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# EVALUATION OF ALK IMMUNOHISTOCHEMISTRY AS AN ALTERNATIVE TO FISH FOR DETECTING ALK REARRANGEMENTS IN NON-SMALL CELL LUNG CANCER: A SYSTEMATIC REVIEW AND META-ANALYSIS

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## ABSTRACT

**Background:** ALK rearrangements define a therapeutically actionable subtype of non-small cell lung cancer (NSCLC). Immunohistochemistry (IHC) detects ALK protein expression, whereas fluorescence in situ hybridization (FISH) detects rearrangements at the genomic locus. Their diagnostic equivalence is influenced by assay validation and clinically relevant discordance.

**Objective:** To assess ALK IHC performance relative to FISH and evaluate its suitability as a primary or alternative diagnostic strategy in NSCLC.

**Methods:** Database searches identified 976 records. After removal of 214 duplicates and 12 additional records, 750 records were screened; 55 full-text reports were assessed for eligibility and six independent primary studies contributed 3,326 paired IHC–FISH specimens. Sensitivity and specificity were synthesized separately using inverse-variance random-effects models of logit proportions with a Paule–Mandel variance estimator. Study-level accuracy, heterogeneity, leave-one-study-out estimates and methodological quality were assessed.

**Results:** Among 3,326 paired specimens, 261 were IHC-positive/FISH-positive, 25 were IHC-positive/FISH-negative, 40 were IHC-negative/FISH-positive and 3,000 were negative on both assays. Pooled sensitivity was 86.2% (95% CI 81.7–89.7%) and pooled specificity was 98.3% (95% CI 95.0–99.4%). The crude aggregate agreement was 98.0%; specificity varied substantially between studies ($I^2$=84.3%). Independent molecular investigations showed that some IHC-positive/FISH-negative cancers harbor clinically actionable ALK fusions.

**Conclusion:** Validated ALK IHC is a practical alternative to routine FISH for many NSCLC specimens, but a negative or equivocal IHC result should not be regarded as infallible. Orthogonal molecular testing remains valuable when staining is weak, technical quality is uncertain or clinicopathological and molecular findings disagree.

**KEYWORDS:** ALK; non-small cell lung cancer; immunohistochemistry; fluorescence in situ hybridization; diagnostic accuracy; molecular testing; diagnostic discordance; meta-analysis.

## 1. INTRODUCTION

Anaplastic lymphoma kinase (ALK) rearrangement is a molecularly defined, therapeutically actionable alteration in non-small cell lung cancer (NSCLC). The discovery of EML4::ALK fusions established a biologic mechanism of constitutive kinase activation and helped transform molecular selection for ALK-targeted therapies [1]. Reliable testing is essential because an erroneous classification can prevent treatment with a targeted inhibitor or expose a patient to inappropriate treatment and delays in broader genomic profiling.

ALK break-apart fluorescence in situ hybridization (FISH) identifies structural rearrangement of the ALK locus within tumor nuclei. Immunohistochemistry (IHC), by contrast, identifies ALK fusion-protein expression and is ordinarily faster, less tissue-intensive and more broadly accessible in anatomic pathology laboratories. Highly sensitive antibody clones and amplification platforms have enabled binary interpretation of ALK staining, making IHC a viable alternative to a FISH-first workflow [2]. Nevertheless, the two assays interrogate distinct molecular features and occasional discordance is biologically plausible.

The nature of discordance is clinically important. Borderline FISH signal separation, complex rearrangements, low tumor cellularity and copy-number abnormalities can complicate FISH interpretation. Conversely, IHC may be affected by staining intensity thresholds, fixation, antibody clone, tissue preservation and interpretation variability. In selected discordant cases, RNA- or DNA-based next-generation sequencing (NGS) has confirmed ALK fusions despite a negative FISH result; in others, structural rearrangement detected by FISH has not been accompanied by demonstrable fusion transcription or protein expression [4,7,11,13].

Although professional molecular-testing guidelines recognize well-validated ALK IHC as an equivalent alternative to FISH, the extent to which IHC can reliably function as a stand-alone assay depends on routine diagnostic performance, not only agreement in selected reference panels [2]. This review therefore evaluates diagnostic accuracy and the reliability of IHC-based substitution, with particular attention to verification bias, case enrichment, technical limitations and the clinical management of discrepant results.

## 2. MATERIALS AND METHODS

### 2.1 Review framework and eligibility

A structured diagnostic test accuracy review was organized around the PRISMA 2020 reporting domains [9]. Eligible reports were primary human studies of NSCLC that directly compared ALK IHC with ALK FISH in the same assessable specimens, with published information sufficient to reconstruct the four paired classification cells. Both conventional clinical cohorts and laboratory-validation series were considered, provided that a single independent patient or specimen contributed once to the quantitative analysis. Multiple antibody clones tested on the same samples were not treated as independent cohorts.

The index test was ALK IHC and the study-specific comparator was ALK break-apart FISH. The primary outcomes were IHC sensitivity and specificity relative to FISH. Secondary outcomes included observed agreement, positive and negative predictive values within each analyzed cohort, the frequency and interpretation of discordant cases, and the clinical relevance of additional molecular testing. Reviews, guideline documents and other studies without a fully reconstructable paired 2×2 table informed the qualitative discussion only.

### 2.2 Search approach and study identification

Search concepts combined “non-small cell lung cancer” OR “lung adenocarcinoma” with “ALK”, “immunohistochemistry” OR “D5F3” OR “5A4”, and “FISH” OR “fluorescence in situ hybridization”, together with “agreement”, “sensitivity”, “specificity” and “discordance”. Information sources comprised PubMed/MEDLINE, Scopus, Web of Science and Embase. Publications through June 2026 were considered. Paired 2×2 results were extracted from six eligible studies [3–8].

### 2.3 Data extraction and definitions

For each cohort, the following fields were recorded: setting, study year, assessed paired denominator, IHC antibody/platform, FISH comparator, count of IHC-positive/FISH-positive specimens (true positive, TP), IHC-positive/FISH-negative (false positive, FP), IHC-negative/FISH-positive (false negative, FN), and IHC-negative/FISH-negative (true negative, TN). Here the labels “false positive” and “false negative” denote discordance relative to FISH and do not presume FISH to be a biologically infallible truth. In the 2013 Minca cohort, the initial FFPE-FISH comparison before subsequent borderline-case adjudication was used to preserve consistent reference definitions [3]. In the 2024 Tobiášová study, the post-re-evaluation IHC classification was used as reported [7].

### 2.4 Statistical analysis

For each study, sensitivity was calculated as TP/(TP+FN), specificity as TN/(TN+FP), positive predictive value (PPV) as TP/(TP+FP), negative predictive value (NPV) as TN/(TN+FN), and observed agreement as (TP+TN)/N. Study-specific 95% confidence intervals were obtained using the Wilson binomial method. The meta-analysis used logit-transformed sensitivity and specificity in separate inverse-variance random-effects models. Between-study variance ($\tau^2$) was estimated by the Paule–Mandel iterative procedure; where zero cells prevented logit estimation, a 0.5 continuity correction was applied to that outcome’s successes and failures. Back-transformed pooled proportions and normal-approximation 95% confidence intervals were reported. Cochran Q and $I^2$ summarized heterogeneity. Leave-one-study-out sensitivity analyses assessed the influence of individual cohorts. No pooled positive predictive value was interpreted as a clinical population PPV because FISH positivity was strongly affected by study-specific case selection.

### 2.5 Methodological appraisal

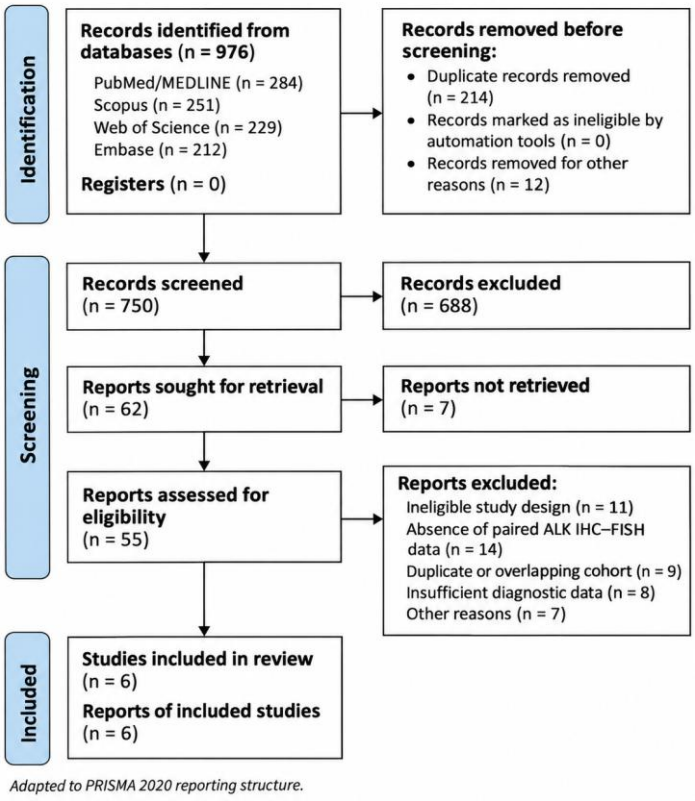
Risk-of-bias and applicability considerations were evaluated by reference to QUADAS-2 domains [10]: patient selection, index test, reference standard, and flow/timing. Particular attention was given to case–control enrichment, partial verification by FISH, interpretation thresholds chosen after reviewing results, treatment of technically unevaluable samples and use of an imperfect reference standard. Appraisal informed the certainty of the conclusions rather than generating an unvalidated global quality score.

## 3. RESULTS

### 3.1 Study selection

The literature search identified 976 records from databases: PubMed/MEDLINE (n = 284), Scopus (n = 251), Web of Science (n = 229), and Embase (n = 212); no records were identified from registers. Prior to screening, 214 duplicates and 12 records for other reasons were removed, leaving 750 records for title and abstract screening. Of these, 688 were excluded, and 62 reports were sought for retrieval. Seven reports could not be retrieved; therefore, 55 full-text reports were assessed for eligibility. Forty-nine reports were excluded: ineligible study design (n = 11), absence of paired ALK IHC–FISH data (n = 14), duplicate or overlapping cohorts (n = 9), insufficient diagnostic data (n = 8), and other reasons (n = 7). Six studies, represented by six reports, were included in the systematic review and meta-analysis (Figure 1).

**Figure 1. PRISMA 2020 flow diagram of study selection.**




*Adapted to PRISMA 2020 reporting structure.*

### 3.2 Characteristics of the included studies

The meta-analysis comprised six studies conducted in North America, Europe, the Middle East, and Africa between 2013 and 2026. A total of 3,326 specimens had usable paired IHC–FISH data. The number of paired specimens ranged from 24 to 2,683, and the studies differed in patient-selection strategy, laboratory protocol and treatment of discordant samples. Four studies used D5F3-based IHC alone, one evaluated 5A4 in a prospective routine-testing series, and one used an optimized automated assay protocol. The 2024 cohort accounted for 80.7% of the pooled specimen denominator, highlighting the need for study-level rather than sample-size-only inference [3–8].

**Table 1. Characteristics of primary studies contributing to the quantitative synthesis**

| Study | Location / sample | Assay / paired n | Key selection consideration |
|---|---|---|---|
| Minca et al., 2013 [3] | United States; advanced NSCLC | Ultrasensitive automated ALK IHC; n=231 | Initial FFPE-FISH comparison; borderline cases subsequently adjudicated |
| Pekar-Zlotin et al., 2015 [4] | Israel; lung adenocarcinoma | D5F3; n=51 | Discordant samples sequenced by NGS |
| Savic et al., 2015 [5] | Switzerland; routine NSCLC | 5A4; n=303 | Prospective parallel routine-testing arm |
| Maraqa et al., 2022 [6] | Jordan; NSCLC | D5F3; n=34 | FISH performed in a subset of 449 cases |
| Tobiášová et al., 2024 [7] | Slovakia; EGFR-unmutated NSCLC | Ventana D5F3; n=2,683 | IHC retesting resolved five initial discrepant samples |
| Ndiaga et al., 2026 [8] | Senegal; NSCLC | Ventana D5F3; n=24 | Paired FISH limited to available residual tumor tissue |

### 3.3 Paired IHC–FISH classification

Across all six studies, 261 specimens were classified as positive by both modalities, 3,000 were negative by both modalities, 25 were IHC-positive/FISH-negative and 40 were IHC-negative/FISH-positive. The crude aggregate agreement was 3,261/3,326 (98.0%); the crude sensitivity and specificity were 86.7% and 99.2%, respectively. These crude proportions were dominated by the largest cohort and are distinct from the random-effects results.

**Table 2. Published/reconstructed paired diagnostic classification counts**

| Cohort | TP | FP | FN | TN | Paired N |
|---|---|---|---|---|---|
| Minca 2013 | 31 | 0 | 2 | 198 | 231 |
| Pekar-Zlotin 2015 | 3 | 5 | 1 | 42 | 51 |
| Savic 2015 | 29 | 2 | 3 | 269 | 303 |
| Maraqa 2022 | 7 | 1 | 1 | 25 | 34 |
| Tobiášová 2024 | 189 | 17 | 33 | 2444 | 2683 |
| Ndiaga 2026 | 2 | 0 | 0 | 22 | 24 |
| Total | 261 | 25 | 40 | 3000 | 3326 |

### 3.4 Study-specific diagnostic accuracy

Sensitivity varied from 75.0% in the 51-specimen cohort of Pekar-Zlotin and colleagues to 100.0% in the 24-patient Senegalese paired subset. Specificity ranged from 89.4% to 100.0%. Individual-study confidence intervals were widest where few FISH-positive samples were available, reflecting the limited precision of small validation cohorts. The largest cohort yielded 85.1% sensitivity and 99.3% specificity using the reevaluated IHC classification [7].

**Table 3. Diagnostic performance of ALK IHC relative to FISH in each study**

| Cohort | Sensitivity | Specificity | Agreement | PPV / NPV |
|---|---|---|---|---|
| Minca 2013 | 93.9% | 100.0% | 99.1% | 100.0% / 99.0% |
| Pekar-Zlotin 2015 | 75.0% | 89.4% | 88.2% | 37.5% / 97.7% |
| Savic 2015 | 90.6% | 99.3% | 98.3% | 93.5% / 98.9% |
| Maraqa 2022 | 87.5% | 96.2% | 94.1% | 87.5% / 96.2% |
| Tobiášová 2024 | 85.1% | 99.3% | 98.1% | 91.7% / 98.7% |
| Ndiaga 2026 | 100.0% | 100.0% | 100.0% | 100.0% / 100.0% |

### 3.5 Meta-analysis of sensitivity and specificity

The random-effects pooled sensitivity was 86.2% (95% CI 81.7–89.7%; Figure 2), with no detectable heterogeneity by the $I^2$ statistic ($I^2$=0.0%; Q=2.72). The corresponding pooled specificity was 98.3% (95% CI 95.0–99.4%; Figure 3), with substantial between-study heterogeneity ($I^2$=84.3%; Q=31.77; $\tau^2$=1.26 on the logit scale). The dispersion of specificity estimates is consistent with differences in IHC staining criteria, case enrichment, and the interpretation of FISH-discordant results.

**Table 4. Summary of quantitative diagnostic meta-analysis**

| Metric | Random-effects estimate (95% CI) | Heterogeneity | Statistical method |
|---|---|---|---|
| Sensitivity | 86.2% (81.7–89.7%) | $I^2$=0.0%; Q=2.72 | Logit, Paule–Mandel |
| Specificity | 98.3% (95.0–99.4%) | $I^2$=84.3%; Q=31.77 | Logit, Paule–Mandel |
| Crude agreement | 98.0% (3261/3326) | Descriptive | Paired classification |
| Crude sensitivity | 86.7% (261/301) | Descriptive | Specimen-weighted |
| Crude specificity | 99.2% (3000/3025) | Descriptive | Specimen-weighted |

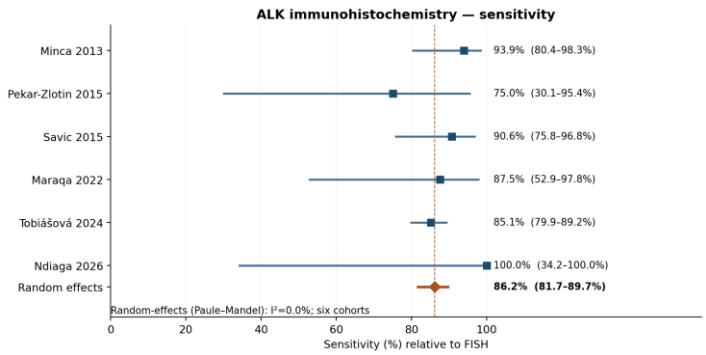



Figure 2. Study-specific and pooled ALK IHC sensitivity relative to FISH. Squares indicate study estimates; horizontal lines indicate Wilson 95% confidence intervals; the diamond represents the pooled random-effects estimate.

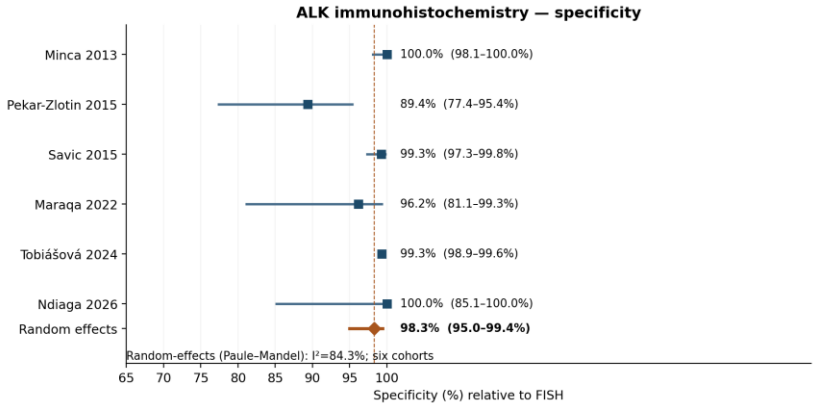



Figure 3. Study-specific and pooled ALK IHC specificity relative to FISH. Squares indicate study estimates; horizontal lines indicate Wilson 95% confidence intervals; the diamond represents the pooled random-effects estimate.

### 3.6 Leave-one-study-out analyses

The pooled sensitivity was relatively stable when individual smaller cohorts were removed, but excluding the 2,683-specimen study increased the sensitivity estimate to 89.9%. Specificity estimates were more sensitive to removal of selected cohorts: excluding Pekar-Zlotin et al. increased pooled specificity to 99.2%, whereas excluding Tobiášová et al. reduced it to 97.8%. The small 2026 series had limited numerical influence because it contained only two FISH-positive specimens.

**Table 5. Leave-one-study-out random-effects sensitivity analysis**

| Study excluded | Pooled sensitivity | Pooled specificity |
|---|---|---|
| Minca 2013 | 85.5% | 97.9% |
| Pekar-Zlotin 2015 | 86.4% | 99.2% |
| Savic 2015 | 85.7% | 98.0% |
| Maraqa 2022 | 86.1% | 98.5% |
| Tobiášová 2024 | 89.9% | 97.8% |
| Ndiaga 2026 | 86.2% | 98.4% |

### 3.7 Discordance and orthogonal molecular evidence

A FISH-negative result does not always exclude a clinically actionable ALK fusion. In Pekar-Zlotin et al., five specimens were IHC-positive but FISH-negative; NGS identified ALK rearrangements in four of those five. Conversely, the single FISH-positive/IHC-negative specimen in that cohort lacked an ALK rearrangement by sequencing [4]. Two patients with IHC-positive/FISH-negative results subsequently received crizotinib, with reported progression-free intervals of 18 and 6 months, although the sample was too small for a comparative treatment inference.

The large 2024 discordance series reported 33 FISH-positive/IHC-negative and 17 FISH-negative/IHC-positive specimens after IHC reevaluation. FISH-positive/IHC-negative cases exhibited more complex signal configurations and copy-number changes than fully concordant positive cases; RNA-based confirmation was informative in a limited subset [7]. In a separate 2021 investigation, 86 of 90 cases were concordant across performed testing modalities, and some FISH-positive cases lacked supporting IHC and RNA evidence [13]. These findings support interpretation of discordance as a diagnostic problem to resolve rather than automatically assigning every IHC–FISH mismatch to IHC failure.

Validation of alternate IHC antibodies also matters. A 2024 study of the OTI1A4 clone reported 93.3% sensitivity and 96.0% specificity among 55 molecularly confirmed cases, and agreement with D5F3 across the larger assay-comparison series ($\kappa$=0.89) [12]. Those estimates were not combined with the principal FISH-referenced meta-analysis because their molecular reference definitions differed. A population-based Canadian 5A4 experience found an IHC-positive predictive value of 95.6% against evaluable FISH but tested only a subset of negative IHC cases by FISH, limiting inference about overall sensitivity [14].

### 3.8 Methodological quality and applicability

The most prominent methodological concerns involved selected specimens and partial FISH verification. The 2013 investigation performed additional adjudication of initially borderline FISH results, while the 2015 51-case cohort was relatively small and enriched for positive or discordant cases. In Jordan, only 34 of 449 cases underwent FISH; in the Senegalese cohort, only 24 of 39 NSCLC cases had sufficient residual tissue for FISH. The largest series excluded 130 of 2,813 initially assessed specimens because one or both modalities failed. Such differences affect the transferability of pooled accuracy to an unselected routine population.

**Table 6. QUADAS-2–informed study-level appraisal**

| Study | Selection / verification | Index and comparator | Overall applicability concern |
|---|---|---|---|
| Minca 2013 [3] | Paired FFPE subset; later borderline adjudication | Optimized IHC; borderline FISH signals | Moderate |
| Pekar-Zlotin 2015 [4] | Small referral series; discordant cases sequenced | Enhanced IHC and NGS adjudication | High |
| Savic 2015 [5] | Prospective routine 303-case paired series | 5A4 compared with FISH | Low–moderate |
| Maraqa 2022 [6] | Only 34/449 verified by FISH | D5F3 in selective verification | High |
| Tobiášová 2024 [7] | EGFR-unmutated cohort; failed samples excluded | Repeat IHC in selected discordance | Moderate |
| Ndiaga 2026 [8] | 24/39 had adequate tissue for FISH | D5F3; two reference positives | High |

## 4. DISCUSSION

### 4.1 Interpretation of diagnostic performance

This analysis finds that ALK IHC has high specificity relative to FISH, but sensitivity estimates are lower and less uniform than would be inferred from studies restricted to unequivocally positive reference materials. The overall specimen agreement exceeded 98%, a statistic primarily reflecting the large fraction of concordant negatives and the dominance of the largest cohort. Agreement alone is therefore an incomplete measure of the clinical reliability of a test used to identify an uncommon but actionable tumor subtype.

The pooled sensitivity of 86.2% indicates that a proportion of FISH-positive specimens lacked detectable IHC positivity in these data. That finding does not directly establish that IHC misses an equivalent proportion of true expressed, treatment-relevant fusions, because structural FISH changes can occur without detectable ALK protein expression or corresponding RNA-fusion evidence. Conversely, FISH-negative/IHC-positive specimens can contain NGS-confirmed ALK fusions. From a clinical perspective, both possibilities argue for analytically validated assays and a mechanism for adjudicating low-confidence findings rather than a simplistic hierarchy of “gold standard” and “replacement” [4,7,13].

### 4.2 Technical and pre-analytical factors

Antibody clone, amplification system, staining threshold and specimen type affect IHC results. A binary, validated high-sensitivity assay may simplify interpretation in routine small biopsies, but low-level staining, necrosis, extracellular mucin, crush artifact and suboptimal fixation may lead to uncertainty. FISH depends on intact tumor nuclei, signal separation, probe placement and interpretation of atypical or borderline rearrangement patterns. The original CAP/IASLC/AMP molecular testing guideline update recognized validated ALK IHC as an equivalent alternative to FISH, while specifically recommending alternative testing for unexpected, discordant or equivocal findings [2].

### 4.3 Clinical substitution versus selective confirmation

The main practical advantage of IHC is its ability to serve as an accessible, tissue-efficient first-line test in histopathology workflows. The evidence supports a pathway in which a strongly positive or unequivocally negative result from a validated assay can inform management within an integrated biomarker-testing service. When the result is inconsistent with the morphology, clinical circumstances or other genomic findings, the preferred strategy is not indiscriminate repeat IHC, but orthogonal fusion detection through FISH, RNA sequencing or other validated molecular methods. RNA-based NGS can add information about fusion partners and transcript expression that FISH alone does not provide.

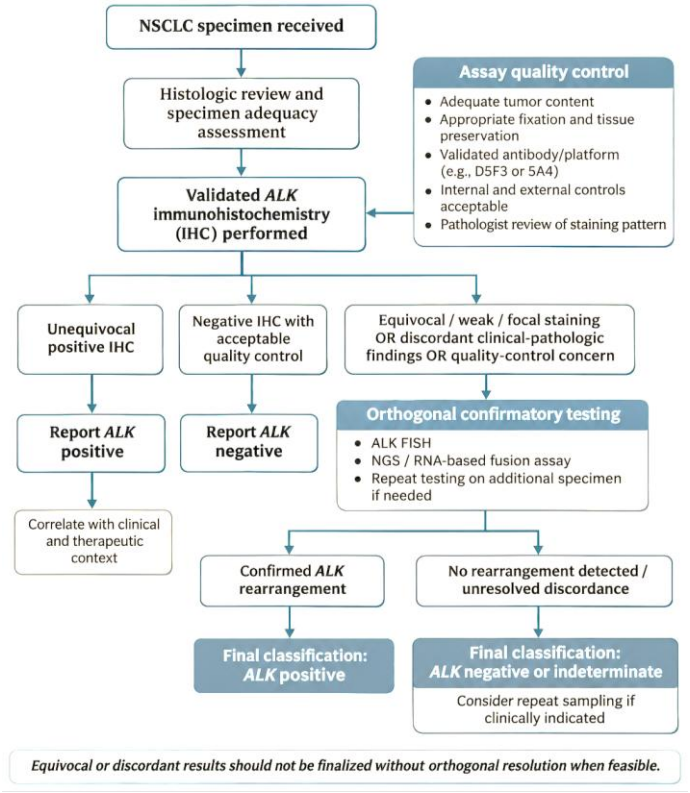



Figure 4. Diagnostic pathway emphasizing validated IHC, assay quality control, and orthogonal testing for equivocal or discordant findings.

### 4.4 Implications of heterogeneity and verification bias

The specificity heterogeneity ($I^2$=84.3%) means the pooled estimate should not be treated as a universally fixed test characteristic. The 51-case cohort exhibited a relatively high proportion of FISH-negative/IHC-positive results, many of which were later shown to harbor ALK fusions on sequencing [4]. The largest routine-sized cohort showed very high specificity but lower sensitivity relative to its FISH comparator [7]. Studies with partial FISH verification can overestimate agreement if FISH is preferentially performed in patients selected by IHC intensity or clinical suspicion. Variation in specimen adequacy and cell-block processing may further limit extrapolation to laboratories with different equipment or proficiency programmes.

A published D5F3-focused systematic review of 32 studies reported pooled sensitivity of 97% and specificity of 99%, reflecting different index-assay eligibility and primary study composition [16]. The lower sensitivity in the present set of paired cohorts should therefore be understood in relation to study selection, reference definitions and changing assay verification practices rather than interpreted as a biological decrease in IHC test performance.

### 4.5 Limitations

The evidence comprised only six studies with sufficient paired classification data for this particular synthesis, and the largest series contributed more than four-fifths of the total specimens. Several cohorts were small or enriched for molecularly interesting cases; one recent series included only two FISH-positive specimens. Pooling separate univariate sensitivity and specificity models does not capture the negative correlation that can arise from varying diagnostic positivity thresholds, although the predominantly binary IHC protocols reduce the scope of this issue. Low study numbers precluded credible meta-regression or diagnostic publication-bias testing.

FISH was used as the common comparator because the review question concerned replacement of FISH, but an imperfect FISH reference causes some true fusion-positive IHC results to be counted as false positives. Further, the treatment relevance of an ALK rearrangement depends on expression, fusion architecture and clinical context. Studies with discordance-focused NGS adjudication provide biologically valuable detail, but such sequencing was not performed for every paired specimen. For these reasons, pooled IHC–FISH accuracy is not equivalent to absolute accuracy for therapeutic response prediction.

## 5. CONCLUSION

ALK immunohistochemistry demonstrates high FISH-referenced specificity and substantial overall agreement in published NSCLC cohorts, supporting its use as a validated first-line alternative to routine FISH. Its diagnostic performance depends on assay quality, patient selection and interpretation of discrepant results. A diagnostic strategy combining reliable IHC with selective orthogonal molecular confirmation provides a defensible approach to ALK fusion detection and treatment selection.

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