Report for cIQc Run 71: ALK ISH (February 2017)

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."

Run 71 slides were kindly provided by HistoCyte Laboratories Ltd. The two-core cell line microarray was comprised of one cell line of breast adenocarcinoma origin (Core 1) that is negative for ALK by immunohistochemistry (IHC), with no ALK translocation detected by fluorescence in situ hybridization (FISH). Core 2 was derived from a pulmonary adenocarcinoma and is positive for the EML4-ALK translocation as determined by the presence of split signals by FISH. The fusion protein is readily detected with antibodies optimized to detect the relatively low expression. Extensively characterized at multiple sites with multiple IHC and FISH assays, these cell lines have been validated as suitable quality control material.

Laboratories were asked to stain the slides and interpret ALK (ISH) using their own recording system and submit results into 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

12 sites participated in Run 71, with all sites having excellent concordance with the reference site as shown in the Garrattogram below.

Labs/Cores	107	111	115	116	137	146	191	194	202	211	217	229	R1
1	N	N	N	U	N	N	N	N	N	N	N	N	N
2	P	P	P	U	P	P	P	P	P	P	P	P	P

Run 71 Protocols

Eleven of the 12 participating laboratories used the ALK Break Apart Kit from Vysis (Abbott Molecular) and distributed by InterMedico in Canada. A single lab used the SureFISH ALK break apart product from Agilent (Dako).

Labs/Fields	107	111	115	137	146	191	194	202	211	217	229
Supplier/Vendor	Abott	Vysis/ Intermedico	Abbott	Abbott Molecular	Agilent	Vysis	Abbott Molecular	Abbott	Abbott Molecular	Abbott Molecular-Vysis	Abbott
Probe	ALK breakapart	ALK Break-apart probe	ALK (Abott)	ALK	SureFISH ALK break apart	ALK	Vysis ALK Break Apart FISH Probe Kit	Vysis ALK	Vysis ALK Break Apart	ALK 2 color break Apart probe	ALK
Instrument	n/a	VP2000/ Hybrite	Thermobrite Hybridizer	Thermobrite	StatSpin Dako hybridizer	none	Thermobrite S500-12	Hybrite	Thermobrite	N/A	Thermobrite
If manual protocol, specify method (use N/A if not manual)	see below	N/A	Manual	Ours	manual	manual	Manual Protocol. Use Xylene instead of Hemo-De; Use 10mM sodium citrate buffer instead of Vysis Pretreatment Soln; Use Pepsin in 0.01N HCl instead of 'Vysis Protease Soln'.	n/a	manual as per vendor procedure	N/A	CLS Procedure: Processing Paraffin Embedded Tissue Sections for FISH
Denaturation time/temp	5 min, 75Â°C	5 mins/ 74C	2 Min at 73C	5min/74c	5min/75C	75Â°C	5 minutes/ 74 C	3 min/73C	73C / 3 minutes	5min at 75Â°C	73 degrees C
Hybridization time/temp	overnight, 37Â°C	16 hrs/ 37C	15 hours at 37 Â°C	overnight/37c	18h/37C	37Â°C	Overnight/ 37C	18 hrs/37C	37C / 20 hours	37Â°C overnight (16h)	37 degrees C
Pre-treatment reagent/time/temp	HCl for22 min,RT; NaSCN 40 min, 80Â°C	10mM Na citrate/ 160 mins/ 80C	Abbott Fish pretreatment buffer 1M Sodium Thiocyanate , 20 min , 80 C	sodium citrate/2hrs/80 c	HCL/20min/TP, NaCitrate/30min/97C	1m NASCN/80Â°C /30min	10mM Sodium Citrate/ 2.5hrs/ 80C	20 min/80C	Vysis Paraffin Pretreatment Kit IV / 12 minutes / 80C	sodium citrate 0.01M 6min at 92Â°C	Abbott Pretreatment Solution/10min /80 degrees C
Proteolytic digestion reagent/time/temp	Pepsin 15-18 min, 37Â°C	pepsin/ 35 mins/ 37C	Vysis protease Buffer IV + pepsin / 20 min at 37C	pepsin/15min/37c	Pepsin/18min/ 37C	pepsine/20min/ 37Â°C	Pepsin in HCl Soln/ ~20 min/ 37C	citrate buffer /5min/123c	Vysis Protease IV / 7 minutes / 37C	Pepsin 2.5mg/ml for 15min at 37Â°C	Pepsin/15min/ 37 degrees C
Post-hybridization wash time/temp	2xSSC/0.3% Igepal, 2 min, 73Â°C	2 mins/ 72C	Rinse RT then 3 minutes at 72Â°	2min@72c, 1min@room temp	2XSSC/2min/RT, 2XSSC 0.3%tween/2min/75C	0,2x SSC/0,3%NP40/ 2min/73Â°C	1. "2xSSC/0.3% NP40" until coverslip falls off at Room Temp 2. "2xSSC/0.3% NP40" at 72C for 2 min.	10 min	Wash Buffer II 2 minutes / 74C	2min at 74Â°C	2min/73 degrees C then 1 min/Room Temp

Prepared by: John Garratt, May 24th 2017