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Assessors' report for CIQC Run 41: ALK Lung Cancer (September 2014)

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Assessment performed on Thursday, November 27, 2014 at Toronto General Hospital, Toronto, Ontario

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-mpps/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 41 consisted of 5 cores of lung cancer tissue with known ALK status. The main purpose of this challenge was to facilitate intra-laboratory calibration of ALK staining, to do this the assessment team evaluated the intensity and specificity of the staining.

The target of 100% agreement (by expert assessment) for both positive and negative results was achieved by all laboratories when the (IHC) is considered a screening test. Five laboratories had optimal staining, which required that labs clearly demarcate positive and negative ALK cases. For eight laboratories, assessors felt that the staining was adequate and showed sufficient staining intensity to call the cases positive or negative without the need for confirmatory FISH testing. These labs were graded as satisfactory. Three laboratories had equivocal results which should reflex for FISH testing. These labs were also graded as satisfactory since patient treatment would not have been withheld because of a false negative result. No false negative results were observed based on expert assessment. One laboratory (114) did call Core 2 negative on self-assessment, but this core was considered equivocal by assessors.

Overall, assessors noted a wide variation in staining in the labs graded as satisfactory. The clean, well-defined staining performed by Labs 102, 112, 123, 189 and 202 does indicate that it is technically possible for the laboratories receiving a "satisfactory" grade to improve their staining.



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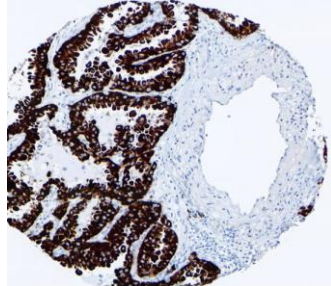
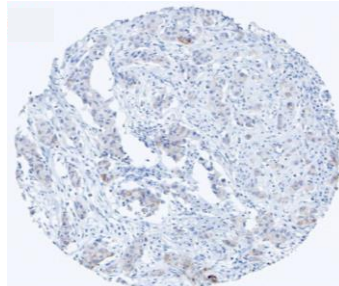
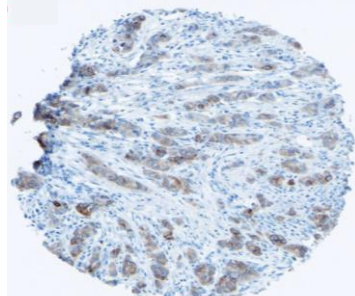
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The assessors evaluated staining intensity of all positive cores, refining the self-assessment scoring parameters as follows:

Score*	Description
4+	Very strong positive
3+	Strong positive
2+	Clearly positive but lacks intensity
1+	Positive but very weak
E	Equivocal
N	Negative

*Background staining was also specifically noted whenever present.

Assessor reassessment scores for Cores 1 to 5 and participant-specific feedback is summarized below:

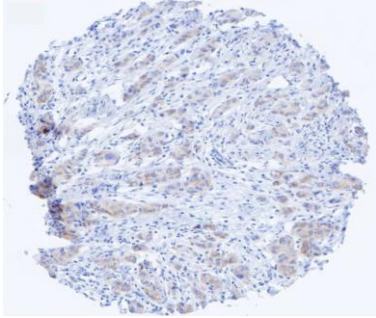
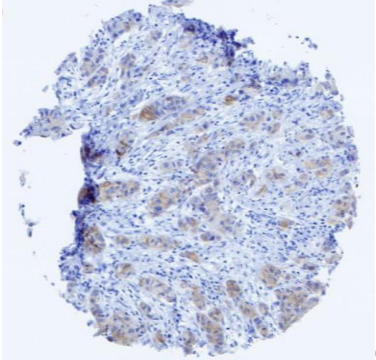
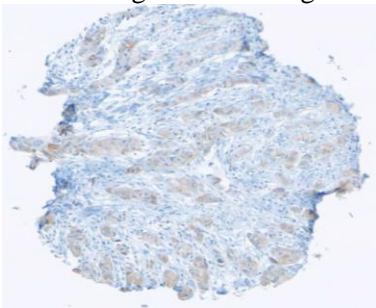
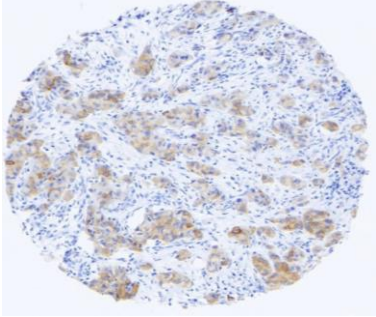
Lab	IHC Status*	Reassessment Score	cIQc Comments
101	Satisfactory	1: Strong staining 4+	<p>Clean with no background. Very strong staining of Core 1 highlighted the weak (equivocal) staining in Core 2. This may be a reflection of the use of amplification with OptiView.</p>  <p>Core 1</p>  <p>Core 2</p>
		2: E	
		3: Weak positive 2+	
		4: N	
		5: N	
102	Optimal	1: 3+ Strong positive	<p>No background staining observed.</p>  <p>Core 2</p>
		2: 2+ Clearly positive with some darker cells	
		3: 2+ Clearly positive	
		4: N	
		5: N	



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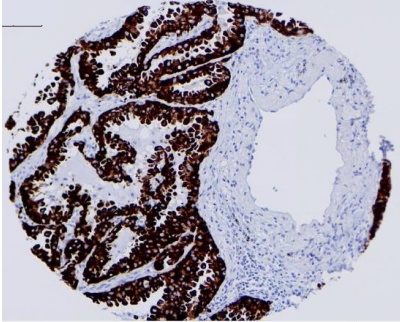
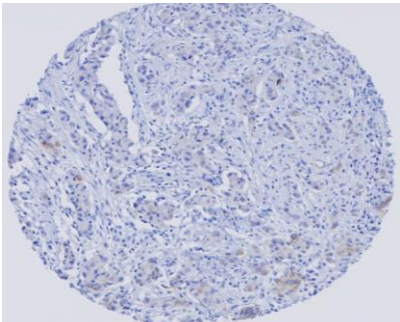
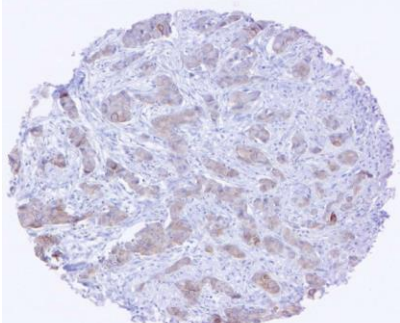
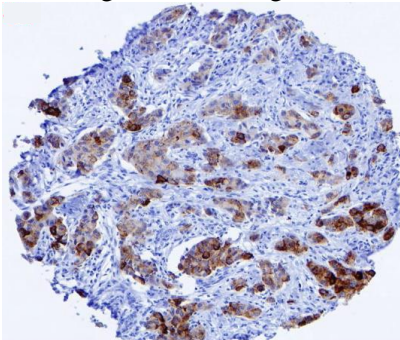
107	Satisfactory	1: 3+	<p>No background staining observed. For clinical purposes it is good but staining could be improved.</p>  <p>Core 2</p>
		2: 1+	
		3: 1+	
		4: N	
		5: N	
110	Satisfactory	1: 3+	<p>No background staining observed.</p>  <p>Core 2</p>
		2: 1+	
		3: 1+	
		4: N	
		5: N	
111	Satisfactory	1: 2+ Staining could be stronger	<p>Some background staining observed.</p>  <p>Core 2</p>
		2: 1+	
		3: 1+	
		4: N	
		5: N	
112	Optimal	1: 3+	<p>No background staining observed.</p>  <p>Core 2</p>
		2: 2+	
		3: 2+	
		4: N	
		5: N	



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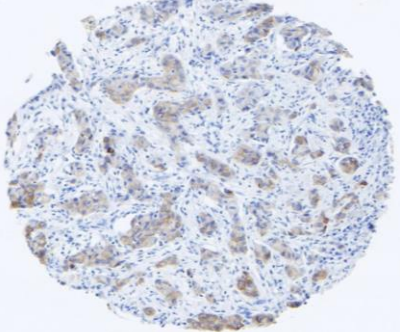
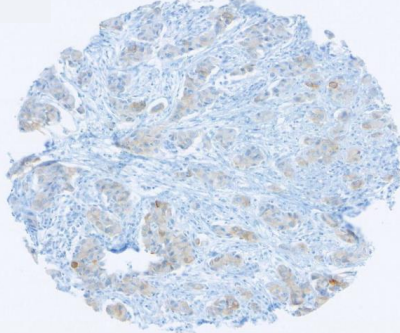
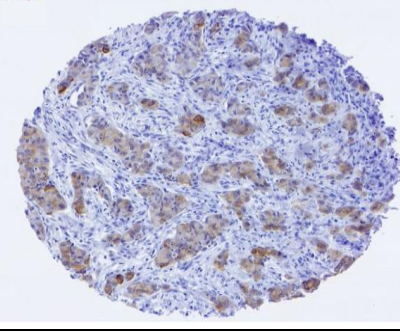
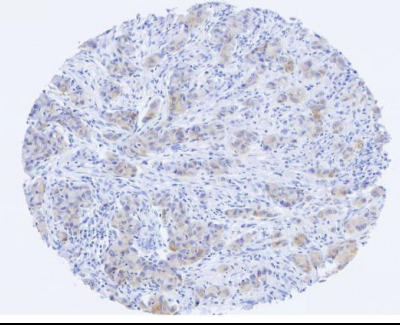
114	Satisfactory	1: 4+	<p>Very strong staining in Core 1 highlighted the weak (equivocal) staining in Core 2. This may be a reflection of the use of amplification with OptiView</p>  <p>Core 1</p>  <p>Core 2</p>
		2: E	
		3: 2+	
		4: N	
		5: N	
115	Satisfactory	1: 3+	<p>Background staining observed.</p>  <p>Core 2</p>
		2: 1+	
		3: 1+	
		4: N but background staining	
		5: N	
116	--	--	Slide not available for review.
123	Optimal	1: 4+	<p>No background staining observed.</p>  <p>Core 2</p>
		2: 3+	
		3: 2+	
		4: N	
		5: N	



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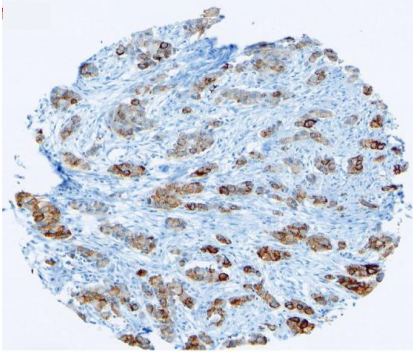
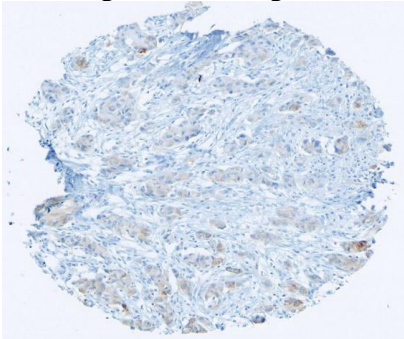
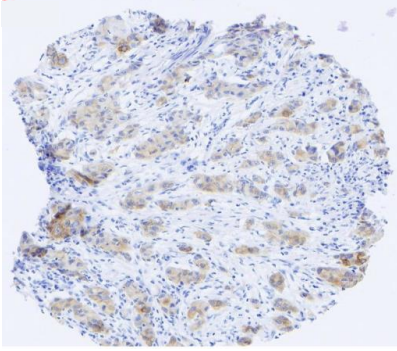
125	Satisfactory	1: 3+	Some background staining observed.  Core 2
		2: 2+	
		3: 2+	
		4: N	
		5: N	
137	Satisfactory	1: 2+	No background staining observed.  Core 2
		2: 1+	
		3: E	
		4: N	
		5: N	
146	Satisfactory	1: 3+	No background staining observed. Hematoxylin was very dark.  Core 2
		2: 2+	
		3: 2+	
		4: N	
		5: N	
149	Satisfactory	1: 3+	No background staining observed.  Core 2
		2: 1+	
		3: 1+	
		4: N	
		5: N	



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189	Optimal	1: 3+	<p>Significant background observed, however, this did not interfere with interpretation.</p>  <p>Core 2</p>
		2: 2+	
		3: 2+	
		4: N	
		5: N	
191	Satisfactory	1: 3+	<p>No background staining observed.</p>  <p>Core 2</p>
		2: 1+	
		3: 1+	
		4: N	
		5: N	
202	Optimal	1: 3+	<p>No background staining observed.</p>  <p>Core 2</p>
		2: 2+	
		3: 2+	
		4: N	
		5: N	

*Based on CIQC expert assessment.



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ALK FISH

The difficulty in running an EQA program was highlighted in the results received for Run 41. cIQc does not review slides from ISH and relies on self-assessment and self-reporting of protocols. Eleven laboratories participated in the survey which, for the most part, matched with the reference facility. However, what was a concern was the large number of “unsatisfactory” results from participants. Since we do not review ISH slides it is difficult to determine if there are physical issues with the tissue (core loss) or interpretative issues (signal loss).

Lab 125 had a negative result for Core 1 which was clearly demonstrated to be positive by other laboratories using both IHC and FISH. It is advised that Lab 125 review this case.

Protocols submitted by laboratories showed a variety of different protocols for what is supposed to be the definitive test for ALK as per Health Canada (see above). cIQc will be reviewing the self-assessment process and requested protocol fields so that we can remove some of the ambiguity. Please contact cIQc (www.ciqc.ca) if you wish to have more sections from Run 41.

Garrattograms for ALK ISH and ALK IHC based on self-assessment and expert assessment are provided in Supplementary Figure 1. Supplementary Table 1 summarizing staining protocols can also be found at the end of this document. Your regular participation in cIQc is greatly appreciated and we look forward to continually working with you and the Canadian Association of Pathologists – Association Canadienne des Pathologistes.

Submitted by J Garratt

Table S1a. Reported ALK IHC staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/ Vendor	Ab Lot #	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
101	CC1	64	5A4	1:50	Leica	6028806	16	OptiView	Y	Y	DAB
102	DAKO 3 IN 1, High pH	10/20/10	5A4	1:50	Novocastra	6017168	1 hour	Dako Flex+Mouse	Yes	Yes	DAB+
107	Benchmark XT cc1	48	5A4	1:20	Novocastra	6028806	48 min at 37 C	Optiview	N	N	DAB
110	PT - pH9	20 min	5A4	1:50	Biocare	51914	30 min	Dako FLEX	15 min LINKER	N	DAB
111	CC1	92 min	5A4	1/10	Leica	6028806	32 min	Ultraview	Yes	Copper	DAB
112	BOND EPITOPE RETRIEVAL 2 pH 9.0	30 minutes	5A4	1:100	Novocastra "Leica"	6028806	15 minutes	BOND Polymer Refine Detection	No	No	DAB
114	CC1	64min	5A4	1:25	Biocare	80513	16	Ventana Optiview	y	y	DAB
115	CC1	90	5A4	1 in 10	Novocastra	6028806	60 min	Ultraview DAB + ampli	yes	no	DAB
116	CC1	64 min	ALK-1	1/25	Cell Marque	8805 H	60min	Optiview DAB Ventana	YES	YES	DAB
123	CC1	92	5A4	1:100	Leica	6028806	60	OptiView	Y	Y	DAB
125	BOND ER2	20	5A4	1/50	Novocastra	6012332	15	BOND Polymer Refine	N	N	DAB
137	CC1	92	5A4	1:30	Leica	6024268	120	Opti-View	N	N	DAB
146	Flex TRS High	20	5A4	1/100	BioCare	21014	25	Envision Flex	N	N	DAB
149	PT Link pH 6.0	20	5A4	1:50	Novocastra	10083723	30	EnVision Flex	Yes	No	DAB
191	CC1	92	D5F3	RTU	Roche	D10417	16	optiview DAB	y	y	
202	ER2	20	SP8	1/10	NOVOCASTRA	6028806	16 MIN	REEFINE DETECTION KIT	N	N	DAB

Table S1b. Reported ALK ISH protocols.

Lab ID	Supplier/Vendor	Probe	Instrument	Hybridization Time	Pretreatment Time	Post-hybridization Wash Reagent	Post-hybridization Wash Time/Temp
107	??	Vysis ALK dual colour break apart	??	18 hrs	Hct:22min, NASCN:40min, Pepsin:15min	??	??
111	Intermedico	ALK break apart	VP 2000	16 hours	160 minutes	2xSSC, 0.3% NP-40	72C, 2 min
115	Abbott	Abbott	Thermabrite	15 hours at 37C	5 min at 73C	Vysis pretreatment solution	20 min at 80C
123	Abbott Molecular/Intermedico	Vysis LSI ALK Dual Colour	Thermobrite/Hybrite	Overnight	2hr NaCitrate/15 mins pepsin	2xSSC/dH2O	5 min @ RT / 5 min @ RT
125	Abbott Vysis	ALK	Thermobrite	16 hours	12 min	protease	20 min
137	Abbott Molecular	LSI ALK Breakapart	Thermobrite	18 hours	2 hrs. sodium citrate buffer; 50 minutes 0.01N HCl with pepsin	none	none
146	Agilen	Sure FISH ALK BA	Hybrideur StatSpin, Dako	18h	30 minutes	N/A	N/A
186	DAKO	ALK	ABBOTT THERMOBRITE	16 HOURS	20 MIN	2XSSC/0.3%NP-40	2.5 min at 73 C
191	Vysis	alk break apart	manual	ON	20min	2xSSC	2x5min 2xSSC
202	VYSIS	ALK BREAK APART FISH PROBE KIT	HYBRITE	73C 3 MIN	37C FOR 18 HRS	post hybridization wash buffer kit (Vysis)	12 min at 80C
211	Abbott Molecular	ALK Breakapart Rearrangement Probe	ThermoBrite	20 hours	12 minutes	sterile water	3 minutes at room temperature