Assessors' report for cIQc Run 67: ALK IHC (September 2016)

Assessors: B Gilks, J Garratt, J English, A Churg, R O'Connor, F Derakhshan, J Flint, J Won (recorder)

Assessment performed on February 24, 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 67 consisted of a single-core tissue microarray containing 30 NSCLC cases with accompanying ALK FISH data, with Core 13 serving as a good on-slide weak positive control for IHC. In total, 21 laboratories participated in Run 67 and all participants returned slides to the cIQc office in time for the assessment meeting. As noted in previous reports, the ALK01 clone was developed primarily for detection of ALK in anaplastic large cell lymphoma and demonstrates low sensitivity in lung adenocarcinoma in our experience. Ganglion cells, present in Core 12 (a chest wall biopsy of lung cancer that is ALK negative) did stain, as expected; these cells were not present on all slides. No labs had misinterpreted these positive cells as tumor cells.

RESULTS

Participant-specific feedback for ALK IHC is summarized below:

Lab ID	IHC Status*	Comment
101	Optimal	
102	Adequate	Slightly weak staining
107	Optimal	Nice crisp staining
109	Optimal	
110	Adequate	Slightly weak staining
111	Optimal	
112	Adequate	Slightly weak staining
114	Adequate	Slightly weak staining
115	Optimal	
116	Optimal	Slight background possibly be due to the run-to-run variation from the amplification step
123	Optimal	
125	Adequate	Slightly weak staining
126	Failed	Many edge artifacts; two false positives and one false negative; generally difficult to interpret staining
146	Suboptimal	Strong counterstain and very weak staining, making interpretation difficult. Good self-assessments indicate that pathologists at the facility have adapted to this staining though.
149	Adequate	Slightly weak staining
176	Suboptimal	Very weak staining likely attributable to the antibody clone used; several equivocal cases
189	Adequate	Strong staining; slight background leading to many equivocal cases
194	Optimal	
202	Adequate	Slightly weak staining
217	Adequate	Slightly weak staining
220	Adequate	Slightly weak staining

*Based on cIQc assessor consensus

Garrattograms from self-assessment and after cIQc assessment of ALK IHC are provided in Supplementary Figure 1. 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.

Figure S1. Garrattogram from self-assessment and after cIQc assessment of ALK IHC.

	Self-assessments																					cIQc Assessment																					
Lab/ Cores	101	102	107	109	110	111	112	114	115	116	125	126	146	149	176	189	194	202	217	220	**FISH**	101	102	107	109	110	111	112	114	115	116	123	125	126	146	149	176	189	194	202	217	220
1	N	U	U	U	U	U	U	U	U	N	N	U	U	N	U	U	U	N	N	U	**N**	N	U	U	U	U	U	U	U	U	N	U	N	U	U	N	U	U	U	N	N	U
2	N	N	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	**N**	N	N	U	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	E	P	P	P	P	P	**P**	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	E	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	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	**N**	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	**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	P	P	P	P	P	P	P	P	P	P	P	P	E	P	P	P	P	P	**P**	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	E	P	P	P	P	P
8	N	N	N	N	N	N	N	N	E	E	N	P	N	P	N	N	E	N	N	N	**N**	N	N	N	N	N	N	N	N	N	E	N	N	P	N	N	N	N	N	N	N	N
9	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N	**N**	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	N	N
10	N	N	N	N	N	N	N	N	N	N	N	U	N	N	N	N	N	N	N	N	**N**	N	N	N	N	N	N	N	N	N	N	N	N	U	N	N	N	N	N	N	N	N
11	P	P	P	P	P	P	P	P	P	P	P	P	P	P	E	P	P	P	P	P	**P**	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	E	P	P	P	P	P
12	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	U	N	N	**N**	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	U	N	N
13	P	P	P	P	P	P	P	P	P	P	P	N	P	P	E	P	P	E	P	P	**P**	P	P	P	P	P	P	P	P	P	P	P	P	N	P	P	E	P	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	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	E	N	N	N	N	N	N	N	N	**N**	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N	N	N	N
16	N	U	U	U	U	U	U	U	U	U	N	U	U	N	U	U	U	N	N	U	**N**	N	U	U	U	U	U	U	U	U	U	U	N	U	U	N	U	U	U	N	N	U
17	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	**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	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	E	N	N	N	N	**N**	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	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	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	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	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
23	N	N	E	E	E	N	N	N	N	N	E	N	N	N	E	E	N	N	N	N	**N**	N	N	N	N	E	N	N	N	N	N	N	E	N	N	N	E	E	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	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
25	N	N	N	U	N	U	N	N	N	N	N	N	N	N	N	E	U	U	N	U	**N**	N	N	N	U	N	U	N	N	N	N	U	N	N	N	N	N	E	U	U	N	U
26	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	**N**	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	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	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
28	P	P	P	P	P	P	P	P	E	P	E	E	P	P	E	P	P	P	P	P	**P**	P	P	P	P	P	P	P	P	P	P	P	P	E	P	P	E	P	P	P	P	P
29	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	**N**	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N
30	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N	N	N	N	**N**	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N	N	N	N

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	6039071	16 min @ 37	Optiview	Y	N	DAB
102	DAKO PT HIGH pH	10/20/10	5A4	1:40	NOVOCASTRA	6044510	60" RT	DAKO FLEX+	YES	CUSO4	DAB+
107	cc1	48	5A4	1:20	Leica/Novocastra	6044510	48	Optiview DAB	Y	Y	DAB
109	HIER HIGH pH CC1	40 MIN	D5F3	RTU	VENTANA	F07106	16 MIN	OPTIVIEW	Y	Y	DAB
110	DAKO PT High ph 9.0@97 C	20 min	5A4	1:50	Biocare	81715	30 min	Dako Envision Flex: Flex+30Ms	Y-Dako Mouse Linker	N	DAB
111	CC1	72 min	5A4	1/25	Leica	6046565	60 min	optiview	y	copper	DAB
112	BOND Epitope Retrieval 2	30 minutes	5A4	1:100	Leica (Novocastra)	6032589	15 minutes	BOND Polymer Refine Detection	none	none	DAB
114	CC1	64	5A4	1/25	Biocare	21014	16	Optiview	Y	Copper	DAB
115	EnVision Flex TRS, High Ph	30 min	D5F3	1/100	cell signaling	5633	30 min	EnVision Flex	N	N	DAB
116	CC1	92 MIN	D5F3	RTU	VENTANA	F 06019	16 MIN	OPTIVIEW DAB IHC	Y	.	DAB
125	HIER-HIGH PH	30	54A	1/50	Leica	6028806	20	Polymer	N	N	DAB
126	Steam, EDTA, ph 8.0	45 minutes	D5F3	1:250	CELL SIGNALING	5	30 MINUTES	MACH-4, HRP	NO	NO	DAB
146	Flex TRS High	20	5A4	1/100	BioCare	12816	25	Flex EnVision	n	n	DAB
149	PT Link high pH	20 min at 97 C	D5F3	RTU	Ventana Roche	00F07106	20	EnVision Flex	Yes	Yes	DAB
176	CC1	32	ALK01	Predilute	Ventana/Roche	F09695	32	Optiview	N	N	DAB
189	CC1	92	D5F3	pre-dilute	Ventana	unknown	16	OptiView	Y	N	DAB
194	HIER	92	D5F3	pre-dilute	Ventana/Roche	F08055	16	Optiview DAB Detection Kit	Y	Y	DAB
202	Leica ER2 citrate Buffer 9.5	20 min	5A4	1/10	Leica	6044510	15 min	Refine Detection kit Leica	no	no	dab
217	HIER	72	D5F3	predilute	Roche Ventana	G01026	36	Optiview	N	Y	DAB
220	CC1	92	5A4	1:30	Leica	6042962	120	Opti-View	No	No	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	Cohen's kappa
101	30	100	30	30/30 (100%)	1	1	1
102	30	93.33	28	28/28 (100%)	1	1	1
107	30	90	27	27/27 (100%)	1	1	1
109	30	90	27	27/27 (100%)	1	1	1
110	30	93.33	28	28/28 (100%)	1	1	1
111	30	90	27	27/27 (100%)	1	1	1
112	30	93.33	28	28/28 (100%)	1	1	1
114	30	93.33	28	28/28 (100%)	1	1	1
115	30	93.33	28	28/28 (100%)	1	1	1
116	30	96.67	29	29/29 (100%)	1	1	1
123	30	90	27	27/27 (100%)	1	1	1
125	30	100	30	30/30 (100%)	1	1	1
126	30	90	27	24/27 (89%)	0.83	0.9	0.7
146	30	93.33	28	28/28 (100%)	1	1	1
149	30	100	30	30/30 (100%)	1	1	1
176	30	93.33	28	28/28 (100%)	1	1	1
189	30	93.33	28	28/28 (100%)	1	1	1
194	30	86.67	26	26/26 (100%)	1	1	1
202	30	93.33	28	28/28 (100%)	1	1	1
217	30	100	30	30/30 (100%)	1	1	1
220	30	90	27	27/27 (100%)	1	1	1