building towards
cIQc
canadian Immunohistochemistry Quality control

Summary of cIQc Run 45: ALK FISH (February 2015)

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."

Overview

cIQc does not review slides from FISH 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. The Garrattogram for ALK FISH based on self-assessment is provided in Supplementary Figure 1. Supplementary Table 1 summarizing 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.

Figure S1. Garrattogram from self-reported assessment of ALK FISH.

Lab/ Core	111	115	123	137	146	162	186	191	197	202	217	R1
1	P	U	P	P	F	P	P	P	P	N	P	P
2	N	U	N	N	N	N	N	N	N	P	N	N
3	P	P	P	P	P	P	P	P	F	P	P	P

Table S1. Reported ALK FISH protocols.

Lab ID	Supplier/Vendor	Probe	Instrument	If manual protocol, please specify (input N/A if not manual)	Denaturation time/temp	Hybridization time/temp	Pre-treatment reagent/time/temp	Proteolytic digestion reagent/time/temp	Post-hybridization wash time/temp
111	Intermedico	Vysis ALK Break Apart Probe	VP 2000, Hybrite	NA	5 mins @ 74C	16 hrs @ 37C	10mM sodium citrate, 160 mins @ 80C	pepsin in 0.01N HCL, 35 mins @ 37C	2 mins @ 72C, then 1 min @ 4C
115	Abbott	ALK (Abbott)	Thermobrite Hybridizer	Manual	5 min at 73C	overnight 37C	Abbott Fish Pretreatment buffer 1M Sodium Thiocyanate, 30min, 80C	20 min at 37C	8 min RT, 5 min at 72C
123	Vysis	ALK	Thermobrite	manual	5min/74C	overnight/37C	NaCitrate/2hr/RT, 2xSSC/5min/RT	Pepsin/15min/37C	2min/72C then 1min/RT
137	Abbott Molecular	LSI ALK break apart	Thermobrite	Manual protocol	5 min at 74C	18 hrs, 37C	2 hrs, sodium citrate buffer, 80C,	0.01N HCl with pepsin, 15 min, 37C	0.4xSSC/0.3% NP40, 72C, 2 min followed by 2xSSC/0.1% NP40, RT, 1 min
146	Agilent	SureFISH ALK break apart	StatSpin Dako hybridizer	manual	5min/75C	18h/37C	HCl/20min/TP, NaCitrate/30min/97C	Pepsin/18min/37C	2XSSC/2min/RT, 2XSSC 0,3% tween/2min/75C
162	Zytovision	ZytoLight ALK/EML4	Hybridizer	dako histology fish accessory kit	75 C 10 min	37 C over night	10min MVO 15 min room temp	10 min room temp	5 min 65 C
186	Dako	Alk	Abbott- Thermobrite	N/A	73 deg for 5 minutes	37 deg for 16 hours	80 deg for 20 minutes	37 deg for 20 minutes	73 deg for 2.5 minutes
191	Abbott/Vysis	ALK/EML4	Manual	Vysis protocol	5min/75°C	37Â°C/ON	NASCN/30min/80°C	Pepsine/20min/37°C	73Â°C/2min
197	Abbott	ALK dual colour breakapart	Hybrite for denaturing & hybridizing	Staining jars in temp controlled H2O baths	5 min at 75°C	18 hours at 37°C	HCl 22min at RT, NaSCN 40 min at 80°C	Pepsin 15 min at 37°C	2xSSC/0.3% Igepal 2 min at 73°C, PBS rinse
202	Abbott	Vysis LSI ALK Dual colour Break apart FISH probe	DAKO Hybridizer	Manual	3 minutes@ 73°C	18 hours@37°C	Vysis pretreatment Solution 12 minutes@ 80C	Protease 20 minutes @ 37 degrees C	2 minutes @74C