25	Biocare	21014	16	Optiview	Y	Y	DAB
115	Envision Flex High pH	30 mins	D5F3	1/100	Cell Signaling	A333	30 mins	Envision Flex	N	N	DAB
116	CC1	92 min	D5F3	RTU	Ventana	F09750	16 min	Optiview DAB	Y	Y	DAB
120	waterbath (TRS High)	20	5A4	1:40	Biocare Medical	22217	30	Dako Envision Flex	Y	N	DAB
146	Flex TRS High	20	5A4	1:100	BioCare	22217	25	EnVision FLEX	N	N	DAB
149	PT Link high pH	20 min at 97 C	5A4	1:50	Novocastra	6047123	30	EnVision Flex	Yes	Yes	DAB
191	cc1 ventana	92 min	D5F3	RTU	Ventana	xxx	16 min	optiview	Y	y	DAB
194	HIER (CC1)	92	D5F3	Predilute	Roche Ventana	F09750	16	Optiview Detection DAB	Y	Y	DAB
194a											
202	HIER citrate pH 9.5	20	5A4	1/10	Leica	6048819	15	Leica Refine detection kit	no	no	DAB
217	HIER CC1	72	D5F3	RTU	Roche Ventana	G05673	36	Optiview	Y	Y	DAB
220	CC1	92 min	5A4	1:30	Leica	6048091	120 min	OptiView	N	N	DAB
222	Ultra CC1	92	D5F3	1:1	Ventana	G07299	16	Ultraview DAB	Y	Y	Copper
234	PT labvision/ HIER M buffer	30	1A4	20	Origene	996.123B-2-4 / 31-08-2018	30	Powervision/poly-HRP-GAMs/RblgG	No	Yes, CuSO4	DAB

Table S2. Descriptive statistics for ALK IHC based on cIQc assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	30	93.33	28	28/28 (100%)	1	1	1	1	1
102	30	90	27	27/27 (100%)	1	1	1	1	1
109	30	83.33	25	25/25 (100%)	1	1	1	1	1
110	30	93.33	28	28/28 (100%)	1	1	1	1	1
111	30	93.33	28	28/28 (100%)	1	1	1	1	1
112	30	93.33	28	28/28 (100%)	1	1	1	1	1
114	30	93.33	28	28/28 (100%)	1	1	1	1	1
115	30	86.67	26	26/26 (100%)	1	1	1	1	1
116	30	100	30	30/30 (100%)	1	1	1	1	1
120	30	100	30	30/30 (100%)	1	1	1	1	1
125	30	100	30	30/30 (100%)	1	1	1	1	1
146	30	86.67	26	26/26 (100%)	1	1	1	1	1
149	30	96.67	29	29/29 (100%)	1	1	1	1	1
191	30	90	27	27/27 (100%)	1	1	1	1	1
194	30	96.67	29	29/29 (100%)	1	1	1	1	1
194a	30	100	30	30/30 (100%)	1	1	1	1	1
202	30	90	27	27/27 (100%)	1	1	1	1	1
217	30	100	30	30/30 (100%)	1	1	1	1	1
220	30	90	27	27/27 (100%)	1	1	1	1	1
222	30	86.67	26	26/26 (100%)	1	1	1	1	1
234	30	100	30	30/30 (100%)	1	1	1	1	1