COLLEGE of AMERICAN PATHOLOGISTS

Principles of Analytic Validation of Immunohistochemical Assays

Summary of Recommendations

Guideline Statement	Strength of Recommendation
1. Laboratories must validate all IHC tests before placing into clinical service. *Note*: Such means include (but are not necessarily limited to): Correlating the new test's results with the morphology and expected results; Comparing the new test's results with the results of prior testing of the same tissues with a validated assay in the same laboratory; Comparing the new test's results with the results of testing the same tissue validation set in another laboratory using a validated assay; Comparing the new test's results with previously validated non-immunohistochemical tests; or Testing previously graded tissue challenges from a formal proficiency testing program (if available) and comparing the results with the graded responses.	Recommendation
2. For initial validation of every assay used clinically, with the exception of HER2/*neu*, ER, and PgR (for which established validation guidelines already exist), laboratories should achieve at least 90% overall concordance between the new test and the comparator test or expected results. If concordance is less than 90%, laboratories need to investigate the cause of low concordance.	Recommendation
3. For initial analytic validation of nonpredictive factor 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. *Note*: 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 quantitatively.	Expert Consensus Opinion
4. For initial analytic validation of all laboratory-developed predictive marker assays (with the exception of HER2/*neu*, ER and PgR), 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. *Note*: Positive cases in the validation set should span the expected range of clinical results (expression levels). This recommendation does not apply to any marker for which a separate validation guideline already exists.	Expert Consensus Opinion
5. For a marker with both predictive and nonpredictive applications, laboratories should validate it as a predictive marker if it is used as such.	Recommendation

Source: Fitzgibbons PL, Bradley LA, Fatheree LA, et al. Principles of analytic validation of immunohistochemical assays: Guideline from the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Pathol Lab Med*. 2014;138(11):1432–1443.

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6. When possible, laboratories should use validation tissues that have been processed using the same fixative and processing methods as cases that will be tested clinically.	Recommendation
7. If IHC is regularly done on cytologic specimens that are not processed in the same manner as the tissues used for assay validation (eg, alcohol-fixed cell blocks, air-dried smears, formalin postfixed specimens), laboratories should test a sufficient number of such cases to ensure that assays consistently achieve expected results. The laboratory medical director is responsible for determining the number of positive and negative cases and the number of predictive and nonpredictive markers to test.	Expert Consensus Opinion
8. 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 issues and the number of predictive and nonpredictive markers to test.	Expert Consensus Opinion
9. Laboratories may use whole sections, TMAs and/or MTBs in their validation sets as appropriate. Whole sections should be used if TMAs/MTBs are not appropriate for the targeted antigen or if the laboratory medical director cannot confirm that the fixation and processing of TMAs/ MTBs is similar to clinical specimens.	Recommendation
10. When a new reagent lot is placed into clinical service for an existing validated assay, laboratories should confirm the assay's performance with at least 1 known positive case and 1 known negative case.	Expert Consensus Opinion
11. Laboratories should confirm assay performance with at least 2 known positive and 2 known negative cases when an existing validated assay has changed in any one of the following ways: Antibody dilution; Antibody vendor (same clone); Incubation or retrieval times (same method).	Expert Consensus Opinion
12. Laboratories should confirm assay performance by testing a sufficient number of cases to ensure that assays consistently achieve expected results when any of the following have changed: Fixative type; Antigen retrieval method (eg, change in pH, different buffer, different heat platform); Antigen detection system; Tissue processing or testing equipment; Environmental conditions of testing (eg, laboratory relocation); Laboratory water supply. The laboratory medical director is responsible for determining how many predictive and nonpredictive markers and how many positive and negative tissues to test.	Expert Consensus Opinion
13. Laboratories should run a full revalidation (equivalent to initial analytic validation) when the antibody clone is changed for an existing validated assay.	Expert Consensus Opinion
14. The laboratory must document all validations and verifications in compliance with regulatory and accreditation requirements.	Expert Consensus Opinion

Abbreviations: IHC, immunohistochemistry; ER, estrogen receptor; PgR, progesterone receptor; TMA, tissue microarray; MTB, multitissue block