Assessors' report for cIQc Run 97: 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 97 consisted of a single-core tissue microarray containing 30 NSCLC cases with accompanying ALK FISH data. With eight FISH-positive cases included in the tissue microarray, a single 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 during CIQC assessment should be designated as "sub-optimal".

RESULTS

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

Lab ID	IHC Status*	Comment
101	Optimal	
102	Optimal	
107	Optimal	
109	Optimal	
110	Optimal	Slightly weak
112	Optimal	Slightly weak
113	Optimal	Slightly weak
114	Optimal	
115	Optimal	Slightly weak
120	Optimal	Slightly weak
123	Optimal	
125	Optimal	Slightly weak

Lab ID	IHC Status*	Comment
136	Optimal	Slightly weak
146	Optimal	
149	Optimal	Nice staining
160	Optimal	
189	Optimal	Background in histiocytes as commonly observed with the VENTANA ALK (D5F3) CDx Assay
194	Optimal	Background in histiocytes as commonly observed with the VENTANA ALK (D5F3) CDx Assay
202	Optimal	
207	Optimal	
217	Adequate	High background (possible issue due to an amp kit?)
220	Optimal	
230	Optimal	

*Based on cIQc assessor consensus

Lab/ Core	101	102	107	109	110	112	113	114	115	120	123	125	136	146	149	160	189	194	202	207	217	220	230	FISH
1	N	U	U	U	U	U	N	U	U	U	U	U	U	U	U	U	N	U	U	N	U	U	U	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	N
3	P	P	P	P	U	E	P	U	U	E	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	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	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
7	P	P	P	P	P	U	P	P	P	E	P	P	E	E	P	P	P	P	P	P	P	P	P	P
8	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	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	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	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	P	P
12	N	U	N	U	N	U	N	N	N	N	U	U	U	N	N	N	N	N	N	N	N	U	N	N
13	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	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	E	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	N	N
16	N	U	U	U	N	U	N	N	U	U	U	U	U	U	N	U	N	U	U	U	U	U	U	N
17	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	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
19	N	U	U	N	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	N	U	U	U	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
21	N	N	N	N	N	N	N	N	N	N	N	N	U	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
23	N	N	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	P	P
25	N	U	U	U	U	U	N	N	U	U	U	U	U	U	N	U	N	U	U	U	U	U	U	N
26	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	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
28	P	P	P	P	P	P	P	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	N	N
30	N	U	U	U	U	U	U	U	U	U	U	U	U	U	N	U	N	U	U	N	U	U	N	N

At the end of this document Supplementary Table 1 summarizes staining protocols, Supplementary Table 2 summarizes descriptive statistics, and Supplementary Table 3 provides the definitions of cIQc IHC Statuses assigned to each participant. 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. 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 Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	EnV FlexTRS, HIgh PH	1 hour	5A4	1:25	Leica	6051144	40 min	DAKO Envision Flex	Y	N	DAB
102	DAKO PT - HIGH PH	20	5A4	1:40	NOVOCASTRA	6062643	60	DAKO ENVISION FLEX+	YES	YES CUSO4	DAB+
107	cc1	64	5A4	1:20	Leica/Novocastra	6064412	48	Optiview DAB	Y	Y	DAB
109	high pH CC1	40 MIN	D5F3	RTU	ROCHE/VENTANA	E11917	16 MIN	OPTIVIEW	Y	Y	DAB
110	DAKO PT High ph 9.0@97 C	20 min	5A4	1:50	Biocare	82718	30 min	Dako Envision Flex	Y-Dako Mouse Linker	N	DAB
112	BOND Epitope Retrieval 2 pH 9.0	30 minutes	5A4	1:25	Leica (Novocastra)	6062643	30 minutes	BOND polymer refine detection	none	none	DAB
113	Dako High pH	20min	5A4	1/25	Leica	6063340	30min	Flex+30	Y	N	DAB
114	Envision Flex TRS, High pH	60	5A4	1:25	Leica (Novocastra)	6062643	40	Envision FLEX DAKO Omnis	N	Mouse Linker	Envision Flex DAB
115	HIER	30 MINS	D5F3	1/100	CELL SIGNALING	6311	30 MINS	ENVISION FLEX	N	N	DAB
120	HIER Waterbath	20	5A4	1:40	Biocare	50418	30	Envision Flex+	y	N	DAB
123	Ventana CC1	92	5A4	1/100	Novocastra/Leica	6062643	60	Ventana OptiView DAB	Y	N	DAB
136	DAKO PT HIGH	20	5A4	1/50	LEICA	6062643	30	DAKO ENVISION FLEX +	Y	N	DAB
146	Flex TRS HIGH	20	5A4	1/100	Biocare	110618	25	EnVision FLEX	N	N	DAB
149	high pH	20 min at 97 C	OTI1A4	1:1000	Origene	F004	30 min	EnVision Flex	Yes	No	DAB
160	CC1	64 MIN 100Â°	5A4	1/10	LEICA	6062643	32 MIN 36Â°	OPTIVIEW	Y (4 MIN)	Y	DAB
189	CC1	92	D5F3	pre-dilute	Ventana	unknown	16	OptiView DAB	Y	Y	OptiView DAB
194	HIER (CC1)	92	D5F3	RTU	Roche/Ventana	E11917	16	Optiview	Y	Y	DAB
202	ER2	20 MIN	SA4	1/10	NCL	6064412	15 MIN	BOND POLYMER REFINE DETECTION	NO	NO	NO
207	HIER	30	OTI1A4	1/100	Cedarlane	W003	30	EnVision Flex	Y	N	DAB
220	CC1	92 min	5A4	1/30	Leica	6058446	80 minutes	OptiView	Y	Y	DAB
230	HIER	80	5A4	PREDILUTE	LEICA	63612	64	OPTIVIEW	Y	N	DAB

Table S2. ALK IHC compared to FISH results based on CIQC expert 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	80	24	24/24 (100%)	1	1	1
107	30	83.33	25	25/25 (100%)	1	1	1
109	30	83.33	25	25/25 (100%)	1	1	1
110	30	83.33	25	25/25 (100%)	1	1	1
112	30	76.67	23	23/23 (100%)	1	1	1
113	30	93.33	28	28/28 (100%)	1	1	1
114	30	86.67	26	26/26 (100%)	1	1	1
115	30	80	24	24/24 (100%)	1	1	1
120	30	83.33	25	25/25 (100%)	1	1	1
123	30	80	24	24/24 (100%)	1	1	1
125	30	80	24	24/24 (100%)	1	1	1
136	30	76.67	23	23/23 (100%)	1	1	1
146	30	83.33	25	25/25 (100%)	1	1	1
149	30	93.33	28	28/28 (100%)	1	1	1
160	30	83.33	25	25/25 (100%)	1	1	1
189	30	96.67	29	29/29 (100%)	1	1	1
194	30	83.33	25	25/25 (100%)	1	1	1
202	30	83.33	25	25/25 (100%)	1	1	1
207	30	93.33	28	28/28 (100%)	1	1	1
217	30	80	24	24/24 (100%)	1	1	1
220	30	80	24	24/24 (100%)	1	1	1
230	30	86.67	26	26/26 (100%)	1	1	1

Table S3. IHC Status definitions.

IHC Status	Definition	cIQc Proficiency Testing Performance
Optimal	The staining was considered of the highest technical quality to allow for accurate readout of the target biomarker.	PASS
Adequate	The staining was considered to be sufficient for the purpose of accurate readout of the target biomarker.	PASS
Sub-optimal	The staining was considered to be of a quality that makes readout of the test challenging, which may lead to inaccurate readout of the target biomarker.	PASS, CONDITIONALLY[1]
Failed	The staining was considered to be of such poor quality that accurate readout of the test is unlikely or impossible.	FAIL[2]

1 – A one-time suboptimal performance qualifies for a "Pass" result. Two successive "sub-optimal" results will be designated as a "Fail".

1,2 – Please contact the cIQc for assistance and, if necessary, inform your regional regulatory body as per the terms of your laboratory's accreditation provider.