Validation of Vysis LSI ALK and ROS1 Break Apart FISH Probes

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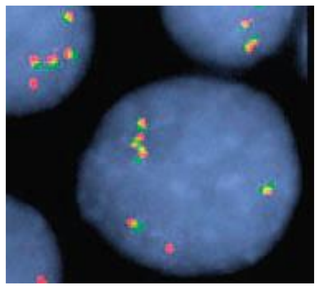
INTRODUCTION

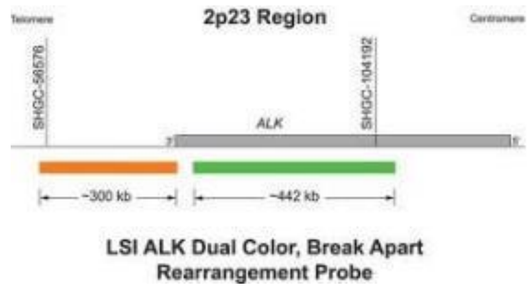
The purpose of this study was to validate two probes used in the detection of two rearrangements found in Non-Small Cell Lung Cancer (NSCLC). Conducted at the cytogenetics lab at Augusta University Medical Center, the validations were a requirement of the American College of Medical Genetics (ACMG) which states that every assay must be validated before implementation. The two probes, manufactured by Abbott Molecular Inc. of Des Plaines, Illinois as part of a FISH kit, detected the ROS1 and ALK rearrangements of NSCLC patients and were a companion diagnostic to XALKORI (trade name crizotinib). The prevalence of Anaplastic Lymphoma Kinase (ALK) gene rearrangements was 2-5% and the prevalence of ROS1 gene rearrangements, was 1-3%. For validations, the sensitivity and specificity of each probe were calculated and compared to the manufacturer's published performance standards.

METHODS

The study population had the following characteristics: diagnosis of non-small cell lung carcinoma and either ROS1 or ALK rearrangement at AUMC and previously tested by Clarient Inc. For the validation, the investigators tested 20 negative and five positive samples for each probe (ACMG, 2016). The samples were de-identified and given random numbers, but coded for proper identification, as such the study was blinded.

Interphase FISH staining was done in accordance with the manufacturer's protocol.





RESULTS

ALK nuclei Enumerations

	True Negative	False Positive
Lab Data	984 (20 specimens x 50 nuclei)	16
Investigator Data	494 (20 specimens x 25 nuclei)	6

	True Positive	False Negative
Lab Data	241 (5 specimens x 50 nuclei)	9
Investigator Data	115 (5 specimens x 25 nuclei)	10

Summary Data

	Investigator's Data	Lab Data (2 Cytogeneticists)
ALK Sensitivity	92%	96.4%
ALK Specificity	98.8%	98.4%
ROS1 Sensitivity	100%	99.4%
ROS1 Specificity	100%	100%

DISCUSSION

The cytogeneticists at AUMC were able to produce results with the required 96.4% sensitivity for both probes, this result is almost identical to the manufacturer's results. The specificities for the ALK probe were greater than 98% from all enumerators, student and cytogeneticists. The student's sensitivity of only 92% can be attributed to lack of experience with enumerations of nuclei but sensitivity can be increased by simply counting greater than the 25 nuclei performed for this study. However, given the >90% sensitivity obtained by the student, it can be argued that the compact fluorescent signal attribute of the LSI (Locus Specific Identifier probes) are so clear and distinct that an inexperienced user can obtain good results. The students' ROS1 calculations for sensitivity and specificity were slightly better than the cytogeneticists but the results from both were obtained by enumerations mostly from the manufacturer's controls. For the ACMG, only 3 positive controls are necessary for the validation to be successful. All of the FISH analyses in this study were perfectly concordant with Clarient Inc. Laboratories.

CONCLUSIONS

Abbott Molecular LSI Vysis probes are highly specific probes, >90% specificity for detecting the presence of the genetic rearrangements of ALK or ROS1. The probes' design made it easy for an inexperienced FISH nuclei enumerator to analyze the signal patterns and get acceptable results.

REFERENCES

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