building towards
canadian Immunohistochemistry Quality control
cIQc

Report for cIQc Run 58 ALK ISH.

July 13, 2016

Run 58 consisted of a slide with a 3-core TMA of Non-Small Cell Lung Cancers seeded with ALK positive cases. Labs were requested to stain the slide as per their usual protocol.

Laboratories were asked to interpret ALK (ISH) using their own recording system and enter the results in TMA Scorer http://www.tmascorersystem.ca/login.php using the cIQc standardized scoring system.

P – Positive: rearranged
N – Negative: non-rearranged
U – Unsatisfactory for analysis due to core loss or disruption
F – Failed staining – unable to interpret

15 sites participated in this challenge, with all sites providing excellent concordance with the reference site.

Run 58 Results

Labs/ Cores	111	115	116	123	137	146	149	160	162	186	191	197	202	211	216	Ref
1	P	P	P	P	P	F	P	P	P	P	P	P	P	P	P	P
2	U	N	N	N	N	N	N	U	N	N	N	N	N	N	N	N
3	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

Run 58 Protocols

11 of the 15 participating laboratories used the ALK Break Apart Kit from Vysis (Abbott Molecular) and distributed by Inter Medico in Canada.
One laboratory used the Zytovision probe. Zytovision is a German company and its products are distributed by Bio SB in the USA
Two labs used the SureFISH ALK break apart product from Agilent (Dako)

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."

Protocols

Laboratories	111	115	116	123	137	146	149	160	162	186	191	197	202	211	216
Supplier/Vendor	Intermedico	Vysis	Vysis	Vysis/Abbot Labs	Abbott	Agilent	Abbott Molecular	Abbott	Zytovision	Dako	Vysis	InterMedico (Abbott)	ABBOTT	Abbott Molecular	Abbott Molecular
Probe	Vysis ALK Break-apart probe	Vysis LSI ALK Dual Color Break Apart FISH probe	LSI ALK Dual Color	LSI ALK	ALK	SureFISH ALK break apart	LSI ALK Dual Color Breakapart Probe	Vysis LSI Dual color breakapart	ALK/ENL4	ALK	ALK FISH	ALK dual colour break apart	alk KIT 06N38-020	ALK Dual Colour Break Apart Probe	Vysis ALK Break Apart FISH Probe Kit
Instrument	VP 2000	Hybridizer	HyBridazer Dako	Thermobrite	Thermobrite	StatSpin Dako hybridizer	Thermobrite for denaturation step only	Manual	Hubridizer	Abbott - Thermobrite	None	n/a	HYBRITE	Thermobrite	Thermobrite S500-12
If manual protocol, please specify (N/A if not manual)	n/a	Manual	NA	manual	yes	manual	Manual protocol according to Abbott protocol	Manual	DAKO	N/A	Manual	Manual with denaturation on Thermobrite S500-12	NA	ALK protocol from Abbott	Protocol. Use Xylene instead of Hemo-De; Use 10mM
Denaturation Time/Temp	5 min/ 74C	2 min @ 73 degrees celcius	NA	5min/74C	5min/74c	5min/75C	73C for 6 minutes	3min/80C	10 min 75 C	73deg fro 5 minutes	5min/75C	5 min, at 75C	73C 3 MIN	3 min / 73C	5 minutes/ 74C
Hybridization Time/Temp	16 hrs/ 37C	15 hours @ 37 degrees celcius	24h /37C	overnight/37C	overnight/37c	18h/37C	Humidified chamber, overnight at 37C	18hr/37C	37 C over night	37 deg for 16 hours	ON/37C	18 hours/overnight, 37C	37C 18 HRS	20 hours / 37C	Overnight/ 37C
Pre-treatment reagent/time/temp	10mM Na citrate/ 160 mins/ 80C	Vysis pretreatment solution 1N NaScn 80 degrees celcius 20 minutes	NA	NaCitrate/2hr/RT, 2xSSC/5min/RT	sodium citrate/2hrs/80c	HCl/20min/TP, NaCitrate/30min /97C	8% sodium thiocyanate at 80C for 6 min	1N Sodium thiocyanate	DAKO 10 min 100 C MWO	80 deg for 20 minutes	NASCN 30min/80Â°C	1) HCl 22min RT, 2) NaSCN 40min 80C	80C 12 MIN	Abbott pretreatment solution / 12 min / 80C	10mM Sodium Citrate/ 2.5hrs/ 80C
Proteolytic digestion reagent/time/temp	pepsin in 0.01N HCl/ 35 mins/ 37C	Vysis Protease Buffer IV and pepsin 37 degrees celcius 20 minutes	NA	Pepsin/15min/37C	pepsin/15min/37c	Pepsin/18min/37C	0.2g pepsin in 50mL 0.2N HCL at 37C for 6 min	Pepsin/50min/37C	DAKO 10 min room temp	37deg for 20 minutes	Pepsisne/20min/37Â°C	Pepsin 15min 37C	PEPSIN 37C 20 MIN	Abbott protease solution / 7 min / 37C	Pepsin in HCl Soln/ ~20 min/ 37C
Post-hybridization wash time/temp	2 mins at 72C, 1 min at 4C	wash buffer 1 @ RT 7 minutes, wash buffer 2 @ 72 degrees celcius 3 minutes	4min/ 70Â°C	2min/72C then 1min/RT	2min@72c, 1min@room temp	2XSSC/2min/RT, 2XSSC 0.3% tween/2min/75C	2xSSC + 0.3% NP-40 at 72C for 2 min	2xSSC pH7.5/4min/65C	DAKO 5 min 65C	73deg for 2.5 minutes	2xSSC/0,3%NP 40/2min/73Â°C	2xSSC 2 min 73C, PBS rinse RT	74C 2 MIN	2 min / 74C	1. "2xSSC/0.3%NP 40" until coverslip fall off at Room Temp 2. "2xSSC/0.3%NP 40" at 72C for 2