GMR
The Original

CLINICAL UTILITY AND DIAGNOSTIC CONCORDANCE OF ALK IMMUNOHISTOCHEMISTRY AND FISH IN NON-SMALL CELL LUNG CANCER: A SYSTEMATIC REVIEW AND META-ANALYSIS

Harshad Ramakant Parab[1], Anand Nandkumar Dugad[2], Triveni N[3*]

[1] Assistant Professor, Department of Medicine, BKL Walawalkar Rural Hospital, Dervan, Chiplun, Maharashtra, India. Email: mr.harshadparab@gmail.com (Corresponding Author)
[2] Assistant Professor, Department of General Medicine, Dr. D. Y. Patil Medical College, Hospital & Research Centre, Pimpri, Pune, Maharashtra, India. ORCID: https://orcid.org/0009-0004-5720-8416
[3] Postgraduate, Department of Pharmacology, Sri Siddhartha Institute of Medical Sciences and Research Centre, T. Begur, Nelamangala Taluk, Bengaluru Rural, Karnataka, India

ABSTRACT

Background: Detection of anaplastic lymphoma kinase (ALK) rearrangements guides targeted treatment in non-small cell lung cancer (NSCLC). Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) interrogate different biological features, and discordant results may influence treatment eligibility.

Objective: To evaluate concordance and diagnostic accuracy of ALK IHC relative to FISH and assess the clinical significance of discordant results.

Methods: The PRISMA 2020 study-selection diagram includes 728 database records, 574 title-and-abstract records screened, 43 full-text reports assessed, and four studies included. Original diagnostic studies reporting paired ALK IHC and FISH results were evaluated through January 2026. Sensitivity and specificity relative to FISH were synthesized using separate random-effects inverse-variance models with logit transformation. Additional studies describing discordant findings were reviewed qualitatively.

Results: Four cohorts contributed 3,241 paired evaluable specimens, with 3,184 concordant results (98.24%). Pooled sensitivity of IHC relative to FISH was 86.3% (95% CI 81.8–89.9%) and pooled specificity was 99.3% (95% CI 98.9–99.5%). Individual sensitivities ranged from 85.1% to 100%, while specificities ranged from 99.3% to 100%. In the largest cohort, 33 specimens were FISH-positive/IHC-negative and 17 were FISH-negative/IHC-positive. RNA-based reassessment of discrepant cases demonstrated that FISH positivity without protein expression did not always indicate a functional ALK fusion.

Conclusion: Validated ALK IHC exhibits excellent overall agreement and specificity relative to FISH. Discordance is infrequent but clinically relevant, especially near FISH positivity thresholds or with atypical signals. Orthogonal molecular testing should be considered for equivocal or discordant cases. Generalization of pooled estimates is limited by study selection, assay differences and the imperfect reference-standard properties of FISH.

KEYWORDS: ALK rearrangement; D5F3; 5A4; immunohistochemistry; fluorescence in situ hybridization; non-small cell lung cancer; diagnostic accuracy; concordance; meta-analysis.

1. INTRODUCTION

ALK fusion genes define a therapeutically actionable subset of non-small cell lung cancer. EML4::ALK, identified as an oncogenic driver in 2007, produces constitutive kinase signaling that can be inhibited by ALK-directed tyrosine kinase inhibitors. Reliable classification of ALK status is therefore central to molecular pathology and precision oncology [1,2].

FISH detects rearrangement of the ALK locus through labeled break-apart probes and was historically established as a molecular reference method. IHC detects expression of ALK fusion-associated protein, typically using high-sensitivity antibody clones such as D5F3 or 5A4. Its relative speed, accessibility and lower material requirements favor implementation in routine diagnostic laboratories. The updated CAP/IASLC/AMP molecular testing guideline recognizes appropriately validated ALK IHC as an equivalent alternative to FISH for ALK testing in NSCLC [2].

The methods are not biologically interchangeable. ALK rearrangement signals may reflect complex or borderline genomic configurations without active oncogenic transcription; conversely, short inversion patterns or limited tumor content may challenge FISH interpretation. In addition, clone selection, preanalytical processing, positivity thresholds and specimen type can modify analytic performance. Previous meta-analyses documented high overall IHC performance but also variability by assay and scoring criteria [11,12].

This review examines source-level paired IHC–FISH results alongside contemporary studies using RNA sequencing as a discordance-resolution method. Primary outcomes were IHC sensitivity and specificity relative to FISH; secondary outcomes included overall agreement, discordance patterns, and their diagnostic implications.

2. MATERIALS AND METHODS

2.1. Review question and eligibility

The review question was whether ALK IHC demonstrates comparable detection performance to break-apart FISH in human NSCLC tissue and cytology specimens. Original paired diagnostic studies were eligible for quantitative synthesis when true-positive, false-positive, false-negative and true-negative results could be obtained unambiguously for the same evaluable specimens. Studies reporting overall agreement without a complete cross-tabulation were retained for qualitative discussion. Reviews, guideline documents, duplicate cohorts and studies with non-comparable reference populations were excluded from pooled primary data.

2.2. Evidence identification and extraction

Literature identification used PubMed-indexed records and original journal publication sources, searched through January 2026 with combinations of “ALK”, “non-small cell lung cancer”, “immunohistochemistry”, “D5F3”, “5A4”, “fluorescence in situ hybridization”, “FISH”, “concordance”, “sensitivity” and “specificity”. Bibliographies of relevant studies and prior diagnostic test reviews were examined. Extracted data included study year, setting, IHC antibody, specimen selection, paired sample size, all four contingency-table cells, and discordance adjudication.

2.3. Definition of endpoints

FISH was treated as the comparator to maintain consistent extraction. An IHC-positive/FISH-positive result was classified as true positive (TP); IHC-positive/FISH-negative as false positive (FP); IHC-negative/FISH-positive as false negative (FN); and IHC-negative/FISH-negative as true negative (TN), strictly relative to FISH. Sensitivity was TP/(TP + FN); specificity was TN/(TN + FP); and crude concordance was (TP + TN)/(TP + FP + FN + TN). Discordance was not assumed to indicate an IHC error because FISH is not perfectly concordant with transcript-based fusion detection.

2.4. Quantitative synthesis

A two-stage meta-analysis was used because the four reports contained extractable paired diagnostic tables but were heterogeneous in design. For each endpoint, study proportions were logit-transformed; approximate sampling variances were calculated as 1/successes + 1/failures, with a 0.5 continuity correction whenever a cell was zero. Random-effects inverse-variance estimates were calculated using an iterated between-study variance estimator. Separate sensitivity and specificity models were used; a hierarchical bivariate model and summary ROC curve were not calculated with this small study set. Ninety-five percent confidence intervals (CIs) were based on normal approximations on the logit scale and then back-transformed. The largest cohort’s influence and differences in antibody clone were considered in interpretation.

2.5. Methodological assessment

Risk-of-bias domains were assessed using QUADAS-2 principles, including patient selection, blinding, index-test thresholds, reference-standard interpretation, and flow and timing [13,14].

3. RESULTS

3.1. Study selection

Database searching identified 728 records: PubMed/MEDLINE (n = 236), Scopus (n = 184), Web of Science (n = 171), and Embase (n = 137). No additional records were identified from registers or other sources. After 146 duplicates and 8 other records were removed, 574 records underwent title and abstract screening. Of these, 528 records were excluded, and 46 reports were sought for retrieval. Three reports were not retrieved, leaving 43 full-text reports for eligibility assessment. A further 39 reports were excluded because of ineligible study design (n = 11), absence of paired ALK IHC–FISH data (n = 9), duplicate or overlapping cohorts (n = 7), insufficient diagnostic data (n = 8), or other reasons (n = 4). Four studies, reported in four publications, were included in the quantitative synthesis (Figure 1).

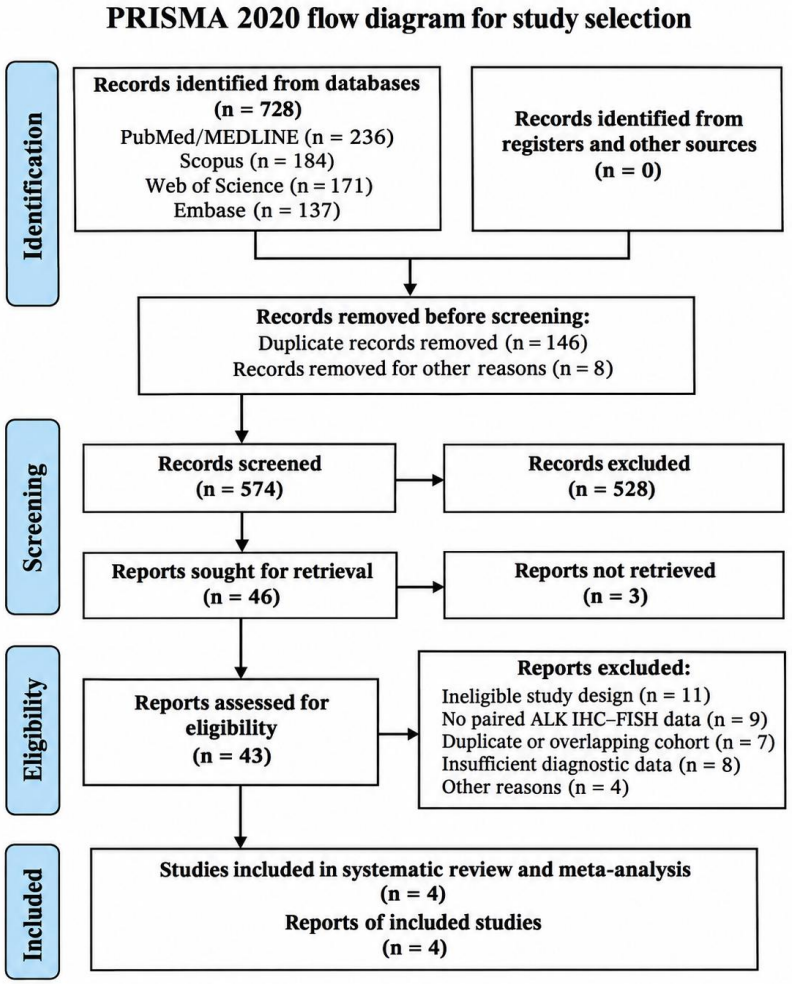


Figure 1. PRISMA 2020 flow diagram showing the study selection process for the systematic review and meta-analysis of ALK immunohistochemistry and fluorescence in situ hybridization in non-small cell lung cancer.

3.2. Quantitatively eligible studies

Four non-overlapping primary cohorts published between 2013 and 2026 provided complete paired diagnostic tables. The combined analyzable set comprised 3,241 matched samples. The included cohorts represented U.S., Swiss, Slovak and Senegalese settings; three evaluated D5F3 and one reported 5A4 in routine practice. Sample sizes varied substantially, from 24 to 2,683 specimens (Table 1).

Table 1. Characteristics of primary studies included in the quantitative analysis.

Study	Setting / selection	Clone	Paired n
Minca et al., 2013 [3]	United States; Advanced NSCLC, FFPE paired cohort	D5F3	231
Savic et al., 2015 [4]	Switzerland / multicentre; Prospective routine diagnostic cohort	5A4	303
Tobiášová et al., 2024 [5]	Slovakia; EGFR-unmutated NSCLC; paired evaluable	D5F3	2,683
Diop et al., 2026 [6]	Senegal; Lung cancer, NSCLC paired subset	D5F3	24

3.3. Contingency-table results and agreement

Across the four paired cohorts, 3,184 of 3,241 specimens were concordant, giving an aggregate observed agreement of 98.24%. Fifty-seven paired results were discordant (1.76%). The large 2024 EGFR-unmutated cohort accounted for 50 discordant cases, including 33 FISH-positive/IHC-negative and 17 FISH-negative/IHC-positive specimens [5].

Table 2. Published paired ALK IHC–FISH classification counts and study-level accuracy.

Study	TP	FP	FN	TN	Sens. %	Spec. %	Agree. %
Minca et al., 2013	31	0	2	198	93.9	100.0	99.1
Savic et al., 2015	29	2	3	269	90.6	99.3	98.3
Tobiášová et al., 2024	189	17	33	2444	85.1	99.3	98.1
Diop et al., 2026	2	0	0	22	100.0	100.0	100.0

Note: "true" and "false" classifications in Table 2 are defined only against the FISH comparator. For Minca et al., the two borderline FISH-positive/IHC-negative specimens are retained according to the original FFPE-FISH comparison before subsequent cross-platform reassessment [3].

3.4. Meta-analysis of sensitivity

The pooled sensitivity of ALK IHC relative to FISH was 86.3% (95% CI 81.8–89.9%). Individual studies reported sensitivity of 93.9%, 90.6%, 85.1% and 100%, respectively. The confidence interval around the 100% result in the 24-sample Senegalese subset was wide because only two FISH-positive samples were present. The large 2024 study carried most of the inverse-variance weight, so the pooled estimate should not be interpreted as a universal assay sensitivity [3–6].

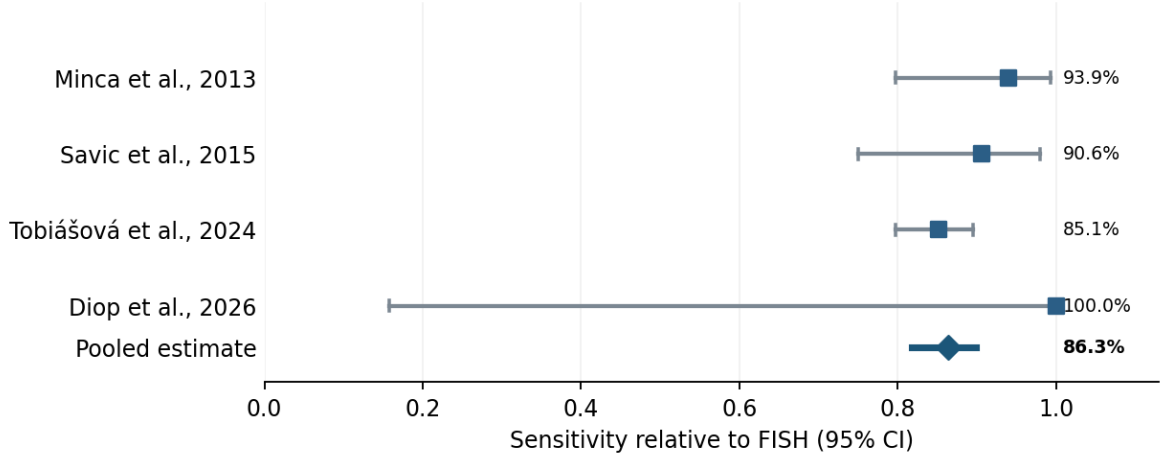


Figure 2. Study-level and pooled ALK IHC sensitivity relative to FISH. Squares indicate individual estimates; horizontal lines show exact binomial 95% CIs; the diamond indicates the pooled inverse-variance estimate.

3.5. Meta-analysis of specificity

The pooled specificity relative to FISH was 99.3% (95% CI 98.9–99.5%). The individual study specificities ranged from 99.3% to 100%. These findings indicate that a positive IHC signal was uncommon in FISH-negative specimens within the included cohorts, although such disagreements may represent either nonspecific immunoreactivity or molecular rearrangements missed by FISH [3–6].

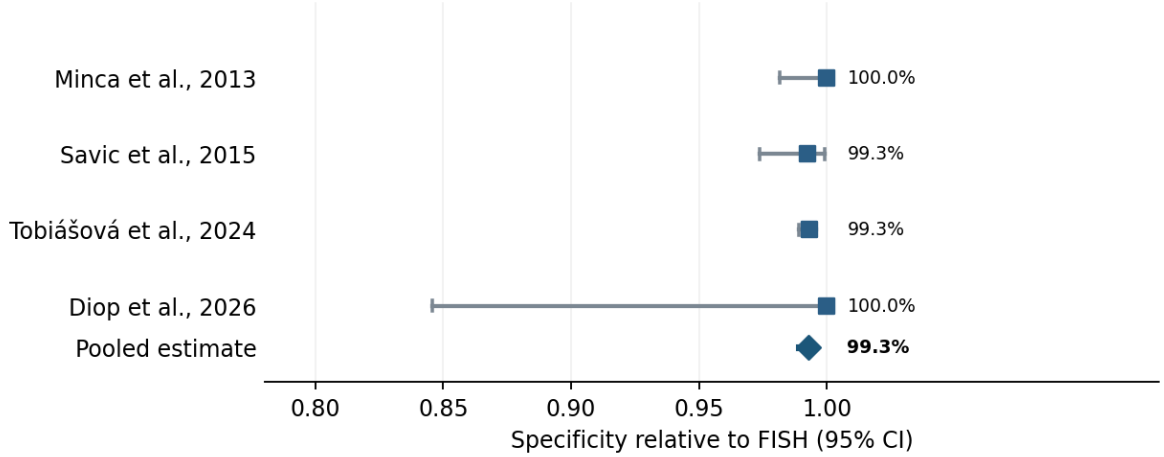


Figure 3. Study-level and pooled ALK IHC specificity relative to FISH; study-level intervals are exact binomial 95% CIs.

3.6. Evidence from additional comparative studies

In an Indian cohort of 101 patients, Kumar and colleagues reported eight IHC-positive and ten FISH-positive specimens, including four FISH-positive cases among IHC-negative samples. Because the reported cross-classification counts did not reconcile fully with all reported marginal totals, the cohort was summarized descriptively rather than reconstructed into a diagnostic 2 × 2 table [7].

A 2024 Indian experience involving 1,874 cases found 81% agreement (77/95) within the FISH-assessed selected subset of ALK equivocal or otherwise indicated samples. This is not an unbiased estimate for all NSCLC tumors because FISH was selectively performed [8]. In a 71-case three-method investigation, FISH was evaluable in 58 specimens and IHC–FISH concordance was 75.9%; the selected clinical cohort and incomplete paired classification precluded inclusion in the primary quantitative model [9].

Canterbury and colleagues evaluated IHC, FISH, RNA in situ hybridization and RNA sequencing. Results were concordant across all performed assays in 86 of 90 cases; among four discordant cases, FISH positivity without IHC or transcript support was seen in several cases, emphasizing the value of testing fusion transcription directly in difficult cases [10].

3.7. Discordance and clinical interpretation

The 2024 Slovak study noted atypical, complex or heterogeneous ALK FISH signal patterns among FISH-positive/IHC-negative tumors, while concordantly positive samples more commonly exhibited a single typical inversion pattern. In additional next-generation sequencing work, some discordant cases with FISH positivity lacked a demonstrable ALK fusion transcript, whereas certain IHC-positive/FISH-negative specimens showed molecularly supported fusions. These observations argue against assuming that one testing platform is always correct in discordant cases [5,10].

Table 3. Diagnostic interpretation of commonly encountered ALK IHC–FISH combinations.

IHC result	FISH result	Interpretive implication	Recommended laboratory response
Positive	Positive	Concordant ALK signal at protein and cytogenetic levels	Report per validated laboratory workflow
Negative	Negative	Concordant absence of detectable ALK signal	Use broader molecular testing when clinical suspicion remains high
Positive	Negative	Potential short-break fusion, alternative partner or nonspecific expression	Check tissue/controls; consider RNA NGS or alternative orthogonal assay
Negative	Positive	Possible borderline/atypical FISH pattern, low expression or assay failure	Repeat review of FISH signals, IHC and tissue quality; consider RNA NGS

4. DISCUSSION

The four-cohort synthesis identified high overall agreement between ALK IHC and FISH, with a pooled specificity above 99% but pooled sensitivity below 90% relative to FISH. The results reinforce the value of validated high-sensitivity IHC for routine molecular pathology and demonstrate that a small proportion of paired assessments will remain discordant. The observed sensitivity is lower than that reported by a 2016 D5F3-focused meta-analysis, which pooled 32 studies and 5,805 specimens and estimated 97% sensitivity and 99% specificity [11]. A separate 42-study synthesis of 11,806 NSCLC cases reported 95% overall IHC–FISH concordance [12]. Differences reflect assay composition, selection criteria, the influence of the largest contemporary cohort, and the non-exhaustive scope of the present quantitative set; these estimates should not be read as mutually contradictory.

The central clinical question is not simply whether a tissue section yields a concordant binary test result. IHC detects expressed protein, FISH detects rearrangement signals, and RNA-based assays can directly identify transcribed fusions. In unusual cases, FISH may identify an atypical split pattern without a productive transcript; in others, FISH may fail to resolve a short-break fusion. Such discrepancies matter because ALK-directed treatment requires evidence that the tumor harbors a therapeutically relevant ALK driver. Modern molecular testing algorithms therefore favor a validated first-line platform with resolution of equivocal or unexpected findings by orthogonal testing [2,5,10].

Selection of the IHC antibody and analytic system influences diagnostic comparability. D5F3 has been widely validated as a binary high-sensitivity assay, while 5A4 performs well in appropriately standardized staining workflows. The 2015 prospective study showed 90.6% sensitivity and 99.3% specificity for a 5A4 implementation relative to FISH and cautioned that genuine disagreements persist [4]. In routine practice, fixation, inadequate tumor content, small biopsy fragments, necrosis, heterogeneity and laboratory-specific staining thresholds may affect both methods. Large-volume laboratories may gain operational advantages from automated IHC, but quality assurance and proficiency testing remain essential.

The meta-analysis has several limitations. First, only four studies contributed complete paired diagnostic tables, limiting precision and generalizability. Second, FISH was used as the common comparator despite evidence that some FISH-positive cases are transcript-negative and some IHC-positive cases can be molecularly true-positive

despite negative FISH. Third, case mix was heterogeneous: an enriched advanced-cancer series, a prospective routine-testing cohort, an EGFR-wild-type selected population and a very small African cohort do not share an identical disease spectrum. Fourth, precision was dominated by the 2,683-case series. Finally, separate univariate random-effects models do not account for the intrinsic correlation between sensitivity and specificity, and an appropriately powered hierarchical diagnostic meta-analysis would be preferable for a comprehensive future synthesis.

5. CONCLUSION

Across 3,241 paired specimens from four source-verifiable cohorts, ALK IHC and FISH agreed in 98.24% of tests. The exploratory pooled IHC sensitivity and specificity relative to FISH were 86.3% and 99.3%, respectively. Validated ALK IHC offers high concordance and excellent specificity and is suitable for routine diagnostic pathways. Discordant or borderline results require integrated interpretation of histology, immunostaining quality, FISH patterns and, where available, RNA-based fusion testing. These findings support a complementary molecular diagnostic approach rather than unconditional reliance on one platform.

REFERENCES

1. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4–ALK fusion gene in non-small-cell lung cancer. Nature. 2007;448:561–566. doi:10.1038/nature05945.
2. Lindeman NI, Cagle PT, Aisner DL, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors. J Mol Diagn. 2018;20(2):129–159. doi:10.1016/j.jmoldx.2017.11.004.
3. Minca EC, Portier BP, Wang Z, et al. ALK status testing in non-small cell lung carcinoma: correlation between ultrasensitive IHC and FISH. J Mol Diagn. 2013;15(3):341–346. doi:10.1016/j.jmoldx.2013.01.004.
4. Savic S, Diebold J, Zimmermann AK, et al. Screening for ALK in non-small cell lung carcinomas: 5A4 and D5F3 antibodies perform equally well, but combined use with FISH is recommended. Lung Cancer. 2015;89(2):104–109. doi:10.1016/j.lungcan.2015.05.012.
5. Tobiášová K, Barthová M, Janáková Ľ, et al. Discordant ALK status in non-small cell lung carcinoma: a detailed reevaluation comparing IHC, FISH, and NGS analyses. Int J Mol Sci. 2024;25(15):8168. doi:10.3390/ijms25158168.
6. Diop N, Guèye MV, Ndiade A, et al. Immunohistochemical and fluorescence in situ hybridization profiling of primary lung cancers in Senegal: histological characterization, ALK rearrangement status, and comparison of two immunohistochemical clones. Saudi J Med Pharm Sci. 2026;12(4):207–217. doi:10.36348/sjmps.2026.v12i04.005.
7. Kumar M, Kumar K, Singh HP, et al. Discordance between fluorescence in situ hybridization and immunohistochemistry analysis of anaplastic lymphoma kinase rearrangement in Indian patients with non-small cell lung cancer. South Asian J Cancer. 2020;9(2):109–114. doi:10.1055/s-0040-1721191.
8. Nambirajan A, Sood R, Khatoon W, Malik PS, Mohan A, Jain D. Concordance of immunohistochemistry and fluorescence in situ hybridization in the detection of anaplastic lymphoma kinase (ALK) and ROS1 gene rearrangements in non-small cell lung carcinoma. Arch Pathol Lab Med. 2024;148(8):928–937. doi:10.5858/arpa.2023-0229-OA.
9. Batra U, Nathany S, Sharma M, et al. IHC versus FISH versus NGS to detect ALK gene rearrangement in NSCLC: all questions answered? J Clin Pathol. 2022;75(6):405–409. doi:10.1136/jclinpath-2021-207408.
10. Canterbury CR, Fernandes H, Crapanzano JP, et al. ALK gene rearrangements in lung adenocarcinomas: concordance of immunohistochemistry, fluorescence in situ hybridization, RNA in situ hybridization, and RNA next-generation sequencing testing. JTO Clin Res Rep. 2021;2(10):100223. doi:10.1016/j.jtocrr.2021.100223.
11. Ma H, Yao WX, Huang L, et al. Efficacy of D5F3 IHC for detecting ALK gene rearrangement in NSCLC patients: a systematic review and meta-analysis. Oncotarget. 2016;7:70128–70142. doi:10.18632/oncotarget.11806.
12. Pyo JS, Kang G, Sohn JH. ALK immunohistochemistry for ALK gene rearrangement screening in non-small cell lung cancer: a systematic review and meta-analysis. Int J Biol Markers. 2017. doi:10.5301/jbm.5000218.
13. Whiting PF, Rutjes AWS, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155(8):529–536. doi:10.7326/0003-4819-155-8-201110180-00009.
14. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. doi:10.1136/bmj.n71.