Summary Report – Run 124 ALK IHC

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Overview

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 non-small cell lung cancer (NSCLC). For Run 124 ALK IHC a 4-core cell line array and a 30-core NSCLC tissue microarray were included. Participants were asked to assess only the 4-core cell line array. CPQA provided assessment of the 30-core tissue microarray for which this summary is based on, using the following score system:

- **Positive (P) –** Intense granular cytoplasmic staining in >10% of tumour cells or smooth moderately intense cytoplasmic staining in >10% of tumour cells
- **Equivocal (E) –** Weak cytoplasmic staining in >10% of tumour cells
- **Negative (N) –** No staining in tumour cells
- **Unsatisfactory (U) –** Tissue core not suitable for assessment e.g. no tumour cells, no core, etc.

If the D5F3 clone from Ventana was used as part of the VENTANA ALK (D5F3) CDx Assay kit, readout in accordance with the readout criteria specified by the manufacturer was applied.

Results

Core 3 contained few tumour cells, but was still sufficient to be included in assessment. All of but one laboratory achieved either an "Adequate" or "Optimal" status. Accurate readout of the single "Sub-optimal" laboratory was still possible, but it is important to be mindful that assessors have an inherent advantage when reviewing participant staining as they become more familiar with expected staining of the challenge material. Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comment
101	Optimal	Slight background in some cores
102	--	Technical issue encoutered; slide withdrawn
107	Optimal	Slightly weak
110	Optimal	
111	Optimal	
112	Adequate	Weak staining
113	Optimal	Slightly weak
114	Optimal	
120	Adequate	Weak staining
123	Optimal	
125	Sub-optimal	Very weak staining
136	Optimal	
146	Optimal	
149	Optimal	Slightly weak
160	Optimal	
194	Optimal	
202	Optimal	Slight background in some cores
207	Optimal	Slight background in some cores
220	Optimal	
230	Optimal	

*based on CPQA assessor consensus

Garrattogram after CPQA assessment:

Lab/ Core	101	107	110	111	112	113	114	120	123	125	136	146	149	160	194	202	207	220	230	FISH
1	U	U	N	N	U	U	U	U	N	N	N	U	N	U	U	N	N	U	U	N
2	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	E	P	E	P	P	E	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
5	U	U	N	N	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	N
6	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	P
8	N	N	N	N	N	N	N	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
10	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
12	U	U	N	U	U	U	U	U	N	U	N	U	U	N	U	U	U	N	N	N
13	P	P	P	P	E	P	P	E	P	E	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
15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
16	N	N	N	N	N	N	N	U	N	N	N	N	N	N	N	N	N	N	N	N
17	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
19	U	U	N	N	U	U	U	U	U	U	U	U	N	N	N	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
21	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
23	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
25	N	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	U	N	U	N
26	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
27	U	U	N	U	U	U	U	U	N	U	U	U	U	N	N	N	U	U	N	N
28	P	E	P	P	P	E	P	E	P	E	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
30	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	N

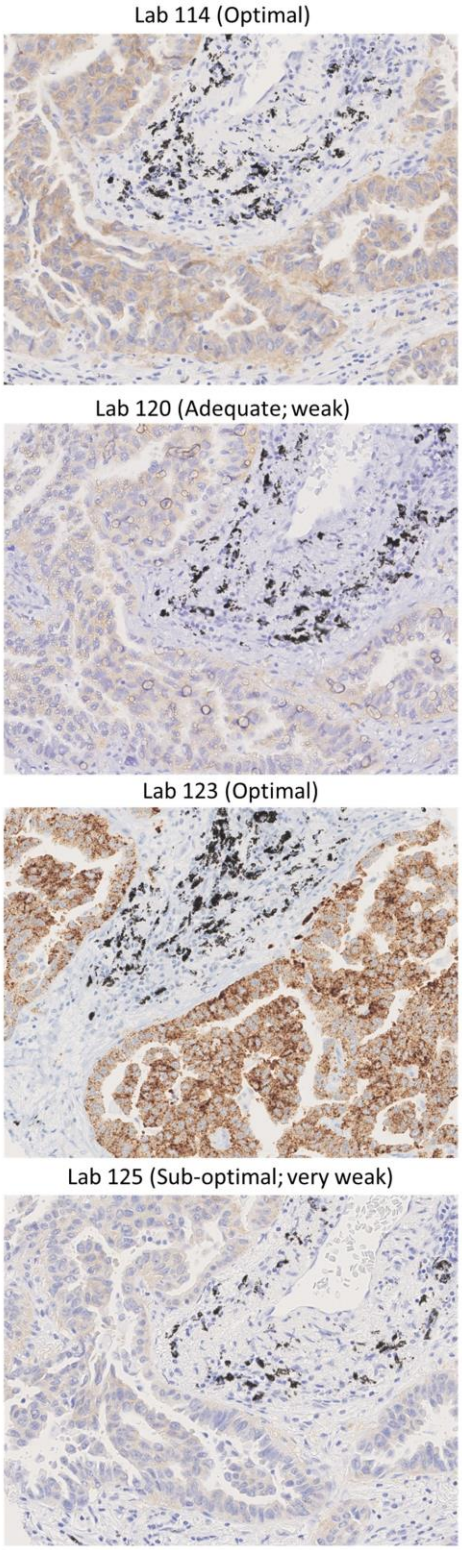

Figure 1. Representative images of the qualitative variability of ALK staining.

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Supplementary Table 1 summarizes the reported staining protocols, which can be referred to during validation or optimization of a staining protocol. Supplementary Table 2 summarizes descriptive statistics based on CPQA assessment. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with this reservation. Supplementary Table 3 provides the definitions of IHC Status and recommended participant action. 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.

Scanned images in this report were acquired using a NanoZoomer SQ that has been graciously loaned to the CPQA-AQCP by Quorum Technologies and Hamamatsu.

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Table S1. Reported ALK IHC staining protocols.

Lab ID	Platform/instrument	LDT or IHC kit	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	DAKO OMNIS	LDT	EnVision FLEX TRS HIGH pH	1 Hr	5A4	1:25	LEICA	6073257	40 MIN	DAKO Envision FLEX	N	N	DAB
107	Dako Omnis	LDT	Dako FLEX TRS High pH	60	5A4	1:25	Leica Novacastra	6077297	40	Dako FLEX	N	N	Dako FLEX DAB
110	DAKO Autostainer Link 48	LDT	DAKO PT High ph 9.0@97 C	20 min	5A4	1:50	Biocare	112019	30 min	Dako Envision Flex	Y	N	DAB
111	ULTRA BENCHMARK	IHC	HIER	72	5A4	1/25	LEICA	6077297	60	OPTIVIEW	YES	YES	DAB
112	BOND III	LDT	BOND Epitope Retrieval 2 pH 9.0	30 minutes	5A4	1:25	Leica (Novacastra)	6077297	30 minutes	BOND polymer refine detection	no	no	DAB
113	Dako OMNIS	LDT	High pH	30min	5A4	1/25	Leica	6073257	27.5	Flex+	Y	N	DAB
114	Dako Omnis	LDT	Envision Flex TRS, high pH	60	5A4	1:25	Leica (Novocastra)	6069219	40	Envision Flex DAKO Omnis	Y	N	Envision FLEX DAB
120	Autostainer Link48	LDT	HIER Waterbath	20	5A4	1:40	Biocare	0042020A	30	Dako Envision Flex	Y	N	DAB
123	Ventana Benchmark Ultra	LDT	Ventana CC1	92	5A4	1/100	Leica	6078682	60	Ventana OptiView DAB	Y	N	DAB
125	Dako Omnis	LDT	HIER	30	5A4	1/50	NCL	6073257	20	EnVision Flex	Y	N	DAB
136	DAKO AS480	LDT	DAKO PT HIGH	20	5A4	1:50	LEICA	6077297	30	DAKO ENVISION FLEX +	Y	N	DAB
146	Autostainer link 48	LDT	HIER	20	5A4	1:100	Biocare medical	042020-A	25	Flex EnVision	n	n	DAB
149	OMNIS	LDC	high pH 97 C	32	OTI1A4	1:1000	Origene	W0030	26	EnVision Flex	Yes	No	DAB
160	VENTANA	LDT	CC1	64MN	5A4	1/10	leica	6075602	32MN	optiview	Y	Y	DAB
194	ULTRA	KIT	CC1	92	D5F3	RTU	Roche	F15304	16	Optiview	Y	Y	DAB
202	LEICA BOND III	LDT	HIER Ph 9.0	20	5A4	1/10	NCL-LEICA	6077297	15	POLYMER REFINE DETECTION KIT	N	N	DAB
207	DAKO OMNIS	ldt	Online-High PH	30 minutes	1A4	1/1000	Cederlane	W0003	30 minutes	Dako Envision DAB	Y	Y	DAB
220	BenchMark ULTRA	LDT	HIER	92	5A4	1:30	Novocastra/Leica	6071624	80	Optiview	Y	N	DAB
230	Benchmark Ultra	LDT	HIER	80	5A4	predilute	LEICA	67447	64	OPTIVIEW	Y	N	DAB

Table S2. Descriptive statistics based on CPQA assessment. An equivocal "E" result is assumed to proceed to further confirmatory testing (i.e. FISH) and is, therefore, considered correct for the purposes of this analysis.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	30	76.67	23	23/23 (100%)	1	1	1
107	30	73.33	22	22/22 (100%)	1	1	1
110	30	93.33	28	28/28 (100%)	1	1	1
111	30	86.67	26	26/26 (100%)	1	1	1
112	30	76.67	23	23/23 (100%)	1	1	1
113	30	76.67	23	23/23 (100%)	1	1	1
114	30	76.67	23	23/23 (100%)	1	1	1
120	30	73.33	22	22/22 (100%)	1	1	1
123	30	86.67	26	26/26 (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	76.67	23	23/23 (100%)	1	1	1
149	30	83.33	25	25/25 (100%)	1	1	1
160	30	86.67	26	26/26 (100%)	1	1	1
194	30	83.33	25	25/25 (100%)	1	1	1
202	30	86.67	26	26/26 (100%)	1	1	1
207	30	76.67	23	23/23 (100%)	1	1	1
220	30	80	24	24/24 (100%)	1	1	1
230	30	80	24	24/24 (100%)	1	1	1

Table S3. Proficiency Testing Definitions of IHC Status.

IHC Status	Definition	Proficiency Testing Performance
Optimal	All expected targets are identified appropriately and demonstrate the expected staining intensity. Absence of non-specific staining (no background staining).	**PASS**
Adequate	All targets are identified, but intensity of staining is weaker than optimal or there is false-positive staining which does not interfere with interpretation.	**PASS**
Sub-optimal	None or only some targets are identified OR all targets are identified, but false-positive staining may interfere with interpretation.	**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]**
Unsatisfactory	Technical issue (e.g. unsuitable antibody selection, etc.)	**N/A**

1 – A one-time sub-optimal 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.