Canadian Pathology Quality Assurance
CPQA
AQCP
Assurance qualité canadienne en pathologie

Assessors' report for Run 107: ALK IHC (January 2020)

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Assessment performed March 12, 2020 at Lions Gate Hospital, North Vancouver, BC.

Health Canada's XALKORI (crizotinib) Product Monograph

Canadian laboratories are required by Health Canada to demonstrate proficiency in using a validated assay for assessment of ALK-positivity in locally advanced or metastatic NSCLC. Regular EQA for ALK (NSCLC) challenges is provided to enable laboratories to comply with Health Canada regulations. ALK validated assays 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 107 ALK IHC was initially distributed by the Canadian Immunohistochemistry Quality Control (CIQC), which has since ceased operations. This report was created and has been distributed by the Canadian Pathology Quality Assurance (CPQA). Run 107 consisted of a 30-core tissue microarray of 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 one or more false-negative result during CPQA assessment should be designated as "sub-optimal". During assessment it was noted that Cores 3 and 7 contained few tumour cells, and that some participants received sections in which the tumour was cut through.

RESULTS

ALK IHC results were excellent, with all participants that submitted slides in time for the assessment meeting having optimal staining. Participant-specific feedback is summarized below:

Lab ID	IHC Status*	Comments
101	Optimal	
102	Optimal	
109	Optimal	
107	Optimal	
110	Optimal	
112	Optimal	
113	Optimal	
114	Optimal	
115	Optimal	
120	Optimal	
125	Adequate	Weak staining

Lab ID	IHC Status*	Comments
123	Optimal	
136	Optimal	
146	Optimal	
149	Optimal	
160	Optimal	
194	Optimal	Slide broken during return shipment
202	Optimal	Slight background in some cores
207	Optimal	
220	Optimal	
230	Optimal	Slight background in some cores

*based on assessor consensus

Garrattogram after CPQA assessment:

Lab/ Core	101	102	107	109	110	112	113	114	115	120	123	125	136	146	149	160	194	202	207	220	230	FISH
1	U	U	U	U	U	N	N	U	U	N	U	U	U	U	N	U	U	U	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
3	P	P	P	U	P	E	U	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	U	N	U	N	N	N	N	N	N	U	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
7	P	P	P	U	U	P	P	P	U	P	P	U	P	P	P	U	U	U	U	P	U	P
8	N	N	N	N	N	N	N	N	E	N	N	N	N	N	N	N	N	N	N	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
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	U	N	U	N	N	N	N	N	N	N	N	N	N	N	N	N	U	N	N	U	N
13	P	P	E	P	P	P	P	P	P	P	P	E	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	E	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	N	U	U	U	N	U	U	N	U	U	U	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	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	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	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
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	U	U	U	N	U	U	N	U	U	N	U	U	U	U	N	U	U	U	N	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
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	P	P	P	P	P	E	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	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	N

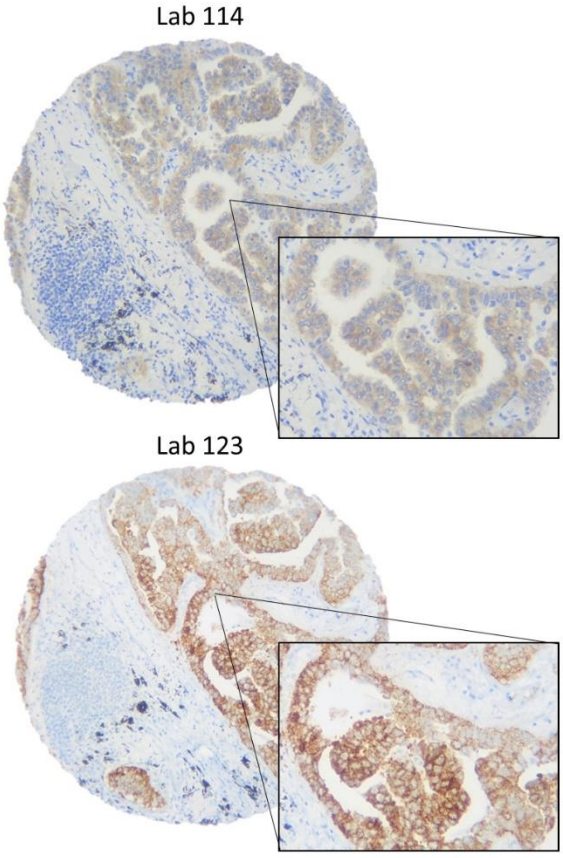

Figure 1. Representative images of the types of staining patterns that can be observed when using an amplification step. Most notably, a very granular pattern is common on Ventana platforms (Lab 123).

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 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 CPQA 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 for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	EnV FlexTRS, High PH	1 hour	5A4	1:25	Leica	6056459	40 min	DAKO Envision Flex	Y	N	DAB
102	DAKO PT - HIGH PH	20	5A4	1:40	LEICA	6064412	60" RT	DAKO ENVISION FLEX+	YES	YES CUSO4	DAB+
107	Dako FLEX TRS High pH	60	5A4	1:25	Novocastra	6071624	40	Dako FLEX	N	N	DAB
110	DAKO PT High ph 9.0@97 C	20 min	5A4	1:50	Biocare	82718	30 min	Dako Envision Flex	Y	N	DAB
112	BOND Epitope Retrieval 2 pH 9.0	30 minutes	5A4	1:25	Leica (Novocastra)	6069219	30 minutes	BOND polymer refine detection	none	none	DAB
113	High pH	30	5A4	1/25	Leica	6071624	27.5	DAKO Envision Flex HRP	N	N	DAB
114	Envision Flex TRS, High pH	60	5A4	1:25	Leica (novocastra)	6065605	40	Envision FLEX DAKO Omnis	Y	N	Envision Flex DAB
115	Envision Flex High PH	30 min	D5F3	1/100	Cell Signaling	3633S	30 min	Envision Flex	Y	N	DAB
120	HIER Waterbath	20	5A4	1:40	Biocare	21219	30	Dako Envision Flex	y	N	DAB
123	Roche CC1	92	5A4	1/100	Novocastra	6071624	60	Roche OptiView	Y	Y	DAB
136	Dako High pH	20	5A4	1:50	Leica	6071624	30	Dako Envision FLEX +	Y	N	DAB
146	FLEX TRS High	20	5A4	1:100	Biocare	112019	25	FLEX	n	n	DAB
149	high pH OMNIS	20 min at 97 C	OTI1A4	1:1000	Origene	0F004	26	EnVision Flex OMNIS	Yes	No	DAB
160	CC1	64 MIN	5A4	1/10	LEICA	6069219	32 MIN	OPTIVIEW	Y	Y	DAB
194	CC1	92	D5F3	RTU	ROCHE	E11917	16	OPTIVIEW	Y	Y	DAB
202	HIER PH9.0	20	5A4	10	NCL	6071624	15	BOND POLYMER REFINE DETECTION KIT	N	N	DAB
207	on line-high PH	30	OTI1A4	1/1000	Cederlane	W003	30	DAB Envision Flex	Y	N	DAB
220	HIER	92	5A4	1/30	NOVOCASTRA/LEICA	6071624	80	VENTANA OPTIVIEW	Y	Y	DAB
230	HIER	80	5A4	predilute	LEICA	66021	64	Optiview	Y	N	DAB

Table S2. ALK IHC compared to FISH results based on CPQA assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	30	83.33	25	25/25 (100%)	1	1	1
102	30	80	24	24/24 (100%)	1	1	1
107	30	86.67	26	26/26 (100%)	1	1	1
109	30	76.67	23	23/23 (100%)	1	1	1
110	30	80	24	24/24 (100%)	1	1	1
112	30	86.67	26	26/26 (100%)	1	1	1
113	30	90	27	27/27 (100%)	1	1	1
114	30	83.33	25	25/25 (100%)	1	1	1
115	30	80	24	24/24 (100%)	1	1	1
120	30	93.33	28	28/28 (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	83.33	25	25/25 (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	80	24	24/24 (100%)	1	1	1
194	30	80	24	24/24 (100%)	1	1	1
202	30	73.33	22	22/22 (100%)	1	1	1
207	30	83.33	25	25/25 (100%)	1	1	1
220	30	83.33	25	25/25 (100%)	1	1	1
230	30	76.67	23	23/23 (100%)	1	1	1

Table S3. IHC Status definitions.

IHC Status	Definition	CPQA 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 CPQA for assistance and, if necessary, inform your regional regulatory body as per the terms of your laboratory's accreditation provider.