



Principles of Analytic Validation of Immunohistochemical Assays: Guideline Update

Statements and Strengths of Recommendations

SUMMARY OF STATEMENTS

Guideline Statement	Category
<p>1. Laboratories must analytically validate all laboratory developed immunohistochemistry (IHC) assays and verify all FDA-cleared IHC assays before reporting results on patient tissues.</p> <p><i>Note:</i> A validation study design may include but is not necessarily limited to, such means as the following:</p> <ul style="list-style-type: none">• Comparing the new assay's results with the expected architectural and subcellular localization of the antigen• Comparing the new assay's results with the results of prior testing of the same tissues with a validated/verified assay in the same laboratory• Comparing the new assay's results with the results of testing the same tissues in another laboratory using a validated/verified assay• Comparing the new assay's results with results of a non-immunohistochemical method• Comparing the new assay's results with the results from testing the same tissues in a laboratory that performed testing for a clinical trial• Comparing the new assay's results against percent positive rates documented in published clinical trials• Comparing the new assay's results to IHC results from cell lines that contain known amounts of protein• Comparing previously graded tissue challenges from a formal proficiency testing program (if available) with the graded responses	Good Practice Statement
<p>2. For initial analytic validation/verification of every assay used clinically, laboratories should achieve at least 90% overall concordance between the new assay and the comparator assay or expected results.</p>	Strong Recommendation
<p>3. For initial analytic validation of nonpredictive laboratory-developed assays, laboratories should test a minimum of 10 positive and 10 negative tissues. When the laboratory medical director determines that fewer than 20 validation cases are sufficient for a specific marker (eg, rare antigen), the rationale for that decision needs to be documented.</p> <p><i>Note:</i> The validation set should include high and low expressors for positive cases when appropriate and should span the expected range of clinical results (expression levels) for markers that are reported using either a semiquantitative or numerical scoring system.</p>	Good Practice Statement

<p>4. For initial analytic validation of all laboratory-developed predictive marker assays, laboratories should test a minimum of 20 positive and 20 negative tissues. When the laboratory medical director determines that fewer than 40 validation tissues are sufficient for a specific marker, the rationale for that decision needs to be documented.</p> <p><i>Note:</i> The validation set should include high and low expressors for positive cases when appropriate and should span the expected range of clinical results (expression levels) for markers that are reported using either a semiquantitative or numerical scoring system.</p>	Good Practice Statement
<p>5. For initial analytic verification of all unmodified FDA-approved predictive marker assays, laboratories should follow the specific instructions provided by the manufacturer. If the package insert does not delineate specific instructions for assay verification, the laboratory should test a minimum of 20 positive and 20 negative tissues. When the laboratory medical director determines that fewer than 40 verification tissues are sufficient for a specific marker, the rationale for that decision needs to be documented.</p> <p><i>Note:</i> The validation set should include high and low expressors for positive cases when appropriate and should span the expected range of clinical results (expression levels) for markers that are reported using either a semiquantitative or numerical scoring system.</p>	Good Practice Statement
<p>6. For initial analytic validation of laboratory-developed assays and verification of FDA-approved/cleared predictive immunohistochemical assays with distinct scoring schemes (eg, HER2, PD-L1), laboratories should separately validate/verify each assay-scoring system combination with a minimum of 20 positive and 20 negative tissues. The set should include challenges based on the intended clinical use of the assay.</p>	Strong Recommendation
<p>7. For laboratory-developed assays with both predictive and nonpredictive applications using the same scoring criteria, laboratories should treat these assays as predictive markers and test a minimum of 20 positive and 20 negative cases.</p> <p><i>Note:</i> See Statement 4 for additional information.</p>	Good Practice Statement
<p>8. Laboratories should use validation tissues that have been processed using the same fixative and processing methods as cases that will be tested clinically, when possible.</p>	Good Practice Statement
<p>9. For analytic validation of IHC performed on cytologic specimens that are not fixed in the same manner as the tissues used for initial assay validation, laboratories should perform separate validations for every new analyte and corresponding fixation method before placing them into clinical service.</p> <p><i>Note:</i> Such cytologic specimens include (but are not necessarily limited to) the following:</p> <ul style="list-style-type: none"> • air-dried and/or alcohol-fixed smears • liquid based cytology preparations • alcohol-fixed cell blocks • specimens collected in alcohol or alternative fixative media that are postfixed in formalin 	Conditional Recommendation
<p>10. A minimum of 10 positive and 10 negative cases is recommended for each validation performed on cytologic specimens, if possible. The medical director should consider increasing the number of cases if predictive markers are being validated. If the minimum of 10 positive and 10 negative cases is not feasible, the rationale for using fewer cases should be documented.</p>	Good Practice Statement

<p>11. If IHC is regularly done on decalcified tissues, laboratories should test a sufficient number of such tissues to ensure that assays consistently achieve expected results. The laboratory medical director is responsible for determining the number of positive and negative tissues and the number of predictive and nonpredictive markers to test.</p>	<p>Good Practice Statement</p>
<p>12. Laboratories should confirm assay performance with at least 1 known positive and 1 known negative tissue when a new antibody lot is placed into clinical service for an existing validated assay (a control tissue with known positive and negative cells is sufficient for this purpose).</p>	<p>Good Practice Statement</p>
<p>13. Laboratories should confirm assay performance with at least 2 known positive and 2 known negative tissues when an existing validated assay has changed in any one of the following ways:</p> <ul style="list-style-type: none"> • Antibody dilution • Antibody vendor (same clone) • Incubation or retrieval times (same method) 	<p>Good Practice Statement</p>
<p>14. Laboratories should confirm assay performance by testing a sufficient number of tissues to ensure that assays consistently achieve expected results when any of the following have changed:</p> <ul style="list-style-type: none"> • Fixative type • Antigen retrieval method (eg, change in pH, different buffer, different heat platform) • Detection system • Tissue processing equipment • Automated testing platform • Environmental conditions of testing (eg, laboratory relocation, laboratory water supply) <p>The laboratory medical director is responsible for determining how many predictive and nonpredictive markers and how many positive and negative tissues to test.</p>	<p>Good Practice Statement</p>
<p>15. Laboratories should run a full revalidation (equivalent to initial analytic validation) when the antibody clone is changed for an existing validated assay.</p>	<p>Good Practice Statement</p>

Abbreviations: FDA, United States Food and Drug Administration; HER2, human epidermal growth receptor 2; PD-L1, programmed death receptor-1

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