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Comparison of the Performance of Two Different ALK Antibody Clones (D5F3 and ALK1) in Anaplastic Large Cell Lymphoma (ALCL)
Dana Martin MD, Steven Smith MD, PhD, Alden Chesney MD, Terra Lewis, HT(ASCP) and Raghavendra Pillappa MD

Background
Anaplastic large cell lymphoma (ALCL) is a T-cell lymphoma characterized by CD30 expression and subdivided into anaplastic lymphoma kinase (ALK) positive and negative subtypes that show clinically significant differences in outcomes. The current standard for evaluating ALK status is immunohistochemistry using the mouse monoclonal anti-human CD246 (ALK1) or fluorescence in situ hybridization. The novel rabbit monoclonal anti-human CD246 (D5F3) is proposed as an alternative to ALK1 and FDA approved for diagnosis of ALK-rearranged lung adenocarcinoma. However, its performance has not been systematically tested and compared to ALK1 in ALCL.

Design
Twenty-seven cases of ALCL were identified from institutional database searches and retrieved. A representative slide from each case was stained using ALK1 and D5F3 in an automated slide stainer. The intensity of cytoplasmic staining (graded 0-3, none, faint, moderate and strong) and percentage of positive cells (0, <5, 5-50%, 50-75% and >75%) were evaluated for each individual clone and subsequently compared between the two clones.

Results
Of the twenty-seven cases, nine were previously diagnosed as ALK expression positive by ALK1 staining. Nine cases were positive for ALK expression by ALK1 staining (34.6%; 1 1+; 0 2+; 8 3+), while fourteen were positive by D5F3 staining (48.1%; 3 1+; 2 2+; 9 3+). There were no cases that were positive by ALK1, but not by D5F3, which had identified the five additional cases. For three of the nine cases (33.3%) positive by both stains, the D5F3 stained slides showed greater percentage of cells stained. The staining intensity was greater by D5F3 in one of nine cases, the other eight cases showed the same (3+) intensity by D5F3 and ALK1. FISH results are available in five cases (19.2%) and demonstrated 100% concordance with ALK expression by both IHC stains (four positive, one negative).

Conclusions
These findings support the use of D5F3 as an equivalent and potentially more sensitive alternative to ALK1 for the evaluation of ALK positivity in ALCL.