Assessors' report for cIQc Run 83: ALK IHC

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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) challenges 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 83 consisted of a single-core tissue microarray containing 30 NSCLC cases with accompanying ALK FISH data. Twenty laboratories participated in Run 83. The assessors blindly reviewed all cores for all available slides returned to the cIQc office in time for the assessment meeting. The tested material included 8 FISH-positive cases. Even one false-negative case results in <90% sensitivity/concordance with FISH results. Therefore, the overall result for any laboratory that produced even a single false-negative result was designated as "sub-optimal".

COMMENTS

The cIQc assessment was performed for the purpose of assessing the agreement of the results obtained by ALK IHC protocols with expert readout compared to results obtained by an ALK FISH protocol as performed by a reference laboratory. The assessment team reviewed all cores for all slides with the consensus readout being recorded. The cIQc assessment was performed in two stages. In **Stage 1**, assessors performed a readout suitable for laboratory developed tests (LDTs) following a recent publication by Marchetti *et al.*[1] based on overall cytoplasmic intensity along with a modification based on the CALK study[2], in which 2+/3+ corresponds to "positive", 1+ corresponds to "equivocal", 0 corresponds to "negative". In **Stage 2**, the assessors performed an additional reading of the slides from laboratories that indicated that they had used clone D5F3 from Ventana; the "cIQc Assessment (Roche Kit Readout)" readout was performed in accordance with the readout criteria specified by the manufacturer of the VENTANA ALK (D5F3) CDx Assay[3]. The reason that two readouts were performed for this subset of laboratories was that it was not possible for the assessment team to be sure whether laboratories using the D5F3 clone from Ventana did so as part of the VENTANA ALK

(D5F3) CDx Assay or as a part of a laboratory developed test. Of note, if your laboratory is using the D5F3 clone, please adjust your performance based on the readout method that is employed by the pathologist(s) at your institution.

RESULTS

Participant-specific feedback for ALK IHC is summarized below:

Lab ID	Readout using established guidelines assessors for LDTs[2]	Readout using Ventana interpretation guidelines for VENTANA ALK (D5F3) CDx Assay[3]	Comments
101	Optimal	N/A	Nice staining, no background.
102	Optimal	N/A	
107	Optimal	N/A	Good staining. See cores 6 and 11 which were upgraded by assessors.
109	Adequate	Sub-optimal	Decrease in analytical sensitivity noted (weak staining). Using the Ventana interpretive guidelines[3] core 6 was a false negative. We recommend that the Ventana package insert staining protocol be followed.
110	Adequate	N/A	Somewhat decreased analytical sensitivity (weak staining), but still acceptable.
111	Optimal	N/A	Good staining. See cores 6 and 11 which were upgraded by assessors.
112	Adequate	N/A	Somewhat decreased analytical sensitivity (weak staining), but still acceptable.
114	Sub-Optimal	N/A	Decrease in analytical sensitivity noted (staining is too weak). The lab is advised to send a stained section of appendix to cIQc for review.
115	Adequate	N/A	Clumps of amorphous stain debris noted and a distracting pink hue present. Second readout using Ventana interpretation guidelines[3] for D5F3 was not done since it was a Cell Signaling antibody and therefor the protocol was considered a LDT.
116	Adequate	Optimal	Recommend follow the package insert for the staining protocol.
123	Optimal	N/A	Hematoxylin is rather strong. Would consider decreasing staining time.
125	Adequate	N/A	
146	Adequate	N/A	Hematoxylin is rather strong. Would consider decreasing staining time.
149	Optimal	N/A	Hematoxylin is rather strong. Would consider decreasing staining time.
189	Adequate	Optimal	LDT readout was labeled as “Adequate” because high background in many cases may result in high number of cases sent for FISH, but patient safety is not compromised if this is done.
194	Adequate	Optimal	LDT readout was labeled as “Adequate” because high background in many cases may result in high number of cases sent for FISH, but patient safety is not compromised if this is done.

202	Optimal	N/A	
217	Adequate	Optimal	It appears that self-assessment readout was not performed using the Ventana guidelines [3] See the cIQc assessment using the Ventana guidelines[3]. It was noted that there is likely a deviation from the package insert protocol from Ventana. We recommend that the Ventana package insert staining protocol be followed. LDT readout was labeled as "Adequate" because high background in many cases may result in high number of cases sent for FISH, but patient safety is not compromised if this is done.
220	Optimal	N/A	
222	Adequate	Sub-optimal	Somewhat decreased analytical sensitivity (weak staining), but still acceptable. Using the Ventana interpretive guidelines [3] core 6 was a false negative. We recommend that the Ventana package insert staining protocol be followed.

References:

[1]Marchetti A, Di Lorito A, Pace MV, Iezzi M, Felicioni L, D'Antuono T, Filice G, Guetti L, Mucilli F, Buttitta F. ALK Protein Analysis by IHC Staining after Recent Regulatory Changes: A Comparison of Two Widely Used Approaches, Revision of the Literature, and a New Testing Algorithm. J Thorac Oncol. 2016 Apr;11(4):487-95.

[2]Cutz JC, Craddock KJ, Torlakovic E, Brandao G, Carter RF, Bigras G, Deschenes J, Izevbaye I, Xu Z, Greer W, Yatabe Y, Ionescu D, Karsan A, Jung S, Fraser RS, Blumenkrantz M, Lavoie J, Fortin F, Bojarski A, Côté GB, van den Berghe JA, Rashid-Kolvear F, Trotter M, Sekhon HS, Albadine R, Tran-Thanh D, Gorska I, Knoll JH, Xu J, Blencowe B, Iafrate AJ, Hwang DM, Pintilie M, Gaspo R, Couture C, Tsao MS. Canadian anaplastic lymphoma kinase study: a model for multicenter standardization and optimization of ALK testing in lung cancer. J Thorac Oncol. 2014 Sep;9(9):1255-63.

[3]http://www.roche-diagnostics.ch/content/dam/corporate/roche-dia_ch/documents/broschueren/tissue_diagnostics/Parameter/lung-pathology/ALK_D5F3_interpretation%20Guide_EN.pdf

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-assessment																				
Lab/ Cores	101	102	107	109	110	111	112	114	115	116	125	149	189	194	202	217	220	222	**FISH**	Lab/ Cores
1	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N	**N**	1
2	U	U	N	N	U	U	U	U	U	N	U	U	P	U	U	E	U	N	**N**	2
3	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	**P**	3
4	P	P	P	P	P	P	P	E	E	P	P	P	P	E	P	P	P	P	**P**	4
5	N	N	N	N	N	N	N	N	N	N	N	N	P	E	N	E	N	N	**N**	5
6	E	P	E	E	E	E	E	N	N	E	E	E	P	P	P	P	P	P	**P**	6
7	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	E	N	N	**N**	7
8	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	**P**	8
9	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	**N**	9
10	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	**P**	10
11	P	P	E	P	E	E	E	E	E	P	E	E	P	P	P	P	P	P	**P**	11
12	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	**P**	12
13	P	P	P	P	P	P	E	E	E	P	P	P	P	P	P	P	P	P	**P**	13
14	U	U	U	U	U	U	U	U	U	N	U	N	P	N	N	U	U	U	**N**	14
15	U	U	U	U	U	U	U	U	U	N	U	U	N	U	N	E	U	U	**N**	15
16	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	**N**	16
17	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	E	N	N	**N**	17
18	U	U	N	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	**N**	18
19	N	U	U	N	U	U	U	N	N	N	N	N	E	N	U	P	N	N	**N**	19
20	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	**N**	20
21	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N	N	**N**	21
22	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	**N**	22
23	U	U	N	U	U	U	U	U	U	N	U	N	U	U	U	U	U	N	**N**	23
24	U	U	U	N	N	U	N	N	N	N	N	N	N	N	N	P	N	N	**N**	24
25	N	E	N	N	N	N	N	N	N	N	N	N	E	N	P	N	N	N	**N**	25
26	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	**N**	26
27	N	N	U	N	N	N	N	N	N	N	N	U	N	N	N	E	N	N	**N**	27
28	U	U	U	U	U	U	U	U	U	U	U	N	N	U	U	P	U	U	**N**	28
29	N	N	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	**N**	29
30	N	N	U	N	N	N	N	N	N	N	U	N	N	N	E	N	N	N	**N**	30

	cIQc Assessment (LDT Readout)																						
Lab/ Cores	101	102	107	109	110	111	112	114	115	116	123	125	146	149	189	194	202	217	220	222	**FISH**	Lab/ Cores
1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	E	N	E	N	N	**N**	1
2	U	U	N	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	**N**	2
3	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	**P**	3
4	P	P	P	P	P	P	E	E	P	P	P	P	E	P	P	P	P	P	P	P	**P**	4
5	N	N	N	N	N	N	N	N	N	E	N	N	N	N	E	E	N	E	N	N	**N**	5
6	E	P	P	E	E	P	E	N	E	P	E	E	E	E	P	P	P	P	P	P	**P**	6
7	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	E	N	E	N	N	**N**	7
8	P	P	P	P	P	P	P	E	P	P	P	P	P	P	P	P	P	P	P	P	**P**	8
9	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	E	N	E	N	N	**N**	9
10	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	**P**	10
11	P	P	P	P	E	P	E	E	E	P	P	E	E	E	P	P	P	P	P	P	**P**	11
12	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	**P**	12
13	P	P	P	P	P	P	E	E	P	P	P	E	E	P	P	P	P	P	P	P	**P**	13
14	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	**N**	14
15	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	**N**	15
16	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	E	N	E	N	N	**N**	16
17	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	E	N	E	N	N	**N**	17
18	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	**N**	18
19	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	**N**	19
20	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	E	N	E	N	N	**N**	20
21	N	N	N	E	N	N	N	N	N	E	N	N	N	N	E	E	N	P	E	E	**N**	21
22	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	E	N	E	N	N	**N**	22
23	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	**N**	23
24	U	U	U	N	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	**N**	24
25	N	E	N	E	N	N	N	N	E	E	N	N	N	N	E	E	E	P	N	N	**N**	25
26	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	E	N	E	N	N	**N**	26
27	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	E	N	E	N	N	**N**	27
28	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	**N**	28
29	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	E	N	E	N	N	**N**	29
30	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	E	N	E	N	N	**N**	30

	cIQc Assessment (Roche Kit Readout)							
Lab/ Cores	109	116	189	194	217	222	**FISH**	Lab/ Cores
1	N	N	N	N	N	N	**N**	1
2	U	U	U	U	U	U	**N**	2
3	P	P	P	P	P	P	**P**	3
4	P	P	P	P	P	P	**P**	4
5	N	N	N	N	N	N	**N**	5
6	N	P	P	P	P	N	**P**	6
7	N	N	N	N	N	N	**N**	7
8	P	P	P	P	P	P	**P**	8
9	N	N	N	N	N	N	**N**	9
10	U	U	U	U	U	U	**P**	10
11	P	P	P	P	P	P	**P**	11
12	P	P	P	P	P	P	**P**	12
13	P	P	P	P	P	P	**P**	13
14	U	U	U	U	U	U	**N**	14
15	U	U	U	U	U	U	**N**	15
16	N	N	N	N	N	N	**N**	16
17	N	N	N	N	N	N	**N**	17
18	U	U	U	U	U	U	**N**	18
19	U	U	U	U	U	U	**N**	19
20	N	N	N	N	N	N	**N**	20
21	N	N	N	N	N	N	**N**	21
22	N	N	N	N	N	N	**N**	22
23	U	U	U	U	U	U	**N**	23
24	N	U	U	U	U	U	**N**	24
25	N	N	N	N	N	N	**N**	25
26	N	N	N	N	N	N	**N**	26
27	N	N	N	N	N	N	**N**	27
28	U	U	U	U	U	U	**N**	28
29	N	N	N	N	N	N	**N**	29
30	N	N	N	N	N	N	**N**	30

P Positive N Negative E Equivocal U Unsatisfactory

Table S1. Self-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	6050551	16 min @ 37	OptiView	Y	Y	DAB
102	DAKO PT - HIGH PH	20	5A4	1:40	NOVOCASTRA	6047123	60"	DAKO ENVISION FLEX+	YES	YES CUSO4	DAB+
107	cc1	48	5A4	1:20	Novocastra	6053444	48	Optiview	Y	Y	DAB
109	HIER high pH(CC1)	40 MIN	D5F3	RTU	ROCHE/VENTANA	F09750	16 MIN	OPTIVIEW	Y	Y	DAB
110	DAKO PT High ph 9.0@97 C	20 min	5A4	1:50	Biocare	22217	30 min	Dako Envision Flex	Y-Dako Mouse Linker	N	DAB
111	CC1	72	5A4	1/25	LEICA	6053444	60	OPTIVIEW	Y	Y	DAB
112	BOND Epitope Retrieval 2	30 minutes	5A4	1:50	Leica (Novocastra)	6036086	30 minutes	BOND Polymer Refine Detection	none	none	DAB
114	CC1	64	5A4	1/25	Biocare	22217	16	Optiview	Y	Y	DAB
115	Envision Flex High pH	30 mins	D5F3	1/100	Cell Signaling	3633S	30 mins	Envision Flex	Y	N	DAB
116	CC1	80 min	D5F3	RTU	Ventana Roche	F09750	16 min	Optiview DAB	Y	Y	DAB
149	PT Link high pH	20 min at 97 C	5A4	1:50	Novocastra	6050551	60	EnVision Flex	Yes	Yes	DAB
189	CC1	92	D5F3	pre-dilute	Ventana	unknown	16	OptiView DAB	Y	N	OptiView DAB
194	HIER (CC1)	92	D5F3	Predilute	Roche	Y10749	16	Optiview DAB	Y	Y	DAB
202	ER2	20	5A4	1/10	novocastra/leica	6050551	16	Refine Detection system	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	6051144	80 min	OptiView	Y	Y	DAB
222	Ultra CC1	92	D5F3	1:1	Roche	G05673	16	Optiview Amplification Kit	Y	Y	Copper

Table S2.
ALK IHC compared to FISH results based on cIQc expert assessment/readout. Analyses for laboratories that used DF53 were based on "cIQc Assessment (Roche Kit Readout)".

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with FISH (%)	Sensitivity	Specificity	Cohen's kappa
101	30	70	21	21/21 (100%)	1	1	1
102	30	70	21	21/21 (100%)	1	1	1
107	30	73.33	22	22/22 (100%)	1	1	1
109	30	73.33	22	21/22 (95%)	0.86	1	0.89
110	30	70	21	21/21 (100%)	1	1	1
111	30	70	21	21/21 (100%)	1	1	1
112	30	70	21	21/21 (100%)	1	1	1
114	30	70	21	20/21 (95%)	0.86	1	0.89
115	30	70	21	21/21 (100%)	1	1	1
116	30	70	21	21/21 (100%)	1	1	1
123	30	70	21	21/21 (100%)	1	1	1
125	30	70	21	21/21 (100%)	1	1	1
146	30	70	21	21/21 (100%)	1	1	1
149	30	70	21	21/21 (100%)	1	1	1
189	30	70	21	21/21 (100%)	1	1	1
194	30	70	21	21/21 (100%)	1	1	1
202	30	70	21	21/21 (100%)	1	1	1
217	30	70	21	21/21 (100%)	1	1	1
220	30	70	21	21/21 (100%)	1	1	1
222	30	70	21	20/21 (95%)	0.86	1	0.89